Review Article

Pre-clinical pharmacology: An important aspect in homoeopathic research

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Abstract

Background: Before the 20th century, research in Homeopathy was limited either for its proving in healthy human volunteers or to its clinical verification/clinical efficacy in diseased individuals. However, in recent years, there has been an increased trend in the number of pre-clinical studies aimed to evaluate the pharmacological activity produced by homeopathic medicines. The objective of this review is to make a systemic compilation of results of experimental pharmacological findings of homeopathic medicines, both *in vitro* and in animal models, and to present the same in a summarised form. **Methods:** Articles published up to March 2017 having information of *in vitro* and *in vivo* studies using homeopathic medicines were collected from the PubMed database, review articles, scientific reports, research articles, thesis, online information extracted from Medline, etc. to compile this review. The articles from the most common therapeutic areas such as antimicrobial effect, central nervous system disorders, anti-inflammatory and analgesic, anticancer, wound healing, antiasthmatic and liver toxicity and other therapeutic areas such as diabetes and malaria were included in this review. **Results:** This review article not only provides the scientific approaches applied in Homeopathy research but also provides evidence-based information on pharmacological effects of dilutions/potencies as well as mother tincture of different homeopathic drugs. This review article also reveals the use of improved methodology, molecular techniques and analytical part adopted in recently published research articles to understand the mechanism of action of homeopathic medicines. **Conclusion:** Homeopathy has substantial scope in pre-clinical research where therapeutic and biological effects of homeopathic medicines with proper mechanism of action can be traced out with the use of modern molecular techniques in *in vivo* and *in vitro* experiments.

Keywords: Basic research, Homeopathy, *In vitro* experiment, Pre-clinical, Review

Introduction

Homeopathic medicines work by following the basic principle of '*Similia Similibus Curantur*' ('let like be cured by like'), according to which a substance taken in large dose that produces the symptoms of an illness in healthy subject will have the reverse effect if taken by ill people in a very minute dose.[1] Preparation of homeopathic medicines plays an important role to achieve better effectiveness. The process of manufacturing homeopathic medicines involves trituration/decoction/maceration in lactose and/or serial dilution in ethanol-water solutions and succession in glass vials. A mother tincture (MT) is the first stage in the preparation of a homeopathic dilution prepared from source materials of typically plant, mineral or animal in nature. Common dilution factors are 1 part source to 9 parts diluent (1/10, decimal, D or X potencies) and 1 part source to 99 parts diluent (1/100, centesimal or C potencies).[2]

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In Homeopathy, there are a number of medicines which are being clinically used/verified for reducing the severity of or completely eliminating a wide variety of ailments including infections, allergies, asthma, autoimmune diseases, attention-deficit hyperactivity disorder, rheumatic problems and metabolic diseases on the basis of subjective and objective symptoms of individual patient.[3] However, before the 20th century, research in Homeopathy was mainly reported in the healthy human volunteers for proving drug symptoms and for clinical verification/clinical efficacy of drugs in diseased individuals, but no effort was made to establish their beneficial

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effect scientifically. However, advancement in scientific knowledge including animal experimentation, *in vitro* and molecular biology techniques as recent evaluating parameters, many scientists are actively involved in conducting both *in vitro* and *in vivo* experimental research and elucidating the therapeutic efficacy by which these medicines produce their effects.

In medical science, one of the most important fundamental prerequisites for the introduction of a new drug or formulation in clinical practice is to evaluate thoroughly the therapeutic efficacy, mode of action, metabolic pathway and the level of safety of the drugs in laboratory animals through pre-clinical trials. In terms of regulatory aspects in the Indian scenario, homeopathic drugs are also included in the Drugs and Cosmetics Act, 1940. Therefore, it becomes imperative/mandatory to generate the data of each drug for its therapeutic/toxic effects in laboratory animals under scheduled 'Y' of Drug Controller General of India before the drug is subjected for drug proving research in healthy human volunteers. Moreover, to establish a scientific basis for actions of these medicines, there is a need for experimental research by conducting a series of experiments both *in vitro* and *in vivo* models.

Therefore, the main objectives of this review are to compile important experimental pharmacological findings and summarise results of both *in vitro* and animal studies of most common therapeutic areas such as antimicrobial effect, central nervous system (CNS) disorders, anti-inflammatory and analgesic effects, anticancer effect, wound healing activity, antiasthmatic activity, effect on liver toxicity and effect on other therapeutic areas such as diabetes, malaria, Leishmaniasis and kidney stone so that information on mechanistic approach of homeopathic drugs could also be traced out. To achieve this target, eighty-nine articles which include reviews, scientific reports, research articles, mostly from PubMed, thesis, online information extracted from Medline published between March 2001 and March 2017 (except a few articles published before 2001) and having data of pre-clinical and *in vitro* studies, where homeopathic medicines had been used in the form of MT and dilutions were collected [Table 1]. Although no definite explanation has been put forward till date about the mechanisms and actions of homeopathic medicines, *in vitro* and *in vivo* studies along with molecular studies discussed in this review article throw some light on the acceptance of health claim of this system scientifically.

Antifungal Effects

In the past few decades, incidences of fungal infections are increasing worldwide. It is not only affecting human health but badly affecting agriculture also. Morbidity and mortality among patients with compromised immune function due to fungal infections are very common. The majority of clinically used antifungals have limitations in terms of toxicity, efficacy, resistance to different fungal strain and cost. To combat this situation, extensive research on alternative medicines such as Homeopathy is increasing very fast. Many studies have been published which projected the role of Homeopathy to overcome the fungal infection. It has been reported that various potencies of *Sulphur iodatum*, *Bacillinum*, *Petroleum* and *Mezereum* were effective against two plant pathogenic fungi, *Alternaria tenuis* and *Curvularia lunata*.[1] The *in vitro* and *in situ* testing of *Sulphur iodatum* 1M and *Petroleum* 30C has been reported to be effective against cellulytic fungi *Aspergillus niger*.[3] *Belladona* 30C, 1M, *Sulphur* 30C, *Bryonia* 200C, *Carbo vegetabilis*, *Graphites* 30C, 1M, *Mercurius solubilis* 6C, *Phosphorus* 30C, *Thuja* 30C and 1M have shown preventive effect against *Aspergillus flavus* strain II, the contaminant of linseed grains under *in vitro*.[4] Out of *Commiphora molmol*, *Hydrastis canadensis* and *Warburgia salutaris* MTs tested against human pathogenic fungi *Candida albicans in vitro,* only the MT of *H. canadensis* showed to inhibit the growth of *C. albicans*.[5] Gupta and Srivastava, 2006, reported the effect of *Thuja occidentalis* MT, 30C, 200C, 1M, 10M and 50M on *in vitro* antifungal effect against human pathogenic fungi, namely *A. flavus* and *A. niger*. *T. occidentalis* 30 and 200C showed maximum inhibitory against *A. flavus* growth as compared to MT, 10M and 50M while 1M was not able to inhibit the growth of tested organism. Against *A. niger*, only *T. occidentalis* 50M has been shown to exhibit inhibitory activity, whereas no effect was seen in MT, 30C, 200C, 1M and 10M.[6] *In vitro* testing of 30C, 200C and 1M potencies of *Tellurium*, *Psorinum*, *Rumex* and *Graphites* and 30C, 200C, 1M and 10M potencies of *Arsenicum* has been shown to exhibit antifungal effect under *in situ* condition, also on paper and textile materials inoculated with spores of cellulolytic fungi, namely *A. niger*, *Chaetomium globosum* and *Emericella nidulans.* Out of five drugs tested, *Arsenicum* 30C, 200C and *Rumex* 200C and 1M showed maximum effectiveness. These two drugs have also shown to control the growth, fungi on the pages of a deteriorated book.[7] *Sulphur, Petroleum*, *Arsenicum* and *Rumex* were found effective in controlling the growth of testing fungi under *in vitro* conditions. These homeopathic drugs were also found highly effective in controlling fungal growth on paper pieces with maida and without maida paste, museum and art objects of organic nature as paper and textile under *in situ* conditions and no adverse effects on the tested materials.[8]

Antibacterial Effects

The activity of many homeopathic medicines against bacterial infection proved clinically; however, only few studies are available *in vitro* which predict the antibacterial potential of homeopathic medicines. *In vitro* antibacterial activity of MTs of *Psidium guajava*, *Eichhornia crassipes, Valeriana officinalis, Alpinia galanga* and *Chenopodium ambrosioides* was screened against *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Citrobacter freundii* and *Staphylococcus aureus*. Among the drugs tested, *P. guajava* MT showed inhibitory activity against the growth of all the bacterial strains, and maximum effect was observed against *S. aureus* whereas

Table 1: Compilation of some of the important homeopathic studies on fundamental research involving animals and cell line

