In-vitro Studies by Date of Publication

Effect of the oral administration homeopathic Arnica montana on mitochondrial oxidative stress.
de Camargo RA, da Costa ED, Catisti R.

Fundação Hermínio Ometto, Uniararas, Araras, SP, Brazil.

Abstract

OBJECTIVE: To analyze the effect of homeopathic Arnica on mitochondrial oxidative stress induced by Ca(2+) plus inorganic phosphate and/or Fe(2+)-citrate-mediated lipid peroxidation through changes in oxygen consumption rates.

METHODS: Mitochondria were isolated by differential centrifugation from the livers of adult male Wistar rats which had been treated with Arnica montana 6cH, 12cH, 30cH or succussed 30% ethanol (control) for 21 days.

RESULTS: In the presence of antimycin-A, electron transport chain inhibitor, as evidenced by antimycin-A insensitive O(2) consumption, Arnica inhibited lipid peroxidation of mitochondrial membranes. In oxidative stress conditions, in the presence of Ca(2+) and inorganic phosphate, animals receiving Arnica 30cH had a significant decrease in mitochondrial O(2) consumption compared to control animals.

CONCLUSION: When administrated orally, Arnica 30cH protects against hepatic mitochondrial membrane permeabilization induced by Ca(2+) and/or Fe(2+)-citrate-mediated lipid peroxidation and fragmentation of proteins due to the attack by reactive oxygen species.

Link to paper: [http://www.homeopathyjournal.net/article/S1475-4916%2812%2900087-2/abstract](http://www.homeopathyjournal.net/article/S1475-4916%2812%2900087-2/abstract)

Dynamized follicle-stimulating hormone affects the development of ovine preantral follicles cultured in vitro.

Laboratory of Manipulation of Oocytes and Preantral Follicles (LAMOFOPA), State University of Ceará, Fortaleza-CE, Brazil. laritza_lima@yahoo.com.br

Abstract

OBJECTIVE: To evaluate the effect of dynamized follicle-stimulating hormone (FSH) on the survival, activation and growth of ovine preantral follicles (PFs) in vitro.

METHODS: Ovarian fragments were cultured for 1 or 7 days in alpha minimum essential medium (α-MEM(+)) control in the absence or presence of alcohol (Al control) or FSH (6cH, 12cH and 30cH) added at intervals of 24 or 48 h. The ovarian fragments were processed, coded and analyzed by a blinded observer by classical histology (CH), fluorescence microscopy (FM) and transmission electron microscopy (TEM).
RESULTS: After 7 days of culture, the group which to which FSH 6cH was added at 24 h intervals showed better rates of follicle survival and activation compared to α-MEM(+) control or Al control (p < 0.05). This group also showed higher follicle and oocyte growth than α-MEM(+) control (p < 0.05). FM and TEM techniques confirmed that FSH 6cH promoted viability and ultrastructural integrity of follicles after 7 days of culture.

CONCLUSIONS: FSH 6cH (24 h) treatment maintained the viability, and promoted the activation and in vitro growth of ovine PFs.

H3N2 homeopathic influenza virus solution modifies cellular and biochemical aspects of MDCK and J774G8 cell lines.

Departamento de Medicamentos-Faculdade de Farmácia, Universidade Federal do Rio de Janeiro - UFRJ, Rio de Janeiro, Brazil.

Abstract
BACKGROUND: Influenza viruses cause highly contagious acute respiratory illnesses with significant mortality, especially among young children, elderly people, and individuals with serious medical conditions. This encourages the development of new treatments for human flu. Biotherapies are diluted solutions prepared from biological products compounded following homeopathic procedures.

OBJECTIVES: To develop a biotherapy prepared from the infectious influenza A virus (A/Aichi/2/68 H3N2) and to verify its in vitro response.

METHODS: The ultradiluted influenza virus solution was prepared in the homeopathic dilution 30dH, it was termed Influenzinum RC. The cellular alterations induced by this preparation were analyzed by optical and electron microscopy, MTT and neutral red assays. Glycolytic metabolism (PFK-1) was studied by spectrophotometric assay. Additionally, the production of tumor necrosis factor-α (TNF-α) by J774.G8 macrophage cells was quantified by ELISA before and after infection with H3N2 influenza virus and treatment.

RESULTS: Influenzinum RC did not cause cytotoxic effects but induced morphological alterations in Madin-Darby canine kidney (MDCK) cells. After 30 days, a significant increase (p < 0.05) in mitosis rate was detected compared to control. MDCK mitochondrial activity was changed after treatment for 10 and 30 days. Treatment significantly diminished (p < 0.05) PFK-1 activity. TNF-α in biotherapy-stimulated J774.G8 macrophages indicated a significant (p < 0.05) increase in this cytokine when the cell supernatant was analyzed.

CONCLUSION: Influenzinum RC altered cellular and biochemical features of MDCK and J774G8 cells.

Homsani F, Barbosa GM, Siqueira CM, Grechi J, dos Santos ALS, Holandino C.

Abstract
Introduction: Candidiasis is an opportunistic infection, caused by yeast of the genus Candida, which emerges as one of the main causes of systemic infections in hospitalized patients. Candida albicans is the most common causing agent of these infections. According to the Brazilian Homeopathic Pharmacopeia, nosodes are medicines compounded from chemically undefined biological products. Living nosodes are prepared using the etiologic agent of an illness in its infective form, were first developed by Brazilian physician Roberto Costa (RC). Roberto Costa’s research indicated that living nosodes present a higher capability to stimulate the host’s immunological system.

Aim: This study aims to evaluate cellular alterations induced in C. albicans yeasts and RAW 264-7 macrophages by Candida albicans RC.

Methodology: To prepare Candida albicans RC, one part of C. albicans infective yeast suspension (10^8 cell/ml) was diluted in 9 parts of sterile distilled water and submitted to 100 mechanical succussions. This process was successively repeated to the potencies of 12x and 30x1. Water 30x was prepared by the same technique, as control. The cell viability of C. albicans previously treated with nosodes in both potencies and respective controls was evaluated using the samples at the concentration of 10% (V/V), in a volume of 1ml, distributed in 1-3 days. The viability of the yeast cells was analyzed by MTT (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolic) (5mg/ml) assay and by Propidium Iodide (PI) incorporation methods. Additionally, using macrophages RAW 264-7 as a cell model, Nitric Oxide (NO) production and cell viability were also evaluated. For this, the following protocol of cell treatment was employed: on each experimental day, RAW 264-7 cells were treated 4 times (4 stimuli) with RC nosode 30x at the concentration of 10% (V/V).

Results: The nosodes (12x and 30x) did not present cytotoxic effects on macrophage cells (n=1), or on C. albicans yeasts (n=2), as detected by MTT and PI methods. Moreover, no statistically significant differences on NO production were detected among the experimental groups (n=6).

Conclusion: Preliminary results of in vitro assays indicate that nosodes (12x and 30x) do not alter mitochondrial activity or cell viability of C. albicans. Similarly, treatment by RC nosodes does not seem to alter NO release and mitochondrial activity of RAW macrophages. New experiments are being performed to confirm these preliminary data.

Link to abstract/paper:

Biochemical responses induced by Biotherapics prepared from intact influenza A (H3N2) and inactivated influenza A (H3N2) virus at 12x and 30x in MDCK cells and RAW-264-7 macrophages.

Abstract
Background: "Roberto Costa’s Biotherapics" are homeopathic remedies prepared from intact microorganisms which have been proposed for treatment of diseases like influenza.

Aim: This study aimed to compare the biochemical effects, in MDCK cells and RAW-264-7 macrophages, of biotherapics prepared from intact influenza virus diluted in water as well as from a sample of the same virus inactivated by ethanol 70% (v/v), both in the homeopathic potencies of 12x and 30x. Water 30x, non-dynamized water and cells without treatment (control cells) were used as control.

Methodology: Treatments were performed by incubating MDCK cells with DMEM medium added in a 1:10 ratio for 6 times (3 different aliquots per day) or 18 times (up to 4 aliquots per day) in each experimental situation. Each aliquot was added with an interval of at least 2 hours. After that, the mitochondrial activity of MDCK cells was analyzed by MTT assay. The effects of treatments with intact biotherapics on MDCK cells respiratory parameters were studied using high resolution respirometry (Oroboros Oxygraph-O2K). RAW-264-7 macrophages were treated with intact and inactivated biotherapic 30x (4 treatments, 24 hours) to verify the nitric oxide production. These macrophages were also submitted to MTT assay.

Results: Both biotherapic preparations 1x (intact and inactivated virus sample) were analyzed by transmission electronic microscopy. The presence of virus particles was detected when water was used as solvent. The use of ethanol as biotherapic solvent induced complete virus lysis. There was no alteration in cell osmolarity revealed by neutral red assay, when 10% of each test solution was used. Cellular viability analyzed by MTT method increased when MDCK cells were treated with 18 stimuli of inactivated biotherapic 30x when compared to intact biotherapic 30x (p<0.05). However, no statistically significant differences (p>0.05) were detected when these cells were compared to control cells. The maximum respiratory capacity of MDCK cells increased after treatment with 18 stimuli of intact biotherapic 30x when compared to control cells. However, no statistically significant differences (p>0.05) induced by biotherapics in macrophage cells were observed by MTT and nitric oxide assays. Moreover, a reduction in nitric oxide was observed in macrophages treated with dynamized water when compared to control cells.

Conclusions: These results indicate that the method of biotherapeutic compounding (intact or inactivated virus as starting point) can modify the cellular parameters with the tendency to increase cellular response with longer treatments and higher potencies.

and its use in spermatozoa requires investigation. It is well established that mitochondrial membrane potential is an important viability parameter of spermatozoa and it is intimately related to reproductive efficiency. In this manner, new technologies in order to improve the activity of sperm cells and, finally, the fecundity of swine herds are of extremely importance. Due to the lack of knowledge of homeopathic treatment effect on spermatozoa, the aim of the present study was to verify the effect of three different homeopathic treatments on viability of boar sperm cells.

Methods: Semen samples were obtained from two sexually mature boars (18 mo of age). The boars were cross bred, with similar genetics of Pietrain versus Duroc, BP 450 progeny from a supplier company of similar reproductive performance animals. The animals were maintained in individual stalls, study conducted in Sao Paulo - Brazil. Three homeopathic treatments: Pulsatilla 6CH, Avena 6 CH or both, compared to placebo treatment (sucrose), the homeopathic medicaments or the control were administrated as globules manipulated according Brazilian Homeopathic Pharmacology. Each globule weighted 30 mg and contained sucrose as vehicle. One dose of two globules was added per 100 mL of diluted boar semen, which were chilled for 24 or 48 hours. All samples were labeled in codes in order to allow all laboratory analysis and evaluations being performed as a blind test. Data were tested for normality of residues and homogeneity of variances using the Guided Data Analysis software. Variables and interactions were analyzed by the PROC MIXED of the SAS package (SAS Institute Ins. Cary, NC). Adjusted least squares means (LSMEANS) of treatments were compared using the Tukey Test.

Results: The different treatments contributed to maintain acrosome integrity for prolonged periods of cooling over 48 hours. The use of Pulsatilla was effective in maintaining high sperm mitochondria activity up to 24 hours from harvesting.

Conclusion: Homeopathic medications can be used in artificial insemination in order to improve the quality of cooled and stored pig semen [1].

Link to abstract/paper:

**Int J High Dilution Res. 2012;11(40):164-165.**

**Effect of two homeopathic remedies at different degrees of dilutions on the wound closure of 3T3 fibroblasts in in vitro scratch assay.**

Hostanska K, Rostock M, Baumgartner S, Saller R.

**Abstract**

Background: Since ancient times, preparations from traditional medicinal plants e.g. *Arnica montana*, *Calendula officinalis* or *Hypericum perforatum* have been used for different wound healing purposes. The aim of this study was to investigate the efficacy of the commercial low dilution homeopathic remedy Similasan® Arnica plus Spray, a preparation of *Arnica montana* 4x, *Calendula officinalis* 4x, *Hypericum perforatum* 4x and *Symphytum officinale* 6x (0712-2) and medium diluted SIM WuS (Petroleum 15x, *Arnica montana* 15x, *Calcium fluoratum* 12x, *Calendula officinalis* 12x, *Hepar sulfuris* 12x and *Mercurius solubilis* 15x; 1101-4), on the wound healing in cultured NIH 3T3 fibroblasts. Both remedies were from Similasan AG (Jonen, Switzerland) and prepared according the German Homoeopathic Pharmacopoeia
Materials and Methods: Cell proliferation, migration and wound closure promoting effect of the preparations (0712-2, 1101-4) and their succussed solvents (0712-1, 1101-3) were investigated on mouse NIH 3T3 fibroblasts. Cell viability was determined by WST-1 assay, cell growth using BrdU uptake, cell migration by chemotaxis assay and wound closure by CytoSelect™ Wound Healing Assay Kit which generated a defined wound area. All assays were performed in three independent controlled experiments. In some experiments diluted unsuccussed alcohol (0712-3) was also investigated.

Results: Preparations (0712-1), (0712-2), (0712-3), (1101-3) and (1101-4) were investigated at decimal dilution steps from 1x to 4x. Cell viability was not affected by any of the substances and (0712-1) and (0712-2) showed no stimulating effect on cell proliferation. Preparation (0712-2) exerted a stimulating effect on fibroblast migration (31.7%) vs 15% with succussed solvent (0712-1) at 1:100 dilutions (p<0.001). Unsuccussed solvent (0712-3) had no influence on cell migration (6.3%; p>0.05). Positive control 2 ng/ml EGF increased migratory activity of cells by 49.8%. Preparation (0712-2) at a dilution of 1:100 promoted in vitro wound closure by 59.5% and differed significantly (p<0.001) from succussed solvent (0712-1), which caused 22.1% wound closure. Medium diluted remedy (1101-4) exerted accelerating effect on wound closure after 14h of treatment. Wounded area was closed by 20% with (1101-4) and 13% by (1101-3) compared to untreated control. Succussed solvent (1101-3) caused about 23% and the remedy (1101-4) about 30% wound closure after 24h. Remedy (1101-4) and succussed solvent (1101-3) modestly stimulated cell growth at dilutions 1:100 and 1:1000 by about 25% and 15%, respectively. No statistically significant differences between preparations 1101-3 and 1101-4 could be detected.

Conclusions: Our results demonstrate that the Similasan® Arnica plus low dilution homeopathic remedy exerted wound healing potential, which is a result of increased ability of fibroblasts to migrate without affecting cell proliferation. Medium diluted preparation SIM WuS exerted stimulating effect on the wound closure accompanied by a cell proliferating effect. Used in vitro wound closure test was sensitive enough for low dilutions preparation, however for medium diluted preparation despite of a trend, no significant differences could be detected.

Link to abstract/paper:

A homeopathic remedy from arnica, marigold, St. John’s wort and comfrey accelerates in vitro wound scratch closure of NIH 3T3 fibroblasts.
Hostanska K, Rostock M, Melzer J, Baumgartner S, Saller R.

Institute for Complementary Medicine, University Hospital Zurich, Raemistrasse 100, Zurich 8091, Switzerland. katarinahostanska@hotmail.com

Abstract
BACKGROUND: Drugs of plant origin such as Arnica montana, Calendula officinalis or Hypericum perforatum have been frequently used to promote wound healing. While their effect on wound healing using preparations at pharmacological concentrations was supported by several in vitro and clinical studies, investigations of herbal homeopathic remedies on wound healing process are rare. The objective of this study was to investigate the effect of a commercial low potency homeopathic remedy Similasan® Arnica plus Spray on wound closure in a controlled, blind trial in vitro.

METHODS: We investigated the effect of an ethanolic preparation composed of equal parts of Arnica montana 4x, Calendula officinalis 4x, Hypericum perforatum 4x and Symphytum officinale 6x (0712-2), its succussed hydroalcoholic solvent (0712-1) and unsuccussed solvent (0712-3) on NIH 3T3 fibroblasts. Cell viability was determined by WST-1 assay, cell growth using BrdU uptake, cell migration by chemotaxis assay and wound closure by CytoSelect™ Wound Healing Assay Kit which generated a defined "wound field". All assays were performed in three independent controlled experiments.

RESULTS: None of the three substances affected cell viability and none showed a stimulating effect on cell proliferation. Preparation (0712-2) exerted a stimulating effect on fibroblast migration (31.9%) vs 14.7% with succussed solvent (0712-1) at 1:100 dilutions (p < 0.001). Unsuccussed solvent (0712-3) had no influence on cell migration (6.3%; p > 0.05). Preparation (0712-2) at a dilution of 1:100 promoted in vitro wound closure by 59.5% and differed significantly (p < 0.001) from succussed solvent (0712-1), which caused 22.1% wound closure.

CONCLUSION: Results of this study showed that the low potency homeopathic remedy (0712-2) exerted in vitro wound closure potential in NIH 3T3 fibroblasts. This effect resulted from stimulation of fibroblasts motility rather than of their mitosis.

Link to paper: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3565897/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3565897/)


**A homeopathic remedy from arnica, margold, St. John's wort and comfrey accelerates in vitro wound scratch closure of NIH 3T3 fibroblasts.**

Hostanska K, Rostock M, Melzer J, Baumgartner S, Saller R.

Abstract

**Background**

Drugs of plant origin such as Arnica montana, Calendula officinalis or Hypericum perforatum have been frequently used to promote wound healing. While their effect on wound healing using preparations at pharmacological concentrations was supported by several in vitro and clinical studies, investigations of herbal homeopathic remedies on wound healing process are rare. The objective of this study was to investigate the effect of a commercial low potency homeopathic remedy Similasan® Arnica plus Spray on wound closure in a controlled, blind trial in vitro.

**Methods**

We investigated the effect of an ethanolic preparation composed of equal parts of Arnica montana 4x, Calendula officinalis 4x, Hypericum perforatum 4x and Symphytum officinale 6x (0712–2), its succussed hydroalcoholic solvent (0712–1) and unsuccussed solvent (0712–3) on NIH 3T3 fibroblasts. Cell viability was determined by WST-1 assay, cell growth using BrdU uptake, cell migration by
chemotaxis assay and wound closure by CytoSelect ™Wound Healing Assay Kit which generated a defined "wound field". All assays were performed in three independent controlled experiments.

Results
None of the three substances affected cell viability and none showed a stimulating effect on cell proliferation. Preparation (0712–2) exerted a stimulating effect on fibroblast migration (31.9%) vs 14.7% with succussed solvent (0712–1) at 1:100 dilutions (p<0.001). Unsuccussed solvent (0712–3) had no influence on cell migration (6.3%; p>0.05). Preparation (0712–2) at a dilution of 1:100 promoted in vitro wound closure by 59.5% and differed significantly (p<0.001) from succussed solvent (0712–1), which caused 22.1% wound closure.

Conclusion
Results of this study showed that the low potency homeopathic remedy (0712–2) exerted in vitro wound closure potential in NIH 3T3 fibroblasts. This effect resulted from stimulation of fibroblasts motility rather than of their mitosis.

Link to paper:


**Effects of two homeopathic complexes on bovine sperm mitochondrial activity.**

Aziz DM, Schnurrbusch U, Enbergs H.

Institute of Physiology, Biochemistry and Hygiene of Animals, Bonn University, Bonn, Germany. dhaferaziz@daad-alumni.de

Abstract
OBJECTIVES: This study was conducted to evaluate the effect of two homeopathic complexes Ubichinon compositum® (Ubi comp) and Coenzyme compositum ad us. vet.® (CoQ10 comp) on bovine sperm mitochondrial activity.

METHODS: Sperm viability, acrosomal integrity and sperm chromatin structure were estimated to detect the possible side effect of complexes on other sperm parameters.

RESULTS: Mitochondrial activity was significantly enhanced by both Ubi comp (P<0.01) and CoQ10 comp (P<0.05). No effects were detected in other tested sperm parameters.

CONCLUSION: The tested homeopathic complex medicines stimulate the mitochondrial activity of bovine sperm without effects on their viability, acrosomal integrity or chromatin structure. The possibility that this translates into improved fertilization capacity in artificial insemination should investigated.

**Abstract**

**INTRODUCTION:** Canova is a complex homeopathic medicine that enhances a specific immunologic responses against several exogenous and endogenous conditions. Canova activates macrophages both in vivo and in vitro.

**AIM AND METHOD:** We evaluated the effects of macrophages activated by Canova in vivo and ex vitro in the proliferation of lymphocytes. Canova was used to activate Cebus apella macrophages in vivo or ex vitro with Canova. Lymphocytes were cultured with the macrophage culture medium. The analysis of Canova effects in cultured lymphocytes was performed according to the cell cycle phase using flow cytometry. The Interferon gamma and Interleukin-5 cytokines quantification in these lymphocyte culture media was performed by Enzyme-linked immunosorbent assay (ELISA).

**RESULTS:**
We observed that Canova actives macrophages in vivo and ex vitro. The lymphocytes cultured in a supplemented medium with macrophages activated by Canova treatment presented a higher number of proliferation cells than lymphocytes not exposed to macrophages activated by Canova. The Interferon gamma and Interleukin-5 cytokines were only observed in the medium of lymphocytes exposed to macrophages activated by Canova. Thus, Canova has potential as a new adjuvant therapy.


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**Mercurius solubilis: actions on macrophages.**

**Abstract**

**BACKGROUND:** Macrophages play central roles in homeostasis as well as host defence in innate and acquired immunity, auto-immunity and immunopathology. Our research group has demonstrated the effects of highly diluted toxic substances in macrophages.

**AIM:** To investigate if highly diluted Mercurius solubilis (Merc sol), can activate or modulate macrophage functions.
METHODS: We evaluated the effects of Merc sol in the 6, 12, 30 and 200 centesimal high dilutions (CH) potencies on mice peritoneal macrophages (in vitro and in vivo). Merc sol was added to mice's drinking water for 7 days (in vivo treatment) and animals were euthanised and cells were collected. In vitro treatment was performed on macrophages and bone-marrow cell cultures.

RESULTS: Macrophages showed activated morphology, both when Merc sol was added directly to the cell culture and to drinking water. The in vitro experiments showed enhanced morphological activation, increased interferon (IFN)γ release in the supernatant at lower dilutions and interleukin (IL)-4 production at higher dilutions. Increase in nitric oxide and decrease in superoxide (O2(-)) and hydrogen peroxide (H2O2) were also observed. In vivo treatment caused a decrease in O2(-) and increase in H2O2 production by macrophages.

DISCUSSION: Taken together, the results allow us to conclude that highly diluted Merc sol modulates reactive oxygen species (ROS), reactive nitrogen species (RNS) and cytokine secretion, which are central mediators of the immune system, wound healing and body homeostasis.


Guedes JR, Carrasco S, Ferreira CM, Bonamin LV, Souza W, Goldenstein-Schainberg C, Parra ER, Capelozzi VL.

Laboratory of Molecular Pathology, Department of Pathology, University of São Paulo School of Medicine, Av. Dr Arnaldo 455, São Paulo, SP, Brazil. centralanimal@uol.com.br

Abstract

BACKGROUND:
Ultra High Dilutions (UHD) are diluted beyond the Avogadro limit with dynamization (dilution with succussion). The process of anuran amphibian metamorphosis is controlled by thyroid hormones, including the resorption of the tadpole tail.