Species	Treatment/potency	Model	Observations	References
Mice	*P. nigricans* 3X and 6X	EPM and OFT models	Anxiolytic activity	[18]
Mice	*Aconitum, N. vomica, G. sempervirens, Belladonna, Argentum nitricum, Tabacum*, all at 5C	Light-dark test and open-field test	*G. sempervirens* showed anxiolytic activity	[20]
Mice	*Gelsemium* 3C	Hole-board test	Anxiolytic activity	[24]
Mice	*Belladonna, Gelsemium* and *Poumon histamine* (5C, 9C, 15C)	Staircase test and light-dark test	Neurotropic and protective effects on behavioural and gastric alterations of low doses of *Gelsemium* and *Belladonna* Immunoprotective and gastroprotective effect by *Poumon histamine*	[26]
Mice	*Argentum metallicum* 30C, 200C and 1M	PTZ, PTX, STR, INH and MES	Anticonvulsant activity of *Argentum metallicum* 200 CH and 1 M	[28]
Mice	*T. aphrodisiaca* MT	Elevated plus-maze model of anxiety	Anxiolytic activity	[29]
Rats	*L. clavatum* (Lyc) MT and 200C	STZ-induced memory impairment	Improved learning and memory	[30]
Rats	*Traumeel*® S containing *Arnica montana*	Adjuvant arthritis model and carrageenan-induced paw oedema	Anti-inflammatory activity	[33]
Cell culture	*Rhus tox* (4X, 30C and 200C)	Primary mouse cultured chondrocytes	Increased mRNA expression of COX-2 and inflammatory responses	[34]
Rat	*Apis, Lachesis* and *Phosphorus*	Carrageenan-induced oedema rat model	No significant effect	[35]
Rat	*Rhus tox* (6, 12, 30 and 200C), *Causticum* (6, 12, 30 and 200C)	Carrageenan-induced paw oedema in rats	Reduced inflammation	[37,38]
Rat	*Guaiacum officinale* MT and two dilutions: *Gua* 30C and *Gua* 200C	FCA, RA model and antioxidant assays	Anti-rheumatic and antioxidant activity	[39]
Rat	*Arnica montana* 6C	Carrageenan-induced oedema	Reduced oedema and decreased signs of inflammation	[40,41]
Mice	Dexamethasone 7C, 15C	Inflammation induced by carrageenan	Partial anti-inflammatory activity	[42]
Rats	MT of *Ricinus communis , Rauwolfia serpentina, Bellis perennis, Curcuma longa, Terminalia arjuna* and *Tribulus terrestris*	Carrageenan-induced inflammation, CFA-induced arthritis	Potent anti-inflammatory activity of *Curcuma longa* and *Tribulus terrestris*	[43]
Naive mice	*Anax-i* 30C and *Anax-i* 200C	FST, EPM test, HP test and open-field test, NPY1 receptor expression	Antidepressant, anxiolytic and analgesic effect	[44]
Mice	1M potency of *Kali muriaticum, Phytolacca, hydrastis, Zincum metallicum, Conium* and *Carcinosin*	Ehrlich tumour cell-induced ascites	Lifespan increased	[45]
Mice	*Ruta graveolens* 200C and HC-30 200C	DLA induced and EAC as well as solid tumours	Increased the lifespan, and reduced in solid tumour volume	[46,47]
Mice	*Thuja* 1M, *Lycopodium* 1M and *Hydrastis* 1M	B16F-10 melanoma-bearing animals	Anti-metastatic activity	[48]
Mice	*Lycopodium* 30C	Chemical-induced (p-DAB and PB) hepatocarcinogenesis	Protection against hepatocarcinoma	[49]
Mice	*Chelidonium* 200C	Carcinogenesis induced by azo dye and p-DAB	Inhibition of carcinogenesis	[50,51]
Mice	*Carcinosin* and *Chelidonium* at 200C	p-DAB-induced hepatocarcinogenesis	Inhibition of hepatocarcinogenesis	[52]
Mice	Combination therapy with *Natrum Sulphuricum* 30C and *Carcinosin* 200C	Hepatocarcinogenesis induced by chronic feeding of p-DAB and PB in mice	Antitumour efficacy decreased the elevated chromosomal, nuclear and sperm head anomalies and also the various toxicity indices	[53]
Rat	*Ruta, Hydrastis, Thuja* and *Lycopodium* at 200C	Hepatocellular carcinoma in rats induced by-NDEA	Inhibit hepatocellular carcinoma	[54]
Mice	*Ruta* 200c and *Phosphorus* 1M	3-methylcholanthrene-induced sarcoma in mice	Reduced incidence of 3-methylcholanthrene-induced sarcomas and increase the lifespan of mice	[54]

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Species	Treatment/potency	Model	Observations	References
Mice	*Thuja occidentalis*, *Carcinosinum* and *Ruta graveolens* 1M, 200C and 30C	Immunomodulatory effect in mice	Increased haematological, haematopoietic parameters, circulating antibody titre, number of PFC. Enhanced proliferation of B- and T-lymphoid cells	[55]
Human renal adenocarcinoma) cells, human colorectal and breast carcinoma cell lines	*Sarsa parilla, Ruta graveolens* and *Phytolacca decandra* MT and various dilutions	*In vitro* cancer cell model	Cytotoxic and anti-proliferative activity	[56]
HeLa cells	*Lycopodium* potency 5C and 15C	*In vitro* cancer cell model	Induce apoptosis in cancer cells	[57,58]
HeLa cells	*Conium maculatum*	MTT Cell Proliferation Assay, LDH enzyme activity, intercellular ROS activity, Morphological study, Fluorescence microscopic study of DNA damage	Potent anticancer by apoptosis through the ROS-mediated pathway.	[59]
HepG2 (liver cancer) cell line and WRL-68 (normal liver cell line)	Hepatitis C 30C (Hep C 30) nosode	MTT Cell Proliferation Assay	Anticancer activity against liver cancer cells	[60]
HepG2 cells	AAI	Cytotoxicity and genotoxicity assay	Inhibited cell proliferation	[61]
Mouse/B16F10 melanoma cells	CHM mixture of *Aconitum napellus, Arsenicum album*, Asafoetida, *Calcarea carbonica, Conium maculatum, Ipecacuanha, Phosphorus, Rhus tox, Silica, Sulphur* and Thuja occidentalis	Mouse lymph node lymphocyte assay	Immunostimulation of lymphocytes, enhanced tumouricidal performance	[62]
Breast cancer (MDAMB231 and MCF7) and noncancerous (HEK 293) cell lines	*Terminalia chebula* (3X, 6C and 30C)	MTT Cell Proliferation Assay	Anticancer activity against breast cancer cell lines	[63]
Normal (peripheral blood mononuclear cells) and A375 skin melanoma cells	*Phytolacca decandra* (MT)	MTT Cell Proliferation Assay LDH enzyme activity, Intercellular ROS activity, Morphological study, Fluorescence microscopic study of DNA damage	Anticancer potentials through activation of caspase-mediated signalling and ROS generation	[64]
HeLa cell	HC-30, *Marsdenia condurango* (Condu-30)	Microarray gene expression studies	High dilutions modified and altered gene expression profiles in HeLa cell lines	[65]
Perfused lung cells of Swiss albino mice	*Thuja* (30 C)	MTT Cell Proliferation Assay Intercellular ROS activity, protein isolation Hsp-90 activity assay morphological study	Increased cell viability of BaP-intoxicated cells, Ameliorated BaP-induced toxicity, stress and DNA damage in lung cells of mice	[66]
Mouse NIH 3T3 fibroblasts	*Arnica montana, Calendula officinalis, Hypericum perforatum* at 4X and *Symphytum officinale* at 6X	*In vitro* scratch assay	*In vitro* wound closure by 59.5% and exerted *in vitro* wound closure potential in NIH 3T3 fibroblasts	[69]
Mice	*Silica* 5C, 30C, 200C	Chronic wound	Wound significantly smaller and healed faster	[70]
Rats	*Hypericum perforatum* and *Arnica montana*	Skin surgical incision surgically induced on the back of Wistar rats	Wound healing activity	[71]
Monocyte macrophage human THP-1 cell line	*Arnica montana* (3C, 5C, 9C, 15C)	Cell viability assay *In vitro* wound healing (scratch) assay	Expression of gene (up- and down-regulation) Accelerating effect on cell migration (bone marrow derived macrophages)	[72]

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Table 1: Contd...