METHODS:
A randomized and blinded study was performed to investigate the influence of triiodothyronine (T3) 5·10(-24)M (10cH) on apoptosis induced by T3 100 nM in Rana catesbeiana tadpoles' tail tips, in vitro. Explants were randomized to three groups: control: no T3 in pharmacological or UHD dose; test: T3 100 nM and challenged with T3 10cH (UHD); positive control: T3 100 nM, treated with unsuccussed ethanol. The apoptotic index and the area of explants of test and control groups at the first and final day of the experiment were compared by t-test.

RESULTS:
There was no difference in tail tip area between test and control groups, but a significantly higher (p<0.01) index of apoptosis in explants of the test group.

CONCLUSION:
This data suggest that T3 10cH modifies the effect of T3 at pharmacological dose, opening new perspectives for further studies and investigation of the dose-effect curve.
Neuroprotective effect of Bellis perennis and Hypericum perforatum on PC12 cells.

Effects of the utilization of homeopathic elements in commercial diluent on swine sperm viability.
Soto FR, Vuaden ER, de Paula Coelho C, Bonamin LV, de Azevedo SS, Benites NR, de Barros FR, Goissis MD, Ortiz D'Ávila Assumpção ME, Visintin JA, Marques MG.

Center for Sanitary Surveillance and Zoonoses Control Tereza Rodrigues de Camargo, Estrada da Vargem do Salto- Km 4,5- Bairro dos Paes, 18150-000 Ibiúna, São Paulo, Brazil.

Abstract
It has been speculated that the homeopathic treatment of sperm cells in order to improve semen quality could be promising. However, few data is available and its use in spermatozoa requires investigation. It is well established that mitochondrial membrane potential is an important viability parameter of spermatozoa and it is intimately related to reproductive efficiency. In this manner, new technologies in order to improve the activity of sperm cells and, finally, the fecundity of swine herds are of extremely importance. Due to the lack of knowledge of homeopathic treatment effect on spermatozoa, the aim of the present study was to verify the effect of three different homeopathic treatments on viability of boar sperm cells. Three homeopathic treatments composed by Pulsatila CH6, Pulsatila and Avena CH6, Avena CH6 and one control treatment (sucrose) were added to diluted boar semen, which were cooled for 24 or 48 h. Interestingly, no positive effect of homeopathic treatments was observed over semen viability. However, it was demonstrated that the 24 h of cooling storage provided more viable sperm cells when compared to the 48-h period. This effect of storage period on sperm viability was assessed by intact plasmatic membrane, intact acrosome and mitochondrial membrane potential evaluation.

In vitro behavior of Mycoplasmagallisepticum live-type nosode.
Abstract
As a step of a doctoral research project, in this study a live-type nosode was prepared from microorganism Mycoplasma gallisepticum strain R (ATCC 93-08/19610) according to Costa model and the rules by Brazilian Homeopathic Pharmacopoeia. Live nosode was tested in vitro to assess safety when used to immunize domestic fowl (Gallus gallus) against infection by this microorganism and to investigate its behavior under laboratory conditions. M. gallisepticum was not shown to grow in fluid (broth) and solid (plate) modified Frey medium with dilutions 11d, 12d, 20d and 30d. Inhibition halos about 2.0 mm were observed around paper disks impregnated with live-type nosode in microorganism-sown Petri dishes, whereas disks impregnated with conventional antibiotic oxytetracycline exhibited 8.0 mm inhibition halos. Protein assessment by Folin-Lowry method showed protein absence in dilutions 12d and 30d and neither microbial DNA traces were found in PCR assay in dilutions 12d, 20d and 30d.

Effects of thymulin 5cH in granuloma evolution and B1 cell differentiation: an experimental model to understand its biological mechanisms.
Sato C, Cardoso TN, Osugui L, Popi AF, Bonamin LV.

Abstract
In previous studies, we found that thymulin (a thymic hormone), when prepared in homeopathic 5cH potency, had the property to improve the productive performance of broiler chickens infected with reovirus, as well as modulate the development of Ehrlich tumor and granuloma inflammatory lesions in mice by immune-mediated mechanisms. The aim of the present work was to study the immunomodulatory mechanisms of thymulin 5cH in a granuloma experimental model, by subcutaneous inoculation of BCG in mice, focusing the B-1 cells and zinc involvement in this process. Three groups of male Balb/c SPF mice (group A treated with thymulin 5cH, group B treated with thymulin 5cH incubated in Chelex ® - a zinc chelant - and group C, control, treated with vehicle) were inoculated with BCG in the left footpad and subcutaneous granuloma and spleen were harvested for histomorphometry analysis, after 7, 14 and 21 days. Ziehl-Neelsen, HE and Prussia Blue staining methods were used. Flow cytometry was also used in the same times to characterize and quantify peritoneal cells. Positive cells for CD11b (activated phagocytes, B-1 cells), CD19 (B-1 and B-2 cells), CD23 (negative B-1 cells, positive B2 cells) and CD5 (B-1a cells) were analyzed in a FACS Calibur (BD) device. Statistical analysis was performed using Kruskal - Wallis / Dunn for nonparametric evaluations and ANOVA / Tuckey-Krammer for the parametric ones. The X² method was used to evaluate the cell count in flow cytometry. P values ≤ 0.05 were considered statistically significant. Mice treated with thymulin 5cH presented higher macrophage activity and increase in the follicular area were seen in spleen after 7 days. Increase in gross lesion diameter and decrease in local BCG infection were seen after 21 days. At this time, the flow cytometry demonstrated the increase in peritoneal phagocytes derived from B-1 cells in thymulin 5cH treated mice, independently of Chelex ® incubation. The incubation of thymulin 5cH with Chelex ® blocked its effects only upon the number of B2 cells in the peritoneum and reduces Mn levels in the medicine solution. We
conclude that thymulin 5CH modulates the BCG-induced granuloma through more than one mechanism, especially by peritoneal B1 cell differentiation into phagocytes.


In Vitro Effects of Natrum muriaticum in Kidney Cell Lines MDCK and LLC-PK1.


Abstract

Previous papers have indicated that homeopathic solutions modify the cellular and biochemical aspects of cells maintained in culture. In this study, the effects of Natrum muriaticum, a medicine used in the homeopathic clinic for the treatment of hypertension, were evaluated in kidney MDCK and LLC-PK1 cell lines. The following cellular parameters were analyzed: viability, morphology and expression of the (Na++K+)-ATPase and the angiotensin II receptors AT1 and AT2. The cell lines were plated (5.0 x 104 cells/mL) in DMEM supplemented with 10% fetal calf serum (FCS). After 24 hours at 37°C, DMEM was re-fed with the addition of 10% (V/V) and 1% (V/V) of the following samples: Natrum muriaticum 30CH, water 30CH and non-dynamized sterile distilled water to do the MTT assay. The results obtained from these groups were compared to those obtained by incubation of the cells in culture medium free of these solutions (Control). Cell viability was assessed by a colorimetric MTT ELISA assay (490nm). The values from four independent experiments performed in quintuplicate were plotted and statistically analyzed by Sigma Plot v.11 (Jandel Scientific). The morphology of MDCK cells was evaluated by optical microscope after Giemsa’s staining. The expression of the (Na++K+)-ATPase and AT1/AT2 of LLC-PK1 cells was evaluated by Western Blot (WB) analysis. For this experimental set, 5.0x104 cells/mL were incubated in DMEM supplemented with 10% FCS and daily culture medium was replaced by a new one, containing: Natrum muriaticum 30CH and water 30CH. Additionally, cells were treated for 5, 10 and 15 days with 1% of specific solutions and the total protein was measured by the Lowry method, after cell lysis. The samples were analyzed by electrophoresis in SDS-PAGE (12% gel) and transferred to nitrocellulose membrane. This membrane was incubated with specific primary antibodies (anti-(Na++K+)-ATPase, anti-AT1 and AT2 or anti-anti-beta-actin). The detection was performed using ECL system and Hyperfilm. MTT assays showed a statistically significant reduction in cellular mitochondrial activity (p<0.001) probably attributed to an osmotic effect due to the use of 10% (V/V) concentration rather than 1% (V/V). The optical microscopy analysis revealed no significant morphological changes in MDCK and LLC-PK1 cells submitted to the different treatments when compared to controls groups. The WB analysis indicated a proportional increase in (Na++K+)-ATPase content according to an increase in the homeopathic stimuli. Although preliminary, these results show for the first time, that the homeopathic medicine is able to modify the expression of important physiological markers for LLC-PK1 cells, which are directly involved in the genesis of hypertension.

Cellular and biochemical responses induced by Biotherapics prepared from intact influenza A (H3N2) and inactivated influenza A (H3N2) virus at 12x and 30x in the MDCK cells.
Siqueira CM, de Mendonça RAF, da Veiga VF, Marcondes M, Zancan P, Couceiro JN, Hollando C.

Abstract
Biotherapics are homeopathic remedies prepared from organic products that are chemically undefined and can be used for treatment of diseases like influenza. There are several classes of biotherapics and, among these, there are some called "living biotherapics" or "Roberto Costa’s Biotherapics". This study aimed to compare the cellular and biochemical effects of biotherapics prepared from intact influenza virus diluted in water and the one obtained from the same viral sample inactivated by ethanol 70% (v/v), both in the potencies of 12x and 30x. Transmission electron microscopy (TEM) analyses were performed on both preparations to assess the integrity of viral particles, which showed that ethanol 70% (v/v) induced a complete denaturation of viral particles. In contrast, the integrity of virus particles was preserved when water was used as the biotherapic solvent. Cellular and biochemical alterations induced by the preparations on MDCK cells were analyzed and compared with those induced by respective controls (water 30x-treated and untreated cells). Cellular viability analyzed by MTT method showed statistically significant differences (p <0.05) in MDCK cells treated with intact biotherapic for 5 (3 stimuli) and 30 (18 stimuli) days in comparison with untreated control. TEM analysis did not show significant cellular changes when the different experimental groups were compared. The enzymatic activity of phosphofructokinase 1 (PFK), an important enzyme in the glycolytic pathway, presented a statistically significant increase (p <0.05) after 30 days of treatment when compared to control groups. The results obtained suggest that inactivation of viral sample with ethanol 70% induces lysis and disruption of viral particles. In addition, preliminary results indicated that treatment with intact biotherapic seems to induce higher variations on MDCK cells responses when compared to inactivated-biotherapic-treated cells. Further analyses are ongoing, including scanning electron microscopy and quantification of the number of mitosis, in order to elucidate the mechanisms involved with biochemical and cellular responses induced by theses biotherapics.

Evaluation of cytokine production by J774.G8 macrophages after treatment with biotherapics.
Siqueira CM, da Lozzo EJ, Kuczera D, de Freitas Buchi D, Couceiro JN, Holandino C.

Abstract
Introduction: Strains of macrophages, such as murine J774.G8 macrophages, are susceptible to influenza A infection [1]. One of the responses to viral infection involves the production of various types of immunostimulatory cytokines by infected cells [2].

Methods: In the present study, the macrophage strain J774.G8, maintained in RPMI medium, was submitted to treatment with 10% V/V of two different biotherapics prepared from influenza H3N2, both at 30x. Additionally, two control groups were analyzed: macrophages stimulated with water 30x and macrophages without any treatment. Biotherapics were prepared from intact H3N2 influenza virus and H3N2 inactivated by alcohol 70%. The compounding of both biotherapics followed this procedure: one part of viral particles was diluted in 9 parts of sterile distilled water. The 1:10 solution was submitted to 100 mechanical succussions using Autic® Brazilian machine, originating the first dilution, named decimal (1x). 1 ml of this solution was diluted in 9 ml of solvent and was submitted to 100 succussions, generating biotherapic 2x. This procedure was successively repeated, according to Brazilian Homeopathic Pharmacopoeia, to obtain the biotherapic 30x. By the same technique, water vehicle was prepared in the potency of 30x to be used as control. All samples were prepared under sterile and aseptic conditions, using laminar flow cabinet, class II, and were stored in the refrigerator (8ºC), to avoid microbiological contamination. J774.G8 macrophages were stimulated for 2 days, in a total of six stimuli. Immediately before infection with 25 µl of H3N2 influenza virus, the supernatants were collected and frozen at -20 ºC for later analysis. Next, 24 hours after the virus infection, the supernatants were aliquoted and frozen under the same conditions. Three independent experiments were done in triplicate. Analysis of supernatants was performed by flow cytometry using the Mouse Inflammation Kit. The cytokines detected in this experiment were IL-10, IL 12, TNF-α and MCP1.

Results: In all cases, there were no significant differences compared to control groups. However, the production of TNF-α detected in macrophages treated by intact and inactivated biotherapics presented a tendency to increase after infection. In fact, similar results were previously detected in other experiments conducted only with the intact biotherapic [3]. The release of the cytokine MCP1 in all experimental situations presented a tendency to decrease after the viral infection when compared to untreated macrophages. No statistically significant difference was detected in the production of IL 12 and IL 10. These experiments will be repeated to confirm the data obtained.

Link to abstract/paper:


Treatment with Candida albicans biotherapic influences in vitro fungal adhesion to Ma-104 cells.
Costa BGB, Siqueira CM, Barbosa GM, da Veiga VF, Portela MB, de Araújo Soares RM, Holandino C.

Abstract
Background: Oral candidiasis is an opportunistic fungal infection in humans, mainly caused by Candida albicans. It occurs when the host presents an imbalance in the immune system and Candida spp., normally found in human flora, become able to
develop the infection [1]. This disease is very common in HIV patients, and in all individuals that present immunosuppression, such as patients treated with chemotherapy. Considering this scenario, the development of new medicines to treat oral candidiasis is mandatory.

Aims: The aim of this study was to evaluate citotoxicity, morphology and quantify the adhesion rates of C. albicans to biotherapic-treated Ma104 cells.

Methodology: The biotherapic was prepared following the Roberto Costa technique and Brazilian Homeopathic Pharmacopeia protocol [2]. Briefly, biotherapic 1X was prepared with 1 mL of aqueous solution containing 108 yeasts of living Candida albicans plus 9 ml of sterile distilled water. This solution was submitted to 100 mechanical succussions. Biotherapic 2X was obtained after addition of 1 ml of 1X solution in 9 ml of sterile distilled water and it was also submitted to 100 mechanical succussions. This procedure was repeated until biotherapic 30X was obtained. As a control, sterile dynamized water (30X) was used. The inhibition of fungal growth induced by biotherapic was evaluated by MTT method after 24 hours of treatment. The morphological aspects of Ma104-biotherapic-treated cells were analyzed by Giemsa staining after 5, 10 and 60 days, and compared with control groups (water 30X and untreated cells). Additionally, Ma104 cells were treated during 5 and 30 days with biotherapic in parallel with respective controls, and the index adhesion of yeast cells was quantified.

Results: The biotherapic was not able to reduce the viability of treated C. albicans when compared with controls. On the other hand, Ma104 treated cells presented important morphological alterations after 60 days, such as: cytoplasmic vacuoles, halos around the nucleolus and elongation of the plasmatic membrane. These changes were not observed in untreated cells nor in ones treated with water 30X. The adhesion index to Ma104 cells was reduced around 27% after 5 and 30 days of treatment when compared to controls.

Conclusion: These results showed that the biotherapic did not present any citotoxicity, but was able to modify the morphological aspects of Ma-104 cells. Additionally, the interaction between host cells and ethilogic agent is directly influenced by biotherapic treatment, suggesting a promising antifungal potential of this medicine.


Triiodothyronine, diluted according homeopathic techniques, modifies the programmed cell death of tadpole tail’s explants.
Guedes JRP, Carrasco S, Mostério CMF, Bonamin LV, Souza W, Schainberg CG, Parra-Cuentas ER, Capelozzi VL.


Int J High Dilution Res. 2011;10(35):75-76.
Effects of 200cH medications on mice bone marrow cells and macrophages.
Paracelsus once wrote: "All things are poison and nothing is without poison, only the dose permits something not to be poisonous." Latter Hahmemann formulated the law of similars, preparations which cause certain symptoms in healthy individuals if given in diluted form to patients exhibiting similar symptoms will cure it. Highly diluted natural complexes prepared according to Hahmemann’s ancient techniques may represent a new form of immunomodulatory therapy. The lack of scientific research with highly diluted products led us to investigate the in vivo and in vitro actions of commonly used medications. Here we describe the results of experimental studies aimed at verifying the effects of Mercurius solubilis, Atropa Belladonna, Lachesis muta and Bryonia alba. All medications were at 200cH dilution. Animals were maintained for 7 days and were allowed to drink the medications, which were prepared in a way that the final dilution and agitation (200cH) was performed in drinking water. The medication bottle was changed and sucussed every afternoon. Co-culture of non treated mice bone marrow cells and in vitro treated peritoneal macrophages were also performed. After animal treatment the bone marrow cells were immunophenotyped with hematopoietic lineage markers on a flow cytometer. We have determined CD11b levels on bone marrow cells after culture and co-culture with treated macrophages and these macrophages were processed to scanning electron microscopy. We have observed by morphological changes that macrophages were activated after all treatments. Mercurius solubilis treated mice showed an increase in CD3 expression and in CD11b on nonadherent bone marrow cells after co-culture with in vitro treatment. Atropa Belladonna increased CD45R and decreased Ly-6G expression on bone marrow cells after animal treatment. Lachesis muta increased CD3, CD45R and, CD11c expression and decreased CD11b ex vivo and in nonadherent cells from co-culture. Bryonia alba increased Ly-6G, CD11c and CD11b expression ex vivo and when in co-culture CD11b was increased in adherent cells as well as decreased in nonadherent cells. With these results we have demonstrated that highly diluted medications act on immune cells activating macrophages, and changing the expression profile of hematopoietic lineage markers. Highly diluted medications are less toxic and cheaper than other commonly used medications and based on our observations, it is therefore conceivable that this medications which are able to act on bone marrow and immune cells may have a potential therapeutic use in clinical applications in diseases were the immune system is affected and also as regenerative medicine as it may allow proliferation and differentiation of progenitor cells.


**Cell alterations induced by a biotherapic for influenza.**  
Abstract
Introduction: Influenza viruses have been responsible for highly contagious acute respiratory illnesses with high mortality, mainly in the elderly, which encourages the development of new drugs for the treatment of human flu. The biotherapics are medicines prepared from biological products, which are not chemically defined. They are compounded following the homeopathic procedures indicated for infectious diseases with known etiology.
Aim: The purpose of the present study is to verify cellular alterations induced by a biotherapeutic prepared from the infectious influenza A virus.
Methodology: This biotherapeutic was prepared for this study in the homeopathic potency of 30X according to the Brazilian Homeopathic Pharmacopeia. The concentration of 10% was not cytotoxic to cells, as verified by neutral red assay. The cellular alterations observed in MDCK cells were analyzed by optical microscopy for the quantification of mitosis, nucleoli and lipid bodies. The mitochondrial activity was assessed by MTT assay and the phosphofructokinase-1 (PFK-1) enzyme activity was analyzed on the MDCK cells treated for 5, 10 and 30 days. Macrophages J778.G8 were treated with this biotherapeutic to evaluate the immunostimulatory cytokine release.
Results: The cellular alterations observed in MDCK cells were verified by optical microscopy. The number of lipid bodies present in MDCK cells stimulated for 10 days was significantly lower (p<0.05) when compared to controls. The biotherapeutic significantly increased (p<0.05) the number of mitosis and the mitochondrial activity of MDCK cells stimulated for 10 and 30 days. These changes were confirmed by a significant reduction (p<0.05) on the PFK-1 activity. These results suggest that the biotherapeutic was able to activate the Krebs cycle and pentose-phosphate metabolism to the generation of amino acids and nucleotides, situations common to cells whose rate of mitosis is increased. The quantification of immunostimulatory cytokines by macrophages J774.G8 indicated that the tumor necrosis factor (TNF-α) production was higher (p<0.05) in the supernatant of the macrophages pre-treated with this biotherapeutic and infected with influenza virus, suggesting an activation of the macrophages by this biotherapeutic.
Conclusion: This biotherapeutic is able to induce some cellular alterations, which show strong evidence that it might be a promising option for the human flu. New experiments are being developed to understand the mechanisms of action of this biotherapeutic.

Putative protective effect of Cadmium chloride high diluted solution on LLC-PK1 cell intoxicated by high concentration of this same metal.
Ghilosso-Bortolini R, Bonamin LV, Holandino C.

Abstract
Cadmium is an important toxic environmental heavy metal. Several studies have demonstrated that a major site of cadmium toxicity in humans and in other animals is the proximal tubule of the kidney. A well established model for nephrotoxicity is the use
of in vitro technique with proximal tubule epithelial cell lines, as LLC-PK1. Herein, we have the intention to study the possible protective effect of highdiluted CdCl₂ solutions. In a blinding way, LLC-PK1 cells were pre-treated with highdiluted cadmium chloride in the potencies 10 cH, 15 cH and 20 cH. After 4 days, these cells have received CdCl₂ in a pre-determined toxic concentration. The cell viability was assessed by MTT assay. We have identified a protective effect of two CdCl₂ highdiluted solutions, 10 cH and 20 cH, when cells were intoxicated by sublethal CdCl₂ concentration. The results indicate that probably the highdilutions have an expressive action on cells in sublethal intoxication.


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**Decreased intensity of Japanese encephalitis virus infection in chick chorioallantoic membrane under influence of ultradiluted Belladonna extract.**

Bandyopadhyay B, Das S, Sengupta M, Saha C, Das KC, Sarkar D, Nayak C.

Abstract

Problem statement: No specific antiviral therapy is currently available despite an emergence and resurgence of Japanese encephalitis in South-East Asian Countries. There are only few recent studies, which were aimed to treat Japanese encephalitis with newer drugs. There is thus a real need for study on antiviral agents that can reduce the toll of death and neurological sequelae resulting from infection with this virus.

Approach: Optimum dilution of the JE virus was determined which could produce significant number of pocks on Chorioallantoic Membrane (CAM). Then ultradiluted belladonna preparations were used to see their inhibitory action on JE virus infection in CAM.

Results: Ultradiluted belladonna showed significantly decreased pock count in CAM in comparison to JE virus control.

Conclusion: Ultradiluted belladonna could inhibit JE virus infection in CAM, which may be mediated through glycosidase inhibitory role of calystegines present in belladonna.


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**Inhibition of basophil activation by histamine: a sensitive and reproducible model for the study of the biological activity of high dilutions.**

Sainte-Laudy J, Belon P.

CHU, Limoges 87042, France. jslaudy@wanadoo.fr

Abstract
BACKGROUND: At the beginning of this series of experiments we were looking for a model based on the use of purified commercially available compounds based on a fully described and accepted pharmacological model to study of the biological effect of high dilutions. Negative feedback induced by histamine, a major pro-inflammatory mediator, on basophils and mast cells activation via an H2 receptor met these criteria. The simplest way of measuring basophil activation in the early 1980’s was the human basophil activation test (HB DT).