Species	Treatment/potency	Model	Observations	References
Guinea pigs	*Blatta orientalis* MT	Anaphylaxis model	Protection against bronchospasm	[73]
Rat	*Blatta orientalis* MT	Anaphylaxis models	Reduction in mesenteric mast cell degranulation, serum IgE level and eosinophil cell count	[73]
Rats	Chel 30C and 200C	p-DAB- and PB-induced hepatotoxicity	Improvement in hepatotoxicity	[74]
Rats	*L. clavatum* 30C	Paracetamol-induced liver toxicity	Reduced hepatic lesions and decrease number of acinar zone 1	[75]
Rats	Combination of potentised *Cholesterinum* (30 and 200C) with *Natrum sulphuricum* (30 and 200C)	p-DAB- and PB-induced hepatotoxicity	Modulation in biochemical parameters, namely AST, ALT, AlkP, GGT, LDH	[76]
Mice	*Arsenicum album* 6C and 30C	Arsenic toxicity	Both the potencies showed potential against arsenic intoxication	[77]
Mice	*Eupatorium perfoliatum* (0/6, 30C) and *Arsenicum album* (0/6, 30C)	Rodent malaria model	*Eupatorium perfoliatum* 60% and *Arsenicum album* (0/6) showed 70% inhibitory effect on parasite multiplication	[78]
Mice	*Thymulin* 5C	Murine Leishmaniasis assay	Improved B1-cell activation and phagocytosis efficiency	[79,80]
Rat	*Berberis vulgaris* 200C	EG-induced urolithiatic	Anti-urolithiasis activity	[81]
Rats	*Syzygium jambolanum* and *Cephalandra indica* in MT, 6C and 30C	High fat and fructose-induced type 2 diabetes mellitus, *In vitro* models	Antidiabetic effects, improved insulin action	[82,83]
BALB/c mice	*Cantharis* 6C	*Escherichia coli*-induced cystitis in female BALB/c mice	*Cantharis* reversed the balance of inflammatory cells and cytokines showed immune modulation	[85]
Mice	*Kalium causticum, Conium maculatum* and *L. clavatum* (13C)	*Trypanosoma cruzi*-infected mice	Significant decreased in blood parasites and increased animal survival by *L. clavatum*	[86]
Mice	Influenza haemagglutinin antigen (7 µg/200 µl)	Influenza antigen challenge in BALB/c mice	Acquired immune antiviral Response regulation changed	[87]
Mouse ES cells	*Nux vomica* and *Sepia* (30C)	Mouse ES cell assay	No toxic effect	[88]

P. nigricans: Pulsatilla nigricans; N. vomica: Nux vomica; G. sempervirens: Gelsemium sempervirens; T. aphrodisiaca: Turnera aphrodisiaca; L. clavatum: Lycopodium clavatum; EPM: Elevated plus maze; OFT: Open-field test; PTZ: Pentylenetetrazole; PTX: Picrotoxin; STR: Strychnine; INH: Isoniazid; MES: Maximal electroshock; MT: Mother tinctures; STZ: Streptozotocin; FCA: Freund's complete adjuvant; RA: Rheumatoid arthritis; CFA: Complete Freund's Adjuvant; FST: Forced swim test; EPM: Elevated plus-maze; HP: Hot plate; DLA: Dalton's lymphoma ascites; EAC: Ehrlich ascites carcinoma; p-DAB: p-dimethylaminoazobenzene; NDEA: N'-nitrosodiethylamine; PFC: Plaque-forming cells; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LDH: Lactate dehydrogenase; ROS: Reactive oxygen species; AAI: Aristolochic acid I; CHM: Brazilian complex homeopathic medication; HC-30: *Hydrastis canadensis*; BaP: Benzo(a)pyrene; Chel: *Chelidonium majus*; PB: Phenobarbital; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltransferase; EG: Ethylene glycol; ES: Embryonic stem; AlkP: Alkaline phosphatase; IgE: Immunoglobulin E

rest of the medicines did not show such effect against any strain of bacteria tested.[9] MT, 1M, 10M and CM (100M) potencies of *Apis mellifica, Arsenic album, Capsicum, Cantharis, Lycopodium, Mercurius solubilis, Medorrhinum* and *Pulsatilla* had variable activity against the bacterial stains, *Escherichia coli, Klebsiella, Proteus* and *Staphylococcus coagulase* negative. Bacterial growth inhibition shown by these medicines was based on strain as well as dose dependent.[10] *In vitro* studies with MTs prepared from desert plants of Pakistan have shown antibacterial activity against two Gram positive (*Bacillus subtilis* and *S. aureus*) and four Gram negative (*E. coli*, *P. aeruginosa*, *Salmonella typhi* and *Shigella sonnei*). *Boerhavia diffusa* MT showed moderate activity against *S. aureus*, *P. aeruginosa* and *S. sonnei* and less activity was observed against *E. coli*. MT of *Chrozophora plicata* showed highly effective results against *S. aureus*, *E. coli* and *P. aeruginosa* whereas *Echinops echinatus* MT showed highly effective against *Salmonella typhi*. *Heliotropium europaeum* and *Tamarix aphylla* MT exhibited maximum activity only against *B. subtilis*. Among the screened drugs, *H. europaeum*, *C. plicata* and *T. aphylla* were more effective against above-tested microorganisms. However, *B. diffusa* and *E. echinatus* were less effective against tested pathogenic bacteria.[10]

Resistance to antibiotics is a major public health concern worldwide. New treatment options such as Homeopathy could be one such option to overcome this problem. In a study where the effect of homeopathic medicine *Belladonna* 6C and a nosode (biotherapy) 30C prepared from a multi-drug resistant bacterial species, methicillin-resistant *S. aureus* (MRSA), on the same bacterium, was tested on MRSA National Collection of Type Cultures 10442. *In vitro* growth of MRSA was significantly inhibited in the presence of *Belladona* and nosode 6C and 30C and

with the combination of *Belladonna* or nosode 6C and 30C and oxacillin. *Belladonna* 30C and nosode 6C and 30C significantly decreased bacterial DNAse production and reduced red blood cell lysis and became more vulnerable to the action of the antibiotic oxacillin.[11]

Antiviral Effects

The homeopathic preparation of Engystol which contains *Vincetoxicum hirundinaria* (swallow-wort) and *Sulphur* showed reductions in DNA viruses adenovirus type 5 and herpes simplex type 1 (HSV-1) and RNA viruses respiratory syncytial virus (RSV) and human rhinovirus (HRV) infections by 73% (A5), 80% (HSV-1), 37% (RSV) and 20% (HRV), respectively.[12] The homeopathic preparation Gripp-Heel which contains fixed combination of homeopathic agents, namely *Aconitum* (monkshood), *Bryonia* (bryony), *Eupatorium perfoliatum* (water hemp), *Lachesis* (bushmaster snake venom) and *Phosphorus* demonstrated dose-dependent *in vitro* antiviral activity (significant reduction of infectivity by 20%–40%) against human pathogenic enveloped and non-enveloped RNA and DNA viruses such as human herpesvirus 1, human adenovirus C serotype 5, influenza A virus, human RSV, human RSV, human parainfluenza virus 3, HRV B serotype 14 and human coxsackievirus serotype A9 in chorioallantoic membrane (CAM) of chick egg.[13] *In vitro* screening of *Belladonna* 3, 12, 30 and 200C showed significant inhibition of Japanese encephalitis virus infection.[14] The homeopathic preparation prepared from a mixture of extract of *Cactus grandiflorus*, *Aloe socotrina, Abies nigra, Arnica, Lycopodium*, Lachesis and mineral (*Calcium carbonate*) products pre-incubated with peripheral blood mononuclear cells (PBMCs) isolated from patients with either chronic fatigue syndrome or acquired immunodeficiency syndrome were found effective in stimulating *in vitro* natural killer function.[15]

Effect on Central Nervous System Activity

Diseases related to CNS are one of the life-threatening problems and high prevalence in modern society. Side effects of allopathic medicines such as psychomotor impairment, dependence and potentiation of effects of other central depressant drugs are sometimes non-reversible and make the condition worse.[16] The lifestyle changes also played a significant role in the development of CNS disorders. Anxiety, depression, memory loss, epilepsy, etc. are among the few diseases which affect human life extensively. Due to the high cost of treatment and side effects of allopathic medicines, attention on alternative medicines is increasing day by day in the scientist due to better effective results and less side effect.[16] Homeopathy is one of the systems in which experimental research on CNS disorders is increasing significantly nowadays due to cost-effectiveness and the least/absence of the side effect. With conventional drugs, dosages and adverse reactions are generally studied in animal models before undertaking human trials. In Homeopathy, the opposite has been true: trials on humans have only recently been followed up with tests on animals. However, to elucidate the indications, limitations and mechanisms of action of homeopathic medicines, scientific fraternity should focus more on pharmacological studies.[17] The past few years have seen an increase in the number of pre-clinical (*in vitro* and animal) studies aimed at evaluating the pharmacological activity or efficacy in different CNS animal models.