OBJECTIVES: Our major goal was first to study the biological effect of centesimal histamine dilutions beyond the Avogadro limit, on the staining properties of human basophils activated by an allergen extract initially house dust mite, then an anti-IgE and N-formyl-Met-Leu-Phe (fMLP). Technical development over the 25 years of our work led us to replace the manual basophil counting by flow cytometry. The main advantages were automation and observer independence. Using this latter protocol our aim was to confirm the existence of this phenomenon and to check its specificity by testing, under the same conditions, inactive analogues of histamine and histamine antagonists. More recently, we developed an animal model (mouse basophils) to study the effect of histamine on histamine release.

METHODS AND RESULTS: For the HBDT model basophils were obtained by sedimentation of human blood taken on EDTA and stained with Alcian blue. Results were expressed in percentage activation. Histamine dilutions tested were freshly prepared in the lab by successive centesimal dilutions and vortexing. Water controls were prepared in the same way. For the flow cytometric protocol basophils were first labeled by an anti-IgE FITC (basophil marker) and an anti-CD63 (basophil activation marker). Results were expressed in percentage of CD63 positive basophils. Another flow cytometric protocol has been developed more recently, based on basophil labeling by anti-IgE FITC (fluorescein isothiocyanate) and anti-CD203 PE (another human basophil activation marker). Results were expressed in mean fluorescence intensity of the CD203c positive population (MFI-CD203c) and an activation index calculated by an algorithm. For the mouse basophil model, histamine was measured spectrofluorimetrically. The main results obtained over 28 years of work was the demonstration of a reproducible inhibition of human basophil activation by high dilutions of histamine, the effect peaks in the range of 15-17CH. The effect was not significant when histamine was replaced by histidine (a histamine precursor) or cimetidine (histamine H2 receptor antagonist) was added to the incubation medium. These results were confirmed by flow cytometry. Using the latter technique, we also showed that 4-Methyl histamine (H2 agonist) induced a similar effect, in contrast to 1-Methyl histamine, an inactive histamine metabolite. Using the mouse model, we showed that histamine high dilutions, in the same range of dilutions, inhibited histamine release.

CONCLUSIONS: Successively, using different models to study of human and murine basophil activation, we demonstrated that high dilutions of histamine, in the range of 15-17CH induce a reproducible biological effect. This phenomenon has been confirmed by a multi-center study using the HBDT model and by at least three independent laboratories by flow cytometry. The specificity of the observed effect was confirmed, versus the water controls at the same dilution level by the absence of biological activity of inactive compounds such as histidine and 1-Methyl histamine and by the reversibility of this effect in the presence of a histamine receptor H2 antagonist.

In vitro examination of potentized atropine sulfate dilutions on the contractility of the isolated rat ileum.
Siegling-Vlitakis C, Martens H, Lüdtke R.

Abstract
OBJECTIVES: Atropine sulphate, a competitive antagonist of acetylcholine (ACh) at muscarinic receptors, was first isolated from Atropa belladonna, one of the most used and best known homeopathic medicines. It has been suggested that high potencies of homeopathic atropine sulphate might have an influence on ACh-induced contraction of smooth muscles. The aim of the study was to determine the effects of homeopathic dilutions of atropine sulphate D6, D32, and D100 compared to the potentized diluents L6, L32, and L100 on ACh-induced contraction of isolated rat ileum.
DESIGN: Forty-eight (48) ileal sections from 12 male Wistar rats were incubated in modified Krebs solutions, and the contractile activity responses to ACh obtained in the absence and presence of the test substances were recorded. Investigators and biometrician were completely blinded.
RESULTS: No significant effects of atropine sulphate D6, D32, or D100 could be found (all p > 0.4 after Bonferoni-Holm correction) compared to the potentized diluents L6, L32, and L100, respectively. These figures did not change considerably even when strict a priori criteria were applied that define a measurement as valid and comparable.
CONCLUSIONS: Our experiments could not replicate previous results on the effects of homeopathic atropine.

Influence of Traumeel on cultured chondrocytes and recombinant human matrix metalloproteinases.
Seilheimer B, Wierzchacz C, Gebhardt R.

Abstract
Background: Chronic joint diseases, such as osteoarthritis, are associated with insults to articular cartilage. Imbalance between extracellular matrix production and degradation as well as defects in chondrocyte proliferation and differentiation lead to progressive degeneration of cartilage. In this study we have investigated whether Traumeel, an anti-inflammatory and wound-healing agent, affects chondrocyte proliferation and differentiation as well as activity of matrix metalloproteinases (MMPs) that are implicated in matrix degradation.
Methods: Chondrocytes from porcine knee joints were cultured in agarose layers in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Foetal Calf Serum (FCS). To maintain the differentiation state of the chondrocytes they were treated with collagen type II in absence of FCS. Viability and proliferation of
chondrocytes upon Traumeel application in combination with transforming growth factor beta (TGF-β) treatment were determined by MTT assay and 3H-thymidine incorporation, respectively. Levels of sulfated glycosaminoglycans (sGAG), which are characteristic of chondrocyte functional activity, were measured with the dimethylmethylene blue assay. Activities of recombinant catalytic domains of human MMPs were determined with a chromogenic assay.

Results: Addition of Traumeel significantly enhanced TGF-β-induced proliferation, but did not affect basal proliferation of chondrocytes in the presence of FCS. In chondrocytes in the differentiated state, Traumeel enhanced viability of the cells and stimulated biosynthesis of sGAG. Several MMPs were screened for inhibition by Traumeel; MMP-13 was found to be most inhibited (by 30%), while MMPs-2, -3, and -9 were not affected.

Discussion: While influence of Traumeel on proliferation of chondrocytes appears to be context-dependent, differentiation was supported under all tested conditions. Since Traumeel is also known to inhibit the production of cytokines which may reduce the functional capacity of chondrocytes, survival and function of these cells is likely to be improved by Traumeel on various levels. Remarkably, Traumeel inhibited MMP-13 which is closely associated with the pathology of joint destruction. Thus, Traumeel might also indirectly slow down the progression of cartilage degeneration.

Conclusion: Our data suggest that Traumeel offers a potential therapeutic option for chronic joint diseases which needs to be further investigated.


Positive influence of Vertigoheel on signalling pathways of smooth muscle cells in vertigo.
Pries A.

Abstract
The incidence of circulatory disorders is increasing against the background of increasing life expectancy. Cardiovascular, metabolic and inflammatory disorders are directly associated with endothelial dysfunction of the small blood vessels. It is possible to influence microcirculation disorders in various ways through the use of drugs. An experimental investigation at the University of Tübingen in cooperation with the Charité Berlin has recently shown that the complex homoeopathic reparation Vertigoheel in vitro influences the contraction of smooth muscle cells. The investigation was performed on sections of rats’ arteries, which were initially contracted and then treated with various concentrations of Vertigoheel. The concentrations used corresponded to the dosages used in clinical therapy. On the one hand the complex remedy induces – through its direct effect on beta-2 receptors and a partial rise in NO – an increase in the synthesis of the messenger substances, while at the same time inhibiting their degradation. The resulting effect is a higher concentration of the molecules, leading to the development of the protective effect of NO. The study showed that under the influence of Vertigoheel there was significant, concentration-dependent relaxation in the vascular wall. In a further fundamental investigation it was possible to show the influence of Vertigoheel on the activity of human adenylate cyclase and phosphodiesterases IV and V. From the combination
of these data it is now possible to describe the signalling pathways of Vertigoheel in a scientifically established manner. However, Vertigoheel not only improves the signalling pathways of smooth muscle cells but also is an effective medication for the therapy of vertigo of varying genesis. Various clinical investigations and cohort studies have shown an equivalent effect to dimenhydrinate \((n=774)\), betahistine \((n=117)\) and ginkgo biloba \((n=170)\). In each case the frequency, duration and intensity of the vertigo attacks were determined, as well as the tolerability of the medication. A meta-analysis of four studies also underlined the efficacy of Vertigoheel in cases of vertigo of varying genesis.


**Einzeller als Testorganismen für den Wirksamkeitsnachweis der Homöopathie.**
[Protozoa as test organisms for the efficacy of homeopathy].
[Article in German]
Schmidt G.

Link to abstract/paper: [http://bibnet.org/vufind/Record/ccmed952162421](http://bibnet.org/vufind/Record/ccmed952162421)


**Stimulation of lymphocyte anti-melanoma activity by co-cultured macrophages activated by complex homeopathic medication.**
Guimarães FS, Abud AP, Oliveira SM, Oliveira CC, César B, Andrade LF, Donatti L, Gabardo J, Trindade ES, Buchi DF.

Departamento de Biologia Celular, Laboratório de Pesquisa em Células Inflamatórias e Neoplásicas, Universidade Federal do Paraná (UFPR), Curitiba - PR, Brazil. ferbiocel@yahoo.com.br

Abstract

**BACKGROUND:** Melanoma is the most aggressive form of skin cancer, and the most rapidly expanding cancer in terms of worldwide incidence. Chemotherapeutic approaches to treat melanoma have been uniformly disappointing. A Brazilian complex homeopathic medication (CHM), used as an immune modulator, has been recommended for patients with depressed immune systems. Previous studies in mice have demonstrated that the CHM activates macrophages, induces an increase in the number of leukocytes and improves the murine response against Sarcoma-180.

**METHODS:** Here we studied the interaction of mouse lymph node lymphocytes, co-cultured in vitro with macrophages in the presence or absence of the CHM, with B16F10 melanoma cells.

**RESULTS:** Lymphocytes co-cultured with macrophages in the presence of the CHM had greater anti-melanoma activity, reducing melanoma cell density and increasing the number of lysed tumor cells. There was also a higher proportion of activated
(CD25+) lymphocytes with increased viability. Overall, lymphocytes activated by treatment destroyed growing cancer cells more effectively than control lymphocytes. CONCLUSION: Co-culture of macrophages with lymphocytes in the presence of the CHM enhanced the anti-cancer performance of lymphocytes against a very aggressive lineage of melanoma cells. These results suggest that non-toxic therapies using CHMs are a promising alternative approach to the treatment of melanomas. In addition, they are attractive combination-therapy candidates, which may enhance the efficacy of conventional medicines by improving the immune response against tumor cells.

Link to paper: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2749867/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2749867/)

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**Dynamized preparations in cell culture.**

Sunila ES, Kuttan R, Preethi KC, Kuttan G.

Research Director, Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala-680 555, India. amalaresearch@rediffmail.com.

Abstract

Although reports on the efficacy of homeopathic medicines in animal models are limited, there are even fewer reports on the in vitro action of these dynamized preparations. We have evaluated the cytotoxic activity of 30C and 200C potencies of ten dynamized medicines against Dalton's Lymphoma Ascites, Ehrlich's Ascites Carcinoma, lung fibroblast (L929) and Chinese Hamster Ovary (CHO) cell lines and compared activity with their mother tinctures during short-term and long-term cell culture. The effect of dynamized medicines to induce apoptosis was also evaluated and we studied how dynamized medicines affected genes expressed during apoptosis. Mother tinctures as well as some dynamized medicines showed significant cytotoxicity to cells during short and long-term incubation. Potentiated alcohol control did not produce any cytotoxicity at concentrations studied. The dynamized medicines were found to inhibit CHO cell colony formation and thymidine uptake in L929 cells and those of Thuja, Hydrastis and Carcinosinum were found to induce apoptosis in DLA cells. Moreover, dynamized Carcinosinum was found to induce the expression of p53 while dynamized Thuja produced characteristic laddering pattern in agarose gel electrophoresis of DNA. These results indicate that dynamized medicines possess cytotoxic as well as apoptosis-inducing properties.

Link to paper: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2686624/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2686624/)

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**Prevention of cadmium-induced toxicity in liver-derived cells by the combination preparation Hepeel(®).**

Gebhardt R.
Institute of Biochemistry, Medical Faculty, University of Leipzig, Johannisallee 30, 04103 Leipzig, Germany.

Abstract
Cadmium is a heavy metal of considerable environmental concern that causes liver damage. This study examined the possible prevention of cadmium toxicity in human HepG2 cells and primary rat hepatocytes by Hepeel(®), a combined preparation of tinctures from seven different plants. Hepeel(®) prevented cadmium chloride (CdCl(2))-induced cell death in both HepG2 cells and hepatocytes, and also reduced the loss of glutathione, lipid peroxidation, nuclear fragmentation, caspase activation and release of mitochondrial cytochrome C. To compare their relative efficacy, the seven constituent plant tinctures of Hepeel(®) were also separately tested. The tinctures China and Nux moschata, which exert solely anti-oxidative effects, failed to reduce cytotoxicity, and only protected against loss of glutathione and lipid peroxidation. In contrast, the tinctures Carduus marianus and Chelidonium, demonstrated anti-apoptotic effects, and protected HepG2 cells and primary hepatocytes against CdCl(2)-induced cell death. These results demonstrate how the effectiveness of Hepeel(®) is determined by the synergistic features of its constituent tinctures. Furthermore, we conclude that cadmium toxicity in the liver is mainly due to stimulation of the intrinsic apoptotic pathway, but may be intensified by increased oxidative stress.


In vitro growth of uropathogenic Escherichia coli isolated from a snow leopard treated with homeopathic and isopathic remedies: a pilot study.
Kawakami AP, Osugui L, César AT, Priven SW, de Carvalho VM, Bonamin LV.

Abstract
This paper reports the results of incubation of a strain of uropathogenic Escherichia coli (UPEC) isolated from a snow leopard - which had died of septicemia secondary to necro-hemorrhagic cystitis - with homeopathic and isopathic remedies. Methods: UPEC was isolated from heart blood and previously typified for virulence factors; it was incubated with homeopathic remedies Cantharis vesicatoria (urinary tract infection affinity), Mercurius solubilis (from symptoms analysis) and nosode prepared from the actual strain, all in dilution 12cH. Results: 2 patterns of bacterial growth were observed, associated to the quality of nutrients in the culture medium; in rich-nutrient medium, nosode of E. coli 12cH had a significant inhibitory effect; in poor-nutrient medium, Merc 12cH exerted significant inhibitory effect. Conclusion: results suggest that the previous conditions of prokaryote systems may influence the in vitro response to homeopathic and isopathic remedies.


Homeopathic drug discovery: theory update and methodological aspect.
Khuda-Bukhsh AR, Pathak S.

University of Kalyani, Department of Zoology, Cytogenetics and Molecular Biology Laboratory, Kalyani-741235, India +91 33 25828768 ; +91 33 25828282 ; prof_arkb@yahoo.co.in khudabukhsh_48@rediffmail.com.

Abstract
Background: Homeopathy treats patients on the basis of totality of symptoms and is based on the principle of 'like cures like'. It uses ultra-low doses of highly diluted natural substances as remedies that originate from plants, minerals or animals.
Objective: The objectives of this review are to discuss concepts, controversies and research related to understanding homeopathy in the light of modern science.
Methods: Attempts have been made to focus on current views of homeopathy and to delineate its most plausible mechanism(s) of action.
Results: Although some areas of concern remain, research carried out so far both in vitro and in vivo validates the effects of highly diluted homeopathic medicines in a wide variety of organisms. Conclusion: The precise mechanism(s) and pathway(s) of action of highly diluted homeopathic drugs are still unknown.

Activation of mononuclear bone marrow cells treated in vitro with a complex homeopathic medication.
Cesar B, Abud AP, de Oliveira CC, Cardoso F, Gremski W, Gabardo J, Buchi Dde F.

Departamento de Biologia Celular, Setor de Ciências Biológicas, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil.

Abstract
Canova is a Brazilian homeopathic medication with immunomodulatory properties, recommended for patients where the immune system is depressed. Previous studies demonstrated that Canova induces up-regulation in numbers of leukocytes. The bone marrow microenvironment is composed of growth factors, stromal cells, extracellular matrix and progenitor cells that differentiate into mature blood cells. We now report the effect of in vitro administration of the medication on the mononuclear differentiation of the bone marrow cell. Swiss mice femurs were dissected cleaned and the cells of the marrow were flushed. The cells were plated, treated or not, incubated for different times and processed for light, transmission and scanning electron, and confocal microscopy analysis. Bone marrow cells showed an enhanced proliferation in vitro in response to Canova medication and Canova plus M-CSF and an increase was also observed in the numbers of the cell niches and ring-shaped nuclei cells. Confocal and transmission and scanning electron microscopy showed the stages of monocyte maturation, with resting and activated cells. With Canova treatment there was a marked increase in cell size, which is mainly attributable to the augmented cytoplasm, an increase in the number of mitochondria, expansion of the RER and an enlarged Golgi. The response to Canova treatment indicates that it influences mononuclear differentiation and activation of bone marrow progenitor and stromal cells.
In vitro evaluation of the antiviral effects of the homeopathic preparation Gripp-Heel on selected respiratory viruses.

Glatthaar-Saalmüller B.

Clinical Immunology, University of Veterinary Medicine Vienna, A-1210 Vienna, Austria. glatthaar@l-d-g.de

Abstract

Gripp-Heel is a homeopathic preparation frequently used in the treatment of respiratory viral infections such as various types of influenza and the common cold. The antiviral activity of Gripp-Heel was studied in vitro on human pathogenic enveloped and nonenveloped RNA and DNA viruses. Before the antiviral assays, in vitro cytotoxicity of Gripp-Heel was determined with cells used for the infection experiments (HeLa, HEP-2, MDCK, BGM) as well as with mitogen-stimulated peripheral blood mononuclear leukocytes. A concentration of 0.5 of the commercially available product slightly reduced cell viability and proliferative capacity, and experiments on antiviral activity were determined starting with a dilution of 0.2 of the commercially available product. The antiviral activity was determined against a broad panel of enveloped and nonenveloped DNA and RNA viruses with plaque reduction assay, cytopathogenic assays, virus titrations, analysis of the viral proteins in virus-specific enzyme immunoassays, and haemagglutination tests. Control substances were acyclovir (10 microg/mL), ribavirin (6 microg/mL), and amantadine hydrochloride (5 microg/mL), depending on the virus type. Gripp-Heel demonstrated dose-dependent in vitro activity (significant reductions of infectivity by 20% to 40%) against Human herpesvirus 1, Human adenovirus C serotype 5, Influenza A virus, Human respiratory syncytial virus, Human parainfluenza virus 3, Human rhinovirus B serotype 14, and Human coxsackievirus serotype A9. The mechanisms of this antiviral activity are still unclear, but type I interferon induction might be a possible explanation. Further research on this homeopathic preparation seems warranted.

Neuroprotective effect of ultra-low doses of antibodies against S100 protein in neuroblastoma culture during oxygen and glucose deprivation.

Pankova TM, Starostina MV, Shtark MB, Epstein OI.

Institute of Molecular Biology and Biophysics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk.

Abstract

Antibodies against S100 protein applied in high and ultra-high dilutions possess neuroprotective activity and maintain survival of neuroblastoma C-1300 cells under conditions of oxygen and glucose deprivation. The examined antibody preparations
stimulated differentiation in neuroblastoma culture thereby demonstrating pronounced neurotrophic activity.

Link to paper:

Effects of Saussurea lappa roots extract in ethanol on leukocyte phagocytic activity, lymphocyte proliferation and interferon-gamma (IFN-gamma).
Sarwar A, Enbergs H.

Department of Veterinary Anatomy, University of Agriculture 38040, Faisalabad, Pakistan. anas_sarwar@hotmail.com

Abstract
Effects of Saussurea lappa root extracts prepared in ethanol according to the homeopathic principles were assessed on leukocyte phagocytic activity, lymphocyte transformation and mitogen-induced interferon-gamma (IFN-gamma) in the cultures of peripheral blood mononuclear cells of goats (PBMC) in vitro. Leukocyte phagocytic activity was measured by flow cytometry, lymphocyte proliferation by MTT and IFN-gamma level in cell culture supernatants was determined by ELISA. The results obtained demonstrated that all test dilutions (D4, D6, D8) of Saussurea lappa in ethanol have exerted a stimulating effect on leukocyte phagocytic activity in dose-dependent manner. A 10 microl dose of Saussurea lappa of each dilution markedly enhanced phagocytic activity, while other doses tested made only a feeble stimulating effect. The increases with 10 microl dose were found significantly (P<0.01) different between each dilution, maximal stimulation was observed by D8 dilution. Different doses (10 microl, 2 microl, 1 microl, 0.5 microl) of all test dilutions (D4, D6, D8) of Saussurea lappa in sterile 0.9% NaCl solution inhibited lymphocyte proliferation. Maximal inhibitory effect was observed with the 2 microl dose. Similarly, Saussurea lappa suppressed the secretion of IFN-gamma by mitogen-activated (PHA; 2.5 microg/ml) of peripheral mononuclear cells in dose-dependent manner. In conclusion these findings suggest that enhanced leukocyte phagocytic activity may be helpful to clear the soluble immune complexes produced during a sustained immune response against self antigens which causes chronic inflammatory injury of tissue. On the other hand, inhibition of lymphocyte proliferation and IFN-gamma by Saussurea lappa may contribute to suppress immune-mediated inflammatory reactions possibly through a cell-mediated cytokine pathway. Thus it is conceivable that ethanolic extracts of Saussurea lappa roots in homeopathic dilutions may be considered as a potential candidate for therapeutic support in autoimmune and chronic inflammatory disorders.

The in vitro evidence for an effect of high homeopathic potencies—a systematic review of the literature.
Abstract

OBJECTIVE: Systematic assessment of the in vitro research on high potency effects.

METHOD: Publications of experiments were collected through databases, experts, previous reviews, citation tracking. Inclusion criteria: stepwise agitated dilutions <10(-23); cells or molecules from human or animal. Experiments were assessed with the modified SAPEH score.

RESULTS: From 75 publications, 67 experiments (1/3 of them replications) were evaluated. Nearly 3/4 of them found a high potency effect, and 2/3 of those 18 that scored 6 points or more and controlled contamination. Nearly 3/4 of all replications were positive. Design and experimental models of the reviewed experiments were inhomogenous, most were performed on basophiles.

CONCLUSIONS: Even experiments with a high methodological standard could demonstrate an effect of high potencies. No positive result was stable enough to be reproduced by all investigators. A general adoption of succussed controls, randomization and blinding would strengthen the evidence of future experiments.


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**Sci Cult.** 2007;73(5-6):173-175.

**Conformational changes of bovine serum albumin in 4M urea and ultra high dilutions of different drugs.**

Sukul NC, Datta S, Sinhababu SP.

Abstract

Conformational changes of bovine serum albumin (BSA) due to homeopathic potencies like Chelidonium 30, Sulphur 30, Nux vomica 30, Santonin 30, Ethanol 30, 90% ethanol (control) and 4M urea (control) were observed by fluorescence emission and electronic circular dichroism (CD) spectra. Fluorescence intensities in urea and homeopathic potencies differed markedly from the control (90% ethanol) by 11.4-29.5. The control showed the highest intensity and 4M urea solution the lowest. The CD maxima and minima varied in different potencies and the control with respect to the wavelength and intensities. The results indicate that different potentized drugs produced different conformations of BSA.

Link to abstract/paper: [http://www.scienceandculture-isna.org/CONFORMATIONAL.htm](http://www.scienceandculture-isna.org/CONFORMATIONAL.htm)

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**Activation of bone marrow cells treated with Canova in vitro.**

Abud AP, Cesar B, Cavazzani LF, de Oliveira CC, Gabardo J, Buchi Dde F.
Setor de Ciências Biológicas, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil.