Several models of anxiety-like behaviours have been developed and described in mice in order to test different anxiolytic drugs related to Homeopathy. Homeopathic preparations of *Pulsatilla nigricans, Arsenicum album, Calcarea carbonica* and *Lycopodium clavatum* are commonly used for the treatment of anxiety and associated disorders.[16-18] Effect of *Sulphur* and *Pulsatilla nigricans* on electroencephalography was used to evaluate the psychophysiological effect in healthy young adults on repeated olfactory administration.[19] The behavioural effects of some of the homeopathic preparations such as *Aconitum, Nux vomica, Belladonna, Argentum nitricum, Tabacum* and *Gelsemium* have been evaluated in mice models by many authors.[20]

The anxiolytic activity of homeopathic preparation *Pulsatilla nigricans* 3X and 6X was reported in Swiss albino mice and compared its activity with the standard anxiolytic drug, diazepam 1 mg/kg in elevated plus maze (EPM) and open-field test (OFT) models. Both diazepam and *Pulsatilla nigricans* showed significant anxiolytic activity in EPM and OFT test compared to control. The total number of entries and time spent in open arm in EPM was increased by both diazepam and *Pulsatilla nigricans*. The effect of 3X dilution of *Pulsatilla nigricans* was found greater than diazepam. In the OFT, the number of squares crossed, rearing and assisted rearing decreased with both diazepam and *Pulsatilla nigricans* compared to control and the anxiolytic effect of diazepam was greater than *Pulsatilla nigricans*. The anxiolytic effect is greater for the 3x dilution than a 6X dilution of *Pulsatilla nigricans*.[21] Magnani *et al*. 2008 reported the effects of *Aconitum, Nux vomica, Gelsemium sempervirens, Belladonna, Argentum nitricum* and *Tabacum*, all at 5C potency in hydroalcoholic (0.3%) solution administered by i. p. injection, screened for their potential effects on animal behaviour.[22] The anxiolytic-like effects of *Gelsemium sempervirens* 5C were reported in various experimental condition (light-dark test and OFT) in mice (CD1 or C57BL/6J). In this study, control mice were treated with 30% ethanol, v/v (vehicle), the same hydroalcoholic (0.3%) solution used to dilute the homeopathic medicines. Diazepam (1 mg/kg) was used as a reference drug. The result showed statistically significant effects of *Gelsemium sempervirens* in several "symptoms" of anxiety-like behaviour. Increased mean time spent in the illuminated compartment in light/dark test, the decrease of resting time and increased in distance travelled in the open arms of the plus maze or in the centre of the OF. In number of cases, the extent of responses to this medicine was comparable to the extent of the responses to diazepam.[23]

The mice tested using the hole-board test, *Gelsemium* 3C, and at a lesser extent, *Gelsemium* 5C reduced the number of exploration attempts, suggesting anxiolytic-like activity.[24] The study also showed that *Gelsemium* 5C, *Sempervirine nitrate* 5C (one of the active principles of *Gelsemium*) and *Argentum nitricum* 9C contrasted the effects of the anxiogenic compound RO 15-3505 (inverse agonist of benzodiazepines) in the labyrinth (plus-maze) test.[24] The same authors reported that RO 15-3505 decreased the affinity of the benzodiazepine receptors in mouse cortex and that this effect was contrasted and reversed by *Sempervirine nitrate* 5C.[25] More recently, it has been reported that in some but not all the experimental conditions *Belladonna*, *Gelsemium* and *Poumon histamine* (5C, 9C, 15C) reduced the stress-induced behavioural alterations of mice in staircase test and light-dark test.[26] However, those results were obtained as reversal of the effects of severe stress (conditioned paradigm) and the findings were highly variable according to the potency used and test performed. Two validated tests on animal models, namely the Light Dark (LD) choice test and the OF test, were used in order to acquire various behavioural parameters widely used in neuropsychopharmacology for drug screening.[24] As reinforced by recent published data, *Gelsemium sempervirens* has been reported for its neurotropic effects in the limbic system (hippocampus and amygdala or H-A) and spinal cord (SC) slices of rat and found at extremely low dilution (5C) of *Gelsemium sempervirens* and of its active principle *Gelsemine* to enhance the production of the neurosteroid allopregnanolone (5a, 3a-tetrahydroprogesterone), a highly active stimulator of GABAa receptors and of inhibitory signaling in the CNS.[27]

Studies on epilepsy have also been done using homeopathic medicines. Anticonvulsant activity of *Argentum metallicum*, a homeopathic preparation, was performed to evaluate the anticonvulsant profile in various models of convulsion by assessing various behavioural and biochemical parameters in laboratory animals. Anticonvulsant activity of *Argentum metallicum* (30C, 200C and 1M) was evaluated against pentylenetetrazole, picrotoxin, strychnine, isoniazid and maximal electroshock (MES)-induced convulsions in mice as well as an electrical kindling model in rats. Brain gamma-aminobutyric acid (GABA) level, nitric oxide (NO), total protein and xanthine oxidase (XO) level were also estimated as *in vitro* parameters. Diazepam and phenytoin were used as reference anticonvulsant drugs for comparison. The study proved that the *Argentum metallicum* exhibits its antiepileptic activity through GABAegic mechanism and by modulation of endogenous antioxidants such as NO and XO.[28]

Another plant *Turnera aphrodisiaca* Ward (Turneraceae) has been traditionally used for the treatment of anxiety, neurosis and as an aphrodisiac. MTs (85% ethanol extracts) of *Turnera aphrodisiaca* have also been used for the treatment of CNS disorders. Kumar *et al*., 2015, reported the anxiolytic activity of *Turnera aphrodisiaca* MTs formulated by three reputed manufacturers, namely National Laboratory, Kolkata, India (NLK), Dr. Willmar Schwabe, Germany (DWSG) and SBL Private Limited, Ghaziabad, India (SBL). Dried MTs of *Turnera aphrodisiaca* were subjected to anxiolytic activity evaluation at various doses, i.e. 50, 75, 100, 125 or 150 mg/kg p.o. in mice using EPM apparatus. Dried MTs exhibited significant anxiolytic activity at 50 mg/kg (NLK), 75 mg/kg (DWSG) and 125 mg/kg (SBL), respectively, with reference to control as well as standard (diazepam, 2 mg/kg p.o).[29]

In one of the study conducted by Kashif *et al*., 2015**,** effect of *Lycopodium clavatum* was explored in animal model of memory impairment and on cerebral blood flow in animal models in intracerebroventricularly (ICV) administered streptozotocin (STZ)-induced memory impairment in rats. STZ (ICV)-treated rats showed impairment in learning and memory. *Lycopodium* MT and 200C treated rats showed improvement in learning and memory which prove that *Lycopodium* may be used as a drug of choice in condition of memory impairment.[30]

Anti-inflammatory and Analgesic Effects

Over the last few years, there has been an increase in the number of pre-clinical (*in vitro* and animal) studies aimed at evaluating the pharmacological activity or efficacy of some homeopathic remedies under potentially reproducible conditions. One of the focuses is on scientific evaluation of homeopathic medicines in inflammatory and analgesic *in vitro* and animal models. Hence, many research studies are published with animal models of inflammation and analgesic.

High dilutions of *Atropa belladonna*[31] and *Phosphorus*[32] have been found to have slight *in vitro* inhibitory effect on neutrophil granulocytes, which play a fundamental role in acute inflammation. *In vitro* effect of *Traumeel*® S (a homeopathic dilution containing *Arnica montana* and other plant extracts as well as minerals) on two important cellular functions, namely superoxide anion production and human platelet were tested and found this drug did not affect either of these cellular functions, suggesting that its anti-inflammatory effects are not due to granulocytes and platelet inhibition.[33]

Homeopathic treatment with *Rhus tox* induced chondrocyte differentiation and inflammatory responses, such as COX-2 expression and PGE2 production, in primary cultured chondrocytes.[34] The induction of COX-2 expression is closely associated with the release of PGE2, and treatment with a COX-2 inhibitor reduces inflammation in animal models. Stimulation with different concentrations of *Rhus tox* increased the mRNA expression of COX-2, and stimulation with 30X *Rhus tox* showed the most prominent RNA expression in both Reverse transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR analyses. It was also observed that homeopathic dilutions of *Rhus tox* inhibited collagen type II expression, suggesting that *Rhus tox* induced the differentiation of chondrocytes. In addition, treatment with 30X *Rhus tox* significantly increased PGE2 release compared with other homeopathic dilutions of *Rhus tox*.