Abstract

Canova is a Brazilian complex homeopathic medication produced from Aconitum, Thuya, Bryonia, Lachesis and Arsenicum. Previous studies demonstrated that Canova induces up-regulation in numbers of leukocytes. The bone marrow microenvironment is composed of growth factors, stromal cells, extracellular matrix, and progenitor cells that differentiate into mature blood cells. As it is the major site of blood cell formation, we studied in vitro Canova effects on bone marrow cells of mice. Swiss mouse femurs were dissected, cleaned, and the marrow was flushed. The cells were plated, treated or not, incubated for different times and processed for light, scanning electron, and confocal microscopy, and also flow cytometry. The treatment did not modify the expression of the analyzed surface markers or cytokine production. All microscopy techniques showed that a monocytic lineage (CD11b(+)) and stromal cells (adherent cells) were activated by treatment. Canova also increased cell clusters over adherent cells, suggesting proliferation areas.


Int J High Dilution Res. 2006;5(17).

Avaliação do Bioterápico Trypanosoma cruzi 30DH: Um Estudo In Vitro. [Evaluation of Trypanosoma cruzi 30dH biotherapic: an in vitro study].
[Article in Portuguese]
Queiroz AO, Xavier SCC, Faria KG, Bernardo RR, Leitão TCA.

English Abstract

In the present study, our main objective was to evaluate the biological activity and the kinetics of the humoral immune response in Swiss Webster mice using the biotherapic agent Trypanosoma cruzi 30DH. This compound was prepared following the pharmacotechnique described by Roberto Costa. Trypanosoma cruzi 30DH was evaluated by the parasitemia parameters and the humoral immune response, performed by Indirect Immunefluorescence Reaction (RIFI) to analyse IgM and IgG antibodies. Based on the biological activity, 50% of the animals in the group treated with the biotherapic agent survived. The group which received treatment and infection simutaneously and in the control group had 100% mortality. Higher IgG levels in the group of animals previously treated with the compound could be observed, presenting 1:80 and with sub-patent parasitemia. Our results suggest that the previous treatment with the biotherapic agent showed a humoral immune response, with higher serological titers and absence of parasites in the blood.


Phagocytosis, endosomal/lysosomal system and other cellular aspects of macrophage activation by Canova medication.

Lopes L, Godoy LM, de Oliveira CC, Gabardo J, Schadeck RJ, de Freitas Buchi D.
Laboratório de Estudos de Células Inflamatórias e Neoplásicas, Departamento de Biologia Celular, Universidade Federal do Paraná, Brazil.

Abstract
Canova is a homeopathic medication with immunomodulatory properties, recommended for diseases where the immune system is depressed. Our research aims to study the activation of mice peritoneal macrophages when submitted to in vivo and in vitro Canova treatment. Morphological parameters and acid phosphatase activity were analyzed using light and transmission electron microscopy. Differential interference contrast microscopy, including serial time acquisition in living cells, was also performed. The results demonstrated a greater spreading ability in Canova treated macrophages, a higher phagocytic activity of non-infective microorganisms (Saccharomyces cerevisiae and Tripanosoma cruzi epimastigotes) and a tendency to lower the phagocytic activity of the infective microorganisms T. cruzi trypomastigotes and Leishmania amazonensis, when compared with control cells. Acid phosphatase activity was analyzed and showed that Canova treatment stimulates an increase of the endosomal/lysosomal system. Treated macrophages that do or do not interact with yeast present a higher number of acid phosphatase marked vesicles compared to control cells. In contrast, the activity of tartrate resistant acid phosphatase (TRAP), is lower in Canova treated macrophages. The net results demonstrate that Canova medication is an effective stimulator of macrophage activity.

Int J High Dilution Res. 2006 Jul-Sep;5(16).
Desenvolvimento de um Novo Bioterápico a partir do Vírus Influenza Infeccioso e Verificação de sua Eficácia in vitro.
[Development of a New Biotherapic from the Infectious Influenza Virus and the Verification of its in vitro Effectiveness].
[Article in Portuguese]
Siqueira CM, Féo da Veiga V, Couceiro JN, Lyrio C, Quaresma CH.

English Abstract
Influenza virus has been responsible for the majority of acute respiratory illnesses all over the world. Biotherapics are remedies prepared following homeopathic procedures from biological products, which are not chemically defined, following the law of similars. They are used in infectious diseases of known etiology. Influenzinum, used in homeopathy medicine, is made from the Influenza vaccine. The purpose of this study is to develop for the first time a biotherapic from the infectious Influenza virus (H1N1 and H3N2) and to verify its in vitro effectiveness. The antiviral power of this biotherapic will be evaluated through the inhibition of the characteristic cytopathic effect (CPE) in MDCK cells pre-treated with different dilutions of this biotherapic and infected by the virus, which will be compared to the CPE caused by other antiviral drugs. Possible morphological modifications will be analyzed through optical and electronic microscopy.
Canova, a Brazilian medical formulation, alters oxidative metabolism of mice macrophages.

**de Oliveira CC, de Oliveira SM, Godoy LM, Gabardo J, Buchi Dde F.**

Laboratório de Estudos de Células Inflamatórias e Neoplásicas, Departamento de Biologia Celular, SCB, Universidade Federal do Paraná, Curitiba, PR, Brasil.
labbiocel@ufpr.br

Abstract

Macrophages play a significant role in the host defence mechanism. When activated they can produce reactive oxygen species (ROS) as well as related reactive nitrogen species (RNS). ROS are produced via NAD(P)H oxidase which catalyzes superoxide (O2-) formation. It is subsequently converted to hydrogen peroxide (H2O2) by either spontaneous or enzyme-mediated dismutation. Nitric oxide synthase (NOS) catalyzes nitric oxide (NO) formation. Canova (CA) is a Brazilian medication produced with homeopathic techniques, composed of Aconitum, Thuya, Bryonia, Arsenicum, Lachesis in distilled water containing less than 1% ethanol. Previous studies demonstrated that CA is neither toxic nor mutagenic and activates macrophages decreasing the tumor necrosis factor-alpha (TNFalpha) production. In this assay we showed that macrophages triggered with Canova increased NAD(P)H oxidase activity as well as that of iNOS, consequently producing ROS and NO respectively. Cytochrome oxidase and peroxisomes activities were inhibited by NO. As NO and O2- are being produced at the same time, formation of peroxynitrite (ONOO-) may be occurring. A potential explanation is provided on how treatment with Canova may enhance immune functions which could be particularly important in the cytotoxic actions of macrophages. CA can be considered as a new adjuvant therapeutic approach to known therapies.


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Bellavite P, Conforti A, Pontarollo F, Ortolani R.

Department of Scienze Morfologico-Biomediche, University of Verona, Italy.
paolo.bellavite@univr.it

Abstract

Here we describe the results of some experimental laboratory studies aimed at verifying the efficacy of high dilutions of substances and of homeopathic medicines in models of inflammation and immunity. Studies carried out on basophils, lymphocytes, granulocytes and fibroblasts are reviewed. This approach may help to test under controlled conditions the main principles of homeopathy such as 'similarity' of drug action at the cellular level and the effects of dilution/dynamization on the drug activity. The current situation is that few and rather small groups are
working on laboratory models for homeopathy. Regarding the interpretation of data in view of the simile principle, we observe that there are different levels of similarity and that the laboratory data give support to this principle, but have not yet yielded the ultimate answer to the action mechanism of homeopathy. Evidence of the biological activity in vitro of highly diluted-dynamized solutions is slowly accumulating, with some conflicting reports. It is our hope that this review of literature unknown to most people will give an original and useful insight into the 'state-of-the-art' of homeopathy, without final conclusions 'for' or 'against' this modality. This kind of uncertainty may be difficult to accept, but is conceivably the most open-minded position now.

Link to abstract/paper: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1375241/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1375241/)

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**Essiac tea: scavenging of reactive oxygen species and effects on DNA damage.**
**Leonard SS, Keil D, Mehlman T, Proper S, Shi X, Harris GK.**

Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, 1095 Willowdale Rd, MS/2015, Morgantown, WV 26505, USA. SEL5@cdc.gov

Abstract
Essiac, a tea reportedly developed by the Ojibwa tribe of Canada and widely publicized as a homeopathic cancer treatment, is prepared from a mixture of four herbs Arctium lappa, Rumex acetosella, Ulmus rubra and Rheum officinale. Each of these herbs has been reported to possess antioxidant and anti-cancer activity. Essiac itself has also been reported to demonstrate anti-cancer activity in vitro, although its effects in vivo are still a matter of debate. We prepared an extract of Essiac tea from a concentration of 25mg/mL and boiled it for 10 min. From this preparation we used concentrations of 5, 10, 25 and 50% to measure Essiac effects. In this study, we examined the effects of Essiac on free radical scavenging and DNA damage in a non-cellular system, as well as the effects Essiac on lipid peroxidation using the RAW 264.7 cell line. We observed, using electron spin resonance, that Essiac effectively scavenged hydroxyl, up to 84% reduction in radical signal at the 50% tea preparation concentration, and superoxide radicals, up to 82% reduction in radical signal also at the 50% tea preparation concentration, as well as prevented hydroxyl radical-induced DNA damage. In addition, Essiac inhibited hydroxyl radical-induced lipid peroxidation by up to 50% at the 50% tea preparation concentration. These data indicate that Essiac tea possesses potent antioxidant and DNA-protective activity, properties that are common to natural anti-cancer agents. This study may help to explain the mechanisms behind the reported anti-cancer effects of Essiac.


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Stimulation of bovine sperm mitochondrial activity by homeopathic dilutions of monensin.
Aziz DM, Enbergs H.

Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Mosul, Mosul, Iraq. dhaferaziz@web.de

Abstract
Mitochondrial activity is an important viability parameter of spermatozoa and is linked to sperm motility. Monensin is commonly used as an inhibitor for sperm mitochondrial activity in the laboratory. This study was conducted to evaluate the influence of some homeopathic dilutions of monensin on sperm mitochondrial activity. Fresh ejaculates from 6 mature bulls were used in the study. Samples of the semen were tested using a flow cytometer for mitochondrial activity and sperm viability using Rhodamine 123 and SYBR-14, respectively. The 9x dilution of monensin resulted in very highly significant (P<0.001) stimulation of mitochondrial activity. Monensin 5x, 7x, 8x and 13x caused highly significant (P<0.01) stimulation of the sperm mitochondrial activity. Other homeopathic dilutions of monensin (6x, 10x, 11x, 12x and 14x) also had a significant (P<0.05) stimulatory effect. The use of monensin did not have any negative effect on sperm viability. We conclude that some homeopathic dilutions of monensin increase mitochondrial activity of bovine spermatozoa without negative effect on sperm viability, the 9x dilution was the most effective. Further in vivo studies are required to estimate the effect of homeopathic dilutions of monensin on semen quality.

FMS*Calciumfluor specifically increases mRNA levels and induces signaling via MAPK 42,44 and not FAK in differentiating rat osteoblasts.
Manduca P, Marchisio S, Astigiano S, Zanotti S, Galmozzi F, Palermo C, Palmieri D.