Carrageenan-induced oedema is an experimental model widely used for the evaluation of anti-inflammatory activity of new medications. This model is used for acute responses because the participation of mediators such as prostaglandins and kinins is intense at the 3rd hafter the stimulus. Many studies reported the effect of widely used homeopathic medicine such as *Rhus tox* and *Causticum* in acute and chronic animal models of inflammation. *Rhus tox* (6,12, 30 and 200C) and *Causticum* (6, 12, 30 and 200C) was reported to be effective in reduction in carrageenan-induced paw oedema in rats interfering with inflammatory processes involving histamine, prostaglandins and other inflammatory mediators.[35-38] Homeopathic preparations of *Guaiacum officinale* MT and two dilutions such as *Gua* 30C and *Gua* 200C were evaluated and found effective in anti-rheumatic and antioxidant activity in experimental rat model.[39] *Arnica montana* also reported to reduce the development of local oedema (such as that seen in the first phase of adjuvant arthritis) and caused a reduction of the carrageenan-induced oedema when administered locally in rats.[33] The positive effects of *Arnica montana* 6C on the individual modulation of acute inflammation kinetics in rats were reported.[40] Carrageenan-induced inflamed rats showed signs of reduced inflammation by showing less intense oedema and lower percentage of mast cell degranulation increase in lymphatic vessels diameter when treated with *Arnica montana* 6C. These symptoms were more prominent at late stage edema. The anti-inflammatory effect of *Arnica montana* 6C was also observed both in acute (carrageenan-induced paw oedema) and chronic inflammation (Nystatin-induced oedema) models in rats. Pre-treatment with *Arnica montana* 6C blocked the action of histamine in increasing vascular permeability.[41] Administration of *Apis, Lachesis* and *Phosphorus* orally in rats had an inhibitory effect on carrageenan oedema whereas the effect was significant only in the case of *Apis* when administered by means of subplanter injections. In blood-induced oedema rat model of inflammation, none of these medicines showed a significant effect when administered orally but showed significant inhibitory effects in subplanter injections.[35] A study on very high dilutions of dexamethasone showed inhibition of its pharmacological effects *in vivo* in adult BALBc mice. The study showed the interaction of dexamethasone at 7C and 15C with dexamethasone in pharmacological concentrations, using as experimental models of acute inflammation induced by carrageenan, *Ehrlich ascites* tumour and migration of tumor-infiltrating leukocytes. Homeopathic dexamethasone partially blocked the anti-inflammatory effect of pharmacological dexamethasone with regard to paw oedema and polymorphonuclear cell migration and demonstrated that a potentised substance may change its own pharmacological effects and suggest that ultra-dilution effects act mostly on host.[42] The anti-inflammatory, analgesic and antiarthritic effect of some homeopathic MTs, namely *Ricinus communis* MT, *Rauwolfia serpentina* MT, *Bellis perennis* MT, *Curcuma longa* MT, *Terminalia arjuna* MT and *Tribulus terrestris* MT was studied in animal models of carrageenan-induced inflammation in hind paw and arthritis by Complete Freund's Adjuvant injection in metatarsal footpad of Wistar albino rats. Out of all the medicines, *Curcuma longa* and *Tribulus terrestris* (MT) reduced hind-paw swelling, decreased the paw volume in carrageenan-treated rats as well as improved symptoms of arthritis and thus revealed potent activity against inflammation. While all homeopathic MTs tested in hot plate-induced thermal analgesia in the mice, assay showed peripheral analgesic activity.[43]

Many studies have been conducted to find out the effect of homeopathic medicines on analgesic effect using animal models. In one of the investigation effect of homeopathic *Anax imperator* (dragonfly) (*Anax-i* 30C and *Anax-i* 200C) in the forced swim test (FST), elevated plus-maze (EPM) test, hot plate (HP) test, open field test and evaluated the expression of NPY1 receptor in naive mice. The results indicated in the FST, treatment with *Anax-i* 30C or *Anax-i* 200C significantly diminished immobility time, while in EPM test, *Anax-i* 200C increased the percentage of time spent in open arms as well as the percentage of open arm/total arms. In the HP test, *Anax-i* 30C or *Anax-i* 200C decreased the total time mice spent licking their hind paws, while in OFT, treatment with *Anax-i* 200C increased the total distance and speed mice travelled compared to the control group indicates the anti-analgesic effect.[44]

Anticancer Effect

Homeopathy therapy in cancer has been a subject of great research interest. The drugs have been proven for their anticancer potential in liver, kidney, colon, breast and lung cancer cell lines as well as in animal models. Numerous publications highlighting positive results in both *in vivo* and *in vitro* experimental models discuss promising efficacy along with major mechanisms underneath the anticancer activity of various drugs.

Swiss albino mice bearing Ehrlich tumour cell-induced ascites were exposed to screen 1M potency of *Kali muriaticum, Phytolacca, Hydrastis, Zincum metallicum, Conium* and *Carcinosin*. All the six medicines were found to be increasing the lifespan of treated animals.[45] *Hydrastis* and *Ruta* were reported to inhibit Dalton's lymphoma ascites (DLA) induced and Ehrlich ascites carcinoma (EAC) as well as solid tumours in mice.[46,47] Both the drugs in 200C significantly increased the lifespan of tumour-bearing mice by 49.75% and 69.4%, respectively, along with a significant reduction in solid tumour volume. 1M dose of *Hydrastis* given orally was also found significant against solid tumours. Significant anti-metastatic activity was found in B16F-10 melanoma-bearing animals treated with *Thuja* 1M, *Lycopodium* 1M and 1M.[48] *Lycopodium* 30C indicated promising protection against chemical-induced (p-dimethylaminoazobenzene [p-DAB] and phenobarbital [PB]) hepatocarcinogenesis in mice,[49] whereas *Chelidonium* 200C showed significant inhibition of carcinogenesis induced by azo dye and p-DAB in mice.[50,51] *Carcinosin* 200 and *Chelidonium* 200 dilution when given alone demonstrated promising inhibition of p-DAB

hepatocarcinogenesis in mice. However, combination therapy of *Carcinosin* 200C and *Chelidonium* 200C was reported to give better results as compared to *Carcinosin* 200C and *Chelidonium* 200C used alone.[50-52] Similarly, combination therapy with *Natrum sulphuricum* 30C and *Carcinosin* 200C demonstrated higher antitumour efficacy compared to their individual treatments, which produced some ameliorative effect against hepatocarcinogenesis induced by chronic feeding of p-DAB and PB in mice. These two drugs effectively reduced the elevated chromosomal, nuclear and sperm head anomalies and also the various toxicity indices, suggesting their ability to combat carcinogenesis at the chromosomal and genomic levels.[53] *Ruta, Hydrastis, Thuja* and *Lycopodium* at 200C potencies were found to inhibit hepatocellular carcinoma in rats induced by N'-nitrosodiethylamine. *Phosphorus* 1M was found to reduce the incidence of 3-methylcholanthrene-induced sarcomas and also increase the lifespan of tumour-bearing mice.[54]

Studies with *T. occidentalis, Carcinosinum* and *Ruta graveolens* 1M, 30C and 200C on the immune system of BALBc/c mice showed significant increase in haematological parameters including total WBC count, haematopoietic parameters such as bone marrow cellularity and the number of a-esterase-positive cells and other parameters of immune response such as circulating antibody titre, and the number of plaque-forming cells, particularly with higher dilutions of *Thuja* and *Ruta,* was observed. Enhanced proliferation of B- and T-lymphoid cells suggests the immunomodulatory activity of homeopathic preparations in high dilution.[55]

In vitro studies in the discipline on similar lines have not only proved the clinical efficacy of the homeopathic medicines but also suggested possible mechanisms involved. Homeopathic medicines such as *Sarsa parilla, R. graveolens* and *Phytolacca decandra* have been reported to exhibit both cytotoxic and anti-proliferative activity in cancer cell lines. *Sarsa parilla* showed specific, irreversible anti-proliferative effects in ACHN (human renal adenocarcinoma) cells sparing normal cells. The efficacy of the medicines, however, decreased with increasing dilution (MT, 30C, 200C, 1M and 10M). *Ruta* and *Phytolacca* were tested in human colorectal and breast carcinoma cell lines in a study.[56] Most studies suggest apoptosis to be the underlying mechanism involved in the anticancer efficacy of homeopathic medicines. Further, involvement of reactive oxygen species (ROS) in possible induction of apoptosis, through release of cytochrome c and activation of caspase-3 after *Lycopodium* treatment in HeLa cells, was reported. *Lycopodium* has rather been extensively investigated both in clinical and pre-clinical studies and suggested as a key supportive medicine in conventional cancer therapy.[57,58] *Lycopodium* potency 5C and 15C were proved to induce chromatin condensation and internucleosomal DNA fragmentation, thus inhibiting the growth of HeLa cells. The study showed *Lycopodium*-induced increase in expression of pro-apoptotic proteins and mRNA of caspase-3 and Bax, along with the decrease in expression of anti-apoptotic proteins such as Bcl2 and Apaf with the release of cytochrome c. These dilutions, however, showed no significant cytotoxic effect in normal PBMCs.[57]

The anticancer activity of *Conium* MT in HeLa cells was evaluated by Khuda-Bukhsh and group. Several methods such as cell viability assay, lactate dehydrogenase (LDH) enzyme activity, intercellular ROS activity, morphological study and fluorescence microscopic study of DNA damage were conducted to ascertain the effects, *Conium* on HeLa cells. *Conium* MT demonstrated its anticancer potentials by inducing apoptosis of cancer cells through the ROS-mediated pathway and has negligible cytotoxicity against normal cells.[59] In another study from the same laboratory, the anticancer effect of *Hepatitis C* 30C (Hep C 30) nosode was evaluated on HepG2 (liver cancer) cell line and one normal liver cell line WRL-68 cells. Hep C 30 induced apoptosis, caused distorted cell morphology typical of apoptotic cells, increased ROS generation and produced increased DNA nicks. The drug also decreased expression of two cancer biomarkers, Top II and telomerase, consistent with its anticancer effect. This finding is highly significant as both these enzymes are actively associated with the divisional activities of cells and DNA.[60]

The cytotoxicity and genotoxicity of aristolochic acid I (AAI), one of the components of the MTs of the *Aristolochia clematitis* extract, were evaluated in HepG2 cells using BrdU-ELISA and colony-forming assay. Cell proliferation was inhibited concentration dependently by AAI. AAI formed DNA adducts, induced chromosomal aberrations (micronuclei) and DNA strand breaks, which led to an arrest of cells in the *S*-phase, which was associated with the increased expression of p53 and p21 proteins.[61] In one of the studies, the interaction of mouse lymph node lymphocytes co-cultured *in vitro* with macrophages in the presence or absence of the CHM (a Brazilian complex homeopathic medication), with B16F10 melanoma cells. Lymphocytes co-cultured with macrophages in the presence of the CHM enhanced the anticancer performance of lymphocytes against a very aggressive lineage of melanoma cells, reducing melanoma cell density and increasing the number of lysed tumour cells indicating the beneficial effect in skin cancer.[62] The anticancer activity of homeopathic preparation of *Terminalia chebula* MT, 3X, 6C and 30C was tested for their effect on the viability of breast cancer cell lines (MDAMB231 and MCF7) and non-cancerous (HEK 293) cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. *Terminalia chebula* MT decreased the viability of breast cancer (MDAMB231 and MCF7) and non-cancerous (HEK 293) cells. However, the other potencies (3X, 6C and 30C) decreased the viability of only breast cancer cells without affecting the viability of the non-cancerous cells. All the potencies, MT, 3X, 6C and 30C, reduced growth kinetics of breast cancer cells.[63]

Cytotoxicity of *P. decandra* MT was tested by conducting MTT assay on both normal (PBMCs) and A375 skin melanoma cells. Results showed that *P. decandra* (PD) administration caused

a remarkable reduction in proliferation of A375 cells, without showing much cytotoxicity on PBMCs. Generation of ROS and DNA damage, which made the cancer cells prone to apoptosis, was found to be enhanced in PD-treated cells. Overall results demonstrate anticancer potentials of PD on A375 cells through activation of caspase-mediated signalling and ROS generation.[64] The ultra-high dilution of plant extracts, *H. canadensis* (HC-30) and *Marsdenia condurango* (Condu-30), was found to work at the gene expression level. It was found to alter in microarray gene expression and triggered epigenetic modification profiles of many genes associated with carcinogenesis in HeLa cells *in vitro*.[65] In another study, the effect of *Thuja* 30 C on benzo(a)pyrene (BaP)-induced DNA damage, stress and viability of perfused lung cells of Swiss albino mice was reported. The cell viability of intoxicated cells was significantly increased with administration of *Thuja* 30C as compared to vehicle, whereas in case of normal lung cells, there was no effect of *Thuja* 30C treatment, which implicates the potentiality of *Thuja* 30C to have a regulatory effect only when the cells were under the carcinogen-induced stress.[66]

Clinical claims and studies with homeopathic drug suggest mixed results yet advocating recovery and relief to cancer patients undergoing chemo- or radiation-therapy confirm their role in improvement of quality of life for cancer patients.[67,68] Thus, potential of homeopathic medicines in prophylactic, therapeutic and as supportive therapy is all worth research investments for a new approach in the fight against cancer.

Wound Healing Activity

Many reports are clinically available which confirm the effectiveness of homeopathic medicines in wound healing which was further confirmed in several animal studies also. Drugs of plant origin, such as *Arnica montana, Calendula officinalis, Hypericum perforatum* at 4X and *Symphytum officinale* at 6X, were evaluated on NIH 3T3 fibroblasts. None of the three substances affected cell viability and none showed a stimulating effect on cell proliferation. However, the study suggested that low-potency Homeopathy preparation promoted *in vitro* wound closure by 59.5% and exerted *in vitro* wound closure potential in NIH 3T3 fibroblasts.[69] Highly diluted solutions of *Silica* 5C, 30C and 200C and of *Saline* 5C were tested in chronic wound in mice. The results showed that in 7/11 experiments, the ear holes of the *Silica*-treated animals were significantly smaller and healed faster than in those treated with *Saline*. Furthermore, the therapeutic effect increased progressively with an increase in dilution of the *Silica* 5C<30C<200C.[70] Significant wound healing effect was seen with of microcurrent application alone or in combination with topical *Hypericum perforatum* and *Arnica montana* on skin surgical incision induced on the back of Wistar rats when compared to the control group.[71] The mechanistic approach of *Arnica montana* at 2C, 3C, 5C, 9C, 15C or control was studied to evaluate the anti-inflammatory and wound healing activity. The effect on gene expression was tested using human THP-1 cell line *in vitro* model, a widely used model for immune modulation. The study revealed that *Arnica montana* 2C stimulated extracellular matrix gene expression significantly as compared to 3C, 5C, 9C, 15C or control. It was also tested in *in vitro* model of wound healing (scratch) assay, in which macrophages migrate through a scratch made in the culture cell monolayer and found evidence of an accelerating effect on cell migration in this system. These findings suggested the new approach of *Arnica montana* for the treatment of tissue healing and wound repair and identify extracellular matrix regulation by macrophages as a new therapeutic target.[72]

Antiasthmatic Activity

Antiasthmatic and anti-anaphylactic activities of *Blatta orientalis* MT, a homeopathic medicine, in experimental animal models (the bronchial hyperactivity models, acetylcholine and histamine-induced bronchospasm, in guinea pigs) was evaluated. Anti-anaphylactic activity was tested by active and passive anaphylaxis models in rats; anti-eosinophilic activity was tested by milk-induced eosinophilia in mice. Significant protection against acetylcholine and histamine aerosol-induced bronchospasm in *Blatta orientalis* MT-treated guinea pigs was seen. Treatment with *Blatta orientalis* MT in albino rat models of active and passive anaphylaxis showed a signifi cant reduction in mesenteric mast cell degranulation, serum IgE level while decreased in the eosinophil cell count were observed in mice when compared with the sensitized control group. These results reveal broad activity of *Blatta orientalis* MT. It may have non-selective antiasthmatic activity. The anti-anaphylactic activity of *Blatta orientalis* MT may be due to mast cell stabilisation, suppression of IgE and eosinophil cell count.[73]

Effect on Liver Toxicity

Homeopathy is a popular form of complementary and alternative medicine used to treat certain liver ailments. *Chelidonium majus* (Chel) 30C and 200C, one of the homeopathic medicines, reported a beneficial effect in experimentally induced hepatotoxicity in rats. Liver toxicity was induced by chronic feeding of p-DAB and PB which elevated the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), LDH, triglyceride, cholesterol, creatinine and bilirubin and lowered the levels of glutathione (GSH), glucose-6-phosphate dehydrogenase (G-6-PD), catalase and HDL-cholesterol. *Chelidonium* treatment modulates these parameters at both the potencies and showed improved signs of hepatotoxicity in rats.[74] Similarly, *Lycopodium clavatum* 30C was evaluated as a hepatoprotector against liver damage experimentally induced by paracetamol (Pct) in Wistar rats. Pre-treatment with *Lycopodium clavatum* 30C reduced hepatic lesions produced by paracetamol overdose and promotes decrease in the number of acinar zone 1 affected by necrosis and inflammatory infiltration.[75] A combination of potentised *Cholesterinum* with *Natrum sulphuricum* has been reported in combating hepatotoxicity generated by chronic feeding of carcinogens,

p-DAB and PB by modulating several biochemical parameters, namely AST, ALT, AlkP, GGT, LDH, etc. in rats.[76] The effect of *Arsenicum album* (*Ars Alb*) 6C and 30C on chronic arsenic toxicity in mice was evaluated by measuring cytogenetical endpoints such as chromosome aberrations, micronuclei, mitotic index, sperm head abnormality and biochemical protocols such as acid and alkaline phosphatase, aspartate and ALT, reduced glutathione, lipid peroxidation, catalase and succinate dehydrogenase. Both the potencies showed potential against arsenic intoxication by reducing chromosome aberrations, micronuclei, sperm head abnormality frequencies and activities of acid and alkaline phosphatase, aspartate and ALT and lipid peroxidation while mitotic index and activities of glutathione, catalase and succinate dehydrogenase were increased.[77]