Laboratorio di Genetica, Dipartimento di Biologia, Università di Genova, C. Europa 26, Italy. man-via@unige.it

Abstract
The homeopathic compound of resonance FMS*Calciumfluor (FMS*) reportedly promotes osteogenic differentiation of rat pre-osteoblasts in vitro. Here, we show that the continuous exposure of differentiating rat osteogenic cells (ROB) to FMS* modulates the level of expression of mRNAs for 7 of the 8 osteogenic markers tested. Alkaline phosphatase (AP), osteocalcin (OC), metalloproteinases (MMP-2 and -14), procollagenase C (BMP-1), biglycan (BG) and integrin 1 are expressed at higher levels in FMS*-treated osteoblasts than in control cultures. MMP-2 and -14 mRNA are not down-modulated at mineralization. Also, the pattern of expression induced by FMS* for some of these genes (BMP-1, BG and integrin 1) is changed, but collagen type I (Coll I) mRNA levels are not affected by treatment with FMS*. This suggests that FMS* modulates mRNA levels and that this is not generalized, but gene(s) specific. We also report that exposure to FMS* rapidly and transiently induces activation of mitogen-activated protein kinases (MAPKs) 42,44 in
populations of early osteoblasts, but not in pre-osteoblasts, with a cell differentiation stage-dependent and pertussis toxin (PTX)-sensitive response. Subsequent to FMS* MAPK signaling activation, an increase in AP and MMP-14 mRNA is detected, which is also inhibited by PTX, suggesting that FMS* activation of MAPK signaling could be an early event required for the induction of these genes. Exposure to FMS* does not cause changes in the activity of p125 (FAK)-mediated signaling.


**Immunomodulatory effect of Canova medication on experimental Leishmania amazonensis infection.**

Pereira WK, Lonardoni MV, Grespan R, Caparroz-Assef SM, Cuman RK, Bersani-Amado CA.

Laboratory of Inflammation, Department of Pharmacy and Pharmacology, University of Maringá, Avenida Colombo, 5790, 87020-900 Maringá-PR, Brazil.

Abstract

This study investigates the action of Canova medication (CM) on experimental infection by Leishmania (Leishmania) amazonensis, utilizing in vitro and in vivo assays. For the in vitro tests, Balb/c mouse peritoneal macrophages (5x10^5) cells in 500 microl of culture medium, supplemented with 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (0.1 mg/ml) (were distributed in 24-well plates and CM was added at concentrations of 20 or 40%. Twenty-four hours later, the macrophages were infected with Leishmania amastigotes in culture medium. The effect of CM on macrophages leishmanicidal activity in 24 and 48 h cultures was evaluated by determining infection index and measuring nitric oxide (NO) production. The in vivo tests were performed in mice infected with 10^7 L. (L.) amazonensis promastigotes injected to the right hind footpad (25 microl in phosphate buffered saline). The progression of the lesions was examined over a 9-week period by measuring footpad swelling, and the parasite load in regional lymph nodes and spleen. The in vitro results showed that at 40% CM reduced the infection index, and induced NO production in the elicited macrophages, which suggests that the inhibitory effect on infection index may be mediated by NO. In the in vivo infection, when administered, orally or subcutaneously in mice, CM reduced infection by L. (L.) amazonensis in the paws, resulting in smaller lesions. CM treatment also decreased parasite load in the regional popliteal lymph nodes and in the spleen. These results suggest that CM modulates experimental infection by L. (L.) amazonensis, controlling infection progression and limiting dissemination.


[An experimental study of potentiated aqueous solutions].
[Article in Russian]
Lobyshev VI, Tomkevich MS, Petrushanko Ilu.
Abstract
A systematic study was undertaken of luminescent aqueous solutions of homeopathic preparation of sodium chloride at a dilution from D1 to D30, produced by "Weleda" company (Moscow) was carried out. It was shown that intensity of luminescence versus the degree of dilution is a non-monotonous function with several maxima, the main maximum corresponds to 13-14 decimal dilution. The dynamics of spectra was registered for several weeks. A systematic study of water samples (D1-D30) exposed to a similar procedure of potentization but without salt addition was also performed. The difference in the luminescence spectra of water of different stages of potentization was shown. The motility of infusoria Spirostoma ambiquum in solutions being examined was studied. A significant negative correlation between the infusoria motility and luminescence intensity was registered. Link to abstract/paper: http://www.carstens-stiftung.de/hombrex/index.php

Potentized Mercuric chloride and Nux vomica facilitate water permeability in erythrocytes of a fresh-water catfish Clarius batrachus under acute ethanol intoxication.
Sukul NC, De A, Sinhababu SP, Sukul A.

Department of Zoology, Visva Bharati University, Santiniketan, and Sukul Institute of Homeopathic Research, Santiniketan, West Bengal, India.
ksh_ncsukul@sancarnet.in

Abstract
OBJECTIVES: The primary biomolecular target of a homeopathic potency is unknown. If it is a plasma membrane protein such as water-channel protein, the drug would alter water permeation in cells. Therefore, the objective is to see if potentized homeopathic drugs like Mercuric chloride 30c and Nux vomica 30c could alter permeation of water through the erythrocytes of a fresh water fish under acute ethanol intoxication.
LOCATION: The work was carried out in the Zoology Laboratory of Visva Bharati University, Santiniketan, West Bengal, India.
SUBJECT: Live freshwater catfish.
DESIGN: Erythrocytes collected from fish with and without ethanol intoxication were incubated in distilled water at 30 degrees C for 30 minutes with Ethanol 30c (control), Merc cor 30c (test 1), and Nux vomica 30c (test 2). Merc cor 30c and Nux vom 30c were prepared by successive dilution of the respective mother tinctures with 90% ethanol (1:100) followed by sonication at 20 kHz for 30 seconds in 30 steps. Ethanol 30c was prepared in the same way from 90% ethanol diluted with 90% ethanol. In another experiment, fish were pretreated with Ethanol 30c and Nux vom 30c followed by ethanol injection at 2 g/kg of body weight. Then their erythrocytes were tested in vitro with the same potencies. After centrifugation of blood samples, fluid part was removed, erythrocyte pellets dried in a BioChemical Oxygen Demand (BOD; Atlas Surgical, New Delhi, India) incubator at 90 degrees C for 12 hours and intracellular water content measured.
RESULTS: Red blood cells (RBCs) from ethanol-injected fish permeated more water than those from normal fish. Water permeation was enhanced with Merc cor 30c and
Nux vom 30c. RBCs from fish pretreated with Nux vom 30c imbibed more water in in vitro treatments than those from fish pretreated with Ethanol 30c.

CONCLUSION: Because water channel proteins or aquaporins are mainly responsible for water transport through the plasma membrane of RBCs, it is thought that potentized drugs interact with these proteins, thereby facilitating water influx in the cells.


**Nonlinear effects of glutamate and KCl on glutamate toxicity in cultured rat cerebellar neurons.**

*Marotta D, Marini A, Banaudha K, Maharaj SV, Jonas WB.*

Samueli Institute for Information Biology, Program on Neuroprotection, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA.

Abstract

Nonlinear responses to toxin exposure have been observed in multiple cell types and organisms across a wide array of phyla. High dose toxin exposures inhibit or kill biological systems, while low dose exposures can stimulate survival mechanisms. We examined the effects of low (10(-3), 10(-5), 10(-7), and 10(-9) M) and ultra-low (10(-25) and 10(-61) M) KCl and glutamate pretreatment (72 h) against glutamate toxicity in rat cerebellar neurons. Ultra-low dilutions (10(-31), 10(-61), and 10(-401)) of an Arnica montana mother tincture were also investigated for their neuroprotective potentials. Viability was significantly enhanced in neurons pretreated with either 10(-3) M glutamate (10.6%) or 10(-9) M KCl (6.3%). None of the toxins evaluated displayed significant toxicity at the concentrations indicated. The protective effect of glutamate is likely mediated through activation of N-methyl-D-aspartate receptors, whereas low dose KCl might confer neuroprotection through enhanced alteration of Na+/K+ receptor dynamics. This is the first time high dose glutamate tolerance has been shown along with low dose KCl, and is consistent with previous reports demonstrating tolerance induced by low dose toxin exposure.


**The influence of very low doses of Cisplatin on tumor cell proliferation in vitro and on some hematological and enzymatic parameters of healthy rats.**

*Malarczyk E, Kandefer-Szerszeń M, Jarosz-Wilkołazka A.*

Department of Biochemistry, M. Curie-Składowska University, pl. M. Curie-Składowska 3, 20-031 Lublin, Poland.

Abstract

Healthy rats had been treated for 2 or 6 weeks with 1.0 mL of 10(-8) and 10(-16) mg/mL of cisplatin. After 2 weeks of treatment, a significant increase in leukocyte and erythrocyte count and also in hematocrit was observed. Among leukocytes the
number of neutrophils and eosinophils significantly increased. Biochemical analyses indicated a decrease in the glycogen content in the liver and kidneys after 2 weeks of treatment with low doses of cisplatin but at the end of the experiment (8th week of experiment) the stores of glycogen increased significantly. Biochemical analyses concerning the activity of some enzymes in the liver revealed a significant increase of peroxidase and acid phosphatase as well as catalase activities after 2 weeks of treatment. However, catalase was induced by a very low concentration of cisplatin, 10(-16) mg/mL. After the cessation of cisplatin treatment the activity of enzymes returned to normal values. Human lung carcinoma cell line A(549) (ECACC No 86012804) was also studied after treatment with the same doses of cisplatin and inhibition of its growth was observed. The results of these experiments strongly indicated that low doses of cisplatin could be stimulating for healthy cells but cytostatic for tumor cells. Possible mechanisms involved in the biological activity of very low cisplatin concentrations are discussed.

Link to paper: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2651610/


Potentized Mercuric chloride and Mercuric iodide enhance alpha-amylase activity in vitro.
Sukul NC, De A, Sukul A, Sinhababu SP.

Department of Zoology, Visva Bharati University, Santiniketan, West Bengal, India. ksh_ncsukul@sancharnet.in

Abstract
Mercuric chloride 30c and Mercuric iodide 30c were prepared by successive dilution in 30 steps of 1:100 followed by sonication at 20KHz for 30s at each step. Both were prepared in two media: 90% ethanol and distilled water. Three preparations of Mercuric chloride 30 in water were used: 12-month old, 1-month old and 4-day old. The controls for the water and ethanol-water preparations were pure water 30c and 90% ethanol 30c, respectively. For the three water preparations there were three matched controls of water 30c of the same ages. Each potentized substance or its control was mixed with distilled water 1:100 before testing. Hydrolysis of starch by alpha-amylase was measured by the standard procedure after incubation for 15 min at 27 degrees C. Mercuric chloride 30c and Mercuric iodide 30c in both water and aqueous ethanol media, enhanced enzyme activity significantly, compared to their respective controls. Mercuric chloride 30c, prepared in water 12 months previously, produced no significant change in the enzyme activity compared to its control. We hypothesize that the structure of the active molecule imprinted on water polymers during the process of dynamization. The specifically structured water interacts with the active sites of alpha-amylase, modifying its activity. Ethanol molecules have large non-polar part stabilizing the water structure and thus retaining activity for a longer time.


**Action of Remedies on Movement of Macrophages and Leucocytes.**
Moss VA, Roberts A, Simpson K.

Abstract
Laboratory measurements of guinea pig macrophages and human leucocytes exposed to the homoeopathic remedies Belladonna, Hepar sulph, Pyrogenium, Silica and Staphylococcinum, showed that these remedies caused an increase in the movement of these cells through a Boyden micropore filter. This may indicate that the remedies produce an increase in this aspect of immune competence.

**Non-linear effects of cycloheximide in glutamate-treated cultured rat cerebellar neurons.**
Marotta D, Marini A, Banaudha K, Maharaj S, Ives J, Morrissette CR, Jonas WB.

Samueli Institute for Informational Biology, Program on Neuroprotection and Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA.

Abstract
Multiple cell types and organisms across a wide array of phyla and a variety of toxins demonstrate non-linear dose responses to low-level chemical exposures with high doses inhibiting cellular function and low doses stimulating