Effect on Other Therapeutic Areas

Malaria is one of the most important parasitic diseases in the world and a major public health problem because of emerging drug-resistant strains of *Plasmodium*.[78] A number of synthetic and natural compounds are now being analysed to develop more effective antimalarial drugs. The focus on homeopathic medicines has been increasing in recent time to explore their role in combating malaria effectively. *Eupatorium perfoliatum* and *Arsenicum album* are two promising homeopathic medicines which were evaluated independently on parasitaemia using a rodent malaria model. *E. perfoliatum* showed 60% at a 30C potency while 70% inhibition was found with *Arsenicum album* at 0/6 potency.[78]

Leishmaniasis is a zoonotic disease caused by protozoan parasites of the mononuclear phagocytic system. The modulation activity of these cells can interfere in the host/parasite relationship and influences the prognosis. Many studies have been conducted clinically to explore the effect of homeopathic medicines in treating Leishmaniasis. Many homeopathic medicines showed promising results clinically. However, pre-clinical evaluation is very limited and very few studies have been reported so far in animals. Homeopathic medicines, *Thymulin* 5C and *Antimonium crudum* 30C, explored in the experimental murine Leishmaniasis model, to elucidate some aspects of the parasite-host relation under this homeopathic treatment. Male BALBc mice were orally treated with *Thymulin* 5C or vehicle during 60 days, after the subcutaneous inoculation of *Leishmania (L.) amazonensis* into the footpad. *Thymulin* 5C is able to improve B1-cell activation and *Leishmania (L) amazonensis* phagocytosis efficiency in mice.[79]

Many homeopathic medicines are very much popular in treating kidney stone and have been proved clinically also. One of the medicines *Berberis vulgaris* is widely used in patients of urolithiasis to treat renal calculi and tested in animal model also. The study reported the anti-urolithiasis potential of ultra-diluted homeopathic potency of *Berberis vulgaris* at 200C. After the 28-day treatment with *Berberis vulgaris* 200C in urolithiatic rats developed by administering 0.75% ethylene glycol to drinking water showed improvement in urolithiatic conditions by decreasing the stone-forming markers in urine and serum.[80]

Many studies clinically or pre-clinical describe the role of Homeopathy in the management of diabetes and its related complications. *Syzygium jambolanum* and *Cephalandra indica* are the two medicines explored widely in clinical aspects as well as in animal models for the beneficial effect in diabetes management. Homeopathic preparations of *Syzygium jambolanum* and *Cephalandra indica* in MT, 6C and 30C were used to examine the molecular mechanism of antidiabetic effects in the skeletal muscle of rats with high fat and fructose-induced type 2 diabetes mellitus. After 30-day treatment, fasting blood glucose, serum insulin and insulin signalling molecules in the skeletal muscle (gastrocnemius) was measured. The study revealed that the homeopathic preparations of *Syzygium jambolanum* and *Cephalandra indica*, including ultramolecular dilutions exhibit antidiabetic effects, improving insulin action through activation of insulin-signalling molecules in skeletal muscle of type 2 diabetic rats.[81] Further, these two medicines were also evaluated the role in glycation-induced structural modifications and further to examine their cellular protective ability. These homeopathic preparations, especially *Syzygium jambolanum,* prevented glycation-induced albumin modifications and subsequent toxicity in human erythrocyte *in vitro*.[82] The protective effect of *Gymnema sylvestre* against advanced glycation end product, sorbitol accumulation and aldose reductase activity in homeopathic formulation was evaluated by Lalit and Randhir, 2015. The investigators suggested that homeopathic preparations of *Gymnema sylvestre* at 6C and 30C had potent antioxidant and antiglycation activity.[83]

Action of homeopathic medicines in many other new therapeutic areas also explored by the scientist; however, the approach is very limited yet. In one of the reported study, the effect of Cantharis 6C on *E. coli*-induced cystitis was evaluated in mice. *Cantharis* 6C increased interleukin (IL)-12 and interferon-γ and decreased IL-10 concentrations in the bladder fluid; in the bladder mucosa, it increased the ratio between B- and T-lymphocytes (31%) and between B-lymphocytes and MIF + macrophages (57%) while the results were opposite. In case of the pelvis, where, decreased in B/T cell ratio (41%) and increased the M1/M2 macrophage ratio (42%) was observed. The inverted balance of inflammatory cells and cytokines in the bladder and pelvis mucosa shows specific local immune modulation induced by *Cantharis* 6C.[84] The effects of *Kalium causticum, Conium maculatum* and *Lycopodium clavatum* 13CH were evaluated in mice infected by *Trypanosoma cruzi*. *Lycopodium clavatum* 13C showed significant benefits in the treatment of mice infected with *Trypanosoma cruzi* by reducing the number of blood parasites and increasing animal survival rate.[85] *Thymulin* 5C and H3N2 30C homeopathic influenza virus solution was evaluated individually considering the inflammatory and behavioural responses against influenza

virus antigens in BALB/c mice. Behavioral response was evaluated using an OF device and inflammation response was evaluated by challenging of subcutaneous influenza haemagglutinin antigen (7 μg/200 μl) at day 21. No behavioural changes were seen in OFTs at any time point after homeopathic treatments; however, subtle changes in acquired immune antiviral response regulation were observed with both homeopathic treatments.[86] The potential effects and safety of homeopathic medicines in pregnancy were evaluated by exposing mouse embryonic stem (ES) cells to 30C potency of *Nux vomica* and *Sepia*. Homeopathic treatment led to modulations in the expression of certain lineage-specific genes, but this difference was not significant with respect to solvent control and showed normal differentiation as demonstrated by the expression of α/β MHC and α-actinin proteins in the differentiated ES cells.[87] The effect of *Arsenicum album* 30 C in *Saccharomyces cerevisiae* yeast exposed to arsenate was evaluated for its antioxidant activity and cell viability. The exposure of *Saccharomyces cerevisiae* to sublethal dose of arsenate generated ROS and subsequent oxidative stress to the organism. *Arsenicum album* 30C significantly attenuated arsenate-induced effects in yeast. It decreased arsenate-induced lipid peroxidation, protein carbonylation, DNA damage, ROS formation, Msn-2, Ysa-1 expression and increased cell viability, GSH, SOD, CAT and Glucose-6-phosphate dehydrogenase (G-6-PD) respectively indicates the effective role of *Arsenicum album* 30 C in reducing the arsenate stress in yeast *S. cerevisiae*.[88]

Conclusion

A positive approach toward the pre-clinical aspect not only opens the way to find the possible mechanistic approach of homeopathic drugs but also justified this alternative system scientifically in the scientific fraternity in a more acceptable way. In conclusion, Homeopathy has immense scope in pre-clinical research. Therapeutic and biological effect of homeopathic medicines could be established with the use of modern approaches of *in vivo* and *in vitro* experiments with inclusion of molecular techniques.

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Conflicts of interest

None declared.

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पूर्व–नैदानिक औषध विज्ञान (फार्माकोलॉजी): होम्योपैथिक अनुसंधान में एक महत्वपूर्ण पहलू

सार

पृष्ठभूमि: 20वीं शताब्दी में, होम्योपैथी में अनुसंधान या तो स्वस्थ मानव स्वयंसेवकों में प्रमाणित होने या रोगग्रस्त व्यक्तियों में नैदानिक सत्यापन/नैदानिक प्रभाव तक ही सीमित था। हाल के वर्षों में, होम्योपैथिक औषधियों द्वारा उत्पादित फार्माकोलॉजिकल कार्यकलाप का मूल्यांकन करने के उद्देश्य से कई पूर्व–नैदानिक अध्ययन किए गए हैं। इस समीक्षा का उद्देश्य इन विट्रो और पशु मॉडल, दोनों में होम्योपैथिक औषधियों के प्रयोगात्मक फार्माकोलॉजिकल निष्कर्षों के परिणामों का व्यवस्थित संकलन करना और इसे संक्षेप में प्रस्तुत करना है।

विधि: मार्च 2017 तक प्रकाशित आलेख जिनमें होम्योपैथिक औषधियों का उपयोग करते हुए इन विट्रो और इन विवो अध्ययनों की जानकारी थी को पबमेड डेटाबेस से एकत्र किया गया। इनमें समीक्षा लेख, वैज्ञानिक रिपोर्ट, अनुसंधान लेख, थीसिस, मेडलाइन इत्यादि से निकाली गई ऑनलाइन जानकारी शामिल थी। इस समीक्षा में सबसे सामान्य उपचारात्मक क्षेत्रों जैसे सूक्ष्मजीवनिवारक प्रभाव, सीएनएस विकार, दाहक विरोधी और पीड़ाहर, कैंसर विरोधी, घाव भरना, दमा विरोधी, यकृत विषाक्तता, मधुमेह, मलेरिया आदि पर लेख शामिल हैं।

परिणाम: होम्योपैथी अनुसंधान में बेहतर कार्य प्रणाली एवं तकनीक पर जानकारी तथा विभिन्न होम्योपैथिक औषधियों के विलयन, पोटेंसी के साथ–साथ मदर टिंचर के औषधीय प्रभावों पर साक्ष्य आधारित जानकारी प्रस्तुत की गई है।

निष्कर्ष: होम्योपैथी में पूर्व–नैदानिक अनुसंधान क्षेत्र में पर्याप्त संभावनाएँ है। इन विट्रो व इन विवो प्रयोगों जैसी आधुनिक आणविक तकनीकों के उपयोग द्वारा होम्योपैथिक औषधियों के चिकित्सीय और जैविक प्रभावों व इनके कार्यकलापों की क्रियाविधि के बारे में का पता लागया जा सकता है।

La pharmacologie pré-clinique : Un aspect important de la recherche homéopathique

Résumé

Contexte: Au XXe siècle, la recherche en homéopathie était limitée à prouver ses effets sur des personnes volontaires en bonne santé ou à sa vérification/efficacité clinique chez des malades. Cependant, ces dernières années, de nombreuses études pré-cliniques ont été effectuées ayant pour objectif d'évaluer l'activité pharmacologique des médicaments homéopathiques. L'objectif de cette revue est de réunir de manière systématique les résultats des expériences pharmacologiques faites avec des médicaments homéopathiques, tant in vitro que dans des études chez les animaux, et de les présenter sous la forme d'un résumé.

Méthodes: Des articles publiés jusqu'à mars 2017 dans des revues, des rapports scientifiques, des articles de recherche, des thèses, des informations en ligne extraites de Medline, etc., contenant des informations relatives aux études *in vitro* et *in vivo* effectuées sur l'utilisation des médicaments homéopathiques ont été recueillis de la base de données pubmed. Les articles concernant les domaines thérapeutiques les plus courants tels que l'action antimicrobienne, les troubles du système nerveux central (SNC), l'action anti-inflammatoire et analgésique, la propriété anticancéreuse, l'efficacité dans la guérison de plaies, l'action antiasthmatique, la toxicité hépatique, les effets sur le diabète, le paludisme etc. ont été inclus dans cette revue.

Résultats: La revue présente les informations sur les méthodologies améliorées, les techniques moléculaires utilisées dans la recherche en homéopathie et des informations fondées sur des preuves quant aux effets pharmacologiques des dilutions, des puissances et de la teinture mère de différents médicaments homéopathiques.

Conclusion: L'homéopathie offre des possibilités importantes dans la recherche préclinique où les effets thérapeutiques et biologiques des médicaments homéopathiques peuvent être suivis grâce aux techniques moléculaires modernes par le biais des expériences *in vivo* et *in vitro*.

Farmacología preclínica: Aspecto importante de la investigación homeopático

Resumen

Fundamento: En el siglo XX, la investigación en homeopatía se limitaba a las patogenesias en voluntarios humanos sanos o a la verificación o eficacia clínica en personas enfermas. Sin embargo, en los últimos años, se han realizadomuchos estudios preclínicos para evaluar la actividad farmacológica generada por los medicamentos homeopáticos. El objetivo de esta revisión es efectuar una recopilación sistémica de los resultadosy hallazgos obtenidos en los experimentos farmacológicos con los medicamentos homeopáticos, tanto *in vitro* como en modelos animales, y presentar los mismos en un esquema resumido.

Métodos: En la base de datos de Pubmed, se recopilaron los artículos publicados hasta marzo de 2017 que contenían información sobre los estudios *in vitroe in vivo*con medicamentos homeopáticos. Se consideraron artículos de revisión, informes científicos, artículos de investigación, tesis, informaciones *online* extraídas de Medline, etc. En esta revisión, se incluyeron los artículos sobre los campos terapéuticos más comunes como el efecto antimicrobiano, los trastornos del SNC, los efectos antiinflamatorios, analgésicos anticancerígenos y antiasmáticos, la curación de heridas, la toxicidad hepática, diabetes, malaria,etc..

Resultados: Se presenta la información sobre las mejoras en la metodología, lastécnicas moleculares en la investigación homeopática y la información basada en evidencias sobre los efectos farmacológicos de las diluciones, las potencias, así como de las tinturas madre de diferentes medicamentos homeopáticos.

Conclusiones: La homeopatía tiene un alcance considerable en la investigación preclínica, en la que, mediante el uso de técnicas moleculares modernas en los experimentos*in vivoein vitro*,se pueden identificar los efectos terapéuticos y biológicos de los medicamentos homeopáticoscon un mecanismo de acción adecuado.

Präklinische Pharmakologie: Ein wichtiger Aspekt in der Homöopathieforschung

Abstrakt

Hintergrund: Im 20. Jahrhundert beschränkte sich die Forschung in der Homöopathie auf die Prüfung am gesunden Probanden oder auf die klinische Verifizierung / klinische Wirksamkeit bei erkrankten Personen. In den letzten Jahren wurden jedoch viele vorklinische Studien durchgeführt, um die pharmakologische Aktivität von homöopathischen Arzneimitteln zu bewerten. Ziel dieser Untersuchung ist es, Ergebnisse experimenteller, pharmakologischer Befunde homöopathischer Arzneimittel sowohl in vitro als auch in Tiermodellen systemisch zusammenzustellen und in zusammengefasster Form darzustellen.

Methoden: Artikel, die bis März 2017 veröffentlicht wurden und Informationen zu In-vitro- und In-vivo-Studien mit homöopathischen Arzneimitteln enthalten, wurden aus der Pubmed-Datenbank gesammelt. Diese bestanden aus Übersichtsartikeln, wissenschaftlichen Berichten, Forschungsartikeln, Abschlussarbeiten, Online-Informationen aus Medline usw. Die Artikel aus den häufigsten therapeutischen Bereichen wie antimikrobielle Wirkung, ZNS-Störungen, entzündungshemmend und schmerzlindernd, Antikrebsmittel, Wundheilung, Antiasthmatika, Lebertoxizität, Diabetes, Malaria etc. wurden in diesen Bericht eingeschlossen.

Ergebnisse: Die Informationen über verbesserte Methodik, molekulare Techniken in der Homöopathieforschung und evidenzbasierte Informationen über pharmakologische Wirkungen von Verdünnungen, Potenzen sowie Urtinkturen verschiedener homöopathischer Arzneimittel werden vorgestellt.

Schlussfolgerung: In der präklinischen Forschung hat die Homöopathie einen substanziellen Anwendungsbereich, in dem therapeutische und biologische Wirkungen homöopathischer Arzneimittel mit geeignetem Wirkungsmechanismus und modernen molekularen Techniken mittels In-vivo- und In-vitro-Experimenten nachgewiesen werden können.

臨床前期的藥理學：順勢療法研究中重要的一面

摘要

背景：在20世紀，順勢療法研究受限於其在健康志願者上的驗證或其在患病個體中的臨床驗證／臨床療效。然而，近年來，已經進行了許多以評估順勢療法藥物產生的藥理學活性為目標的臨床前期研究。本綜述的目的是有系統地彙編順勢療法藥物在體外和動物模型中的實驗藥理結果，並以總結的形式呈現其結果。

方法：截至2017年3月發表的文章，從PubMed資料庫中收集使用順勢療法藥物的體外和體內研究資訊。這些內容包括文獻回顧、科學報告、研究文獻、論文、從Medline取得的線上資訊……等。本綜述包括最常見治療領域的文章，如：抗菌作用、中樞神經系統疾病、抗炎和鎮痛、抗癌、傷口癒合、抗哮喘、肝毒性、糖尿病、癆疾……等。

結果：介紹了順勢療法研究已改進的方法學、分子技術，以及關於不同順勢療法藥物的稀釋液、加能藥物和母酊的藥理作用的循證資訊。

結論：順勢療法在臨床前期研究中具有相當廣泛的應用範圍，其中可以通過在體內和體外實驗，使用現代分子技術追蹤，了解順勢療法藥物治療和生物學效應的正式作用機制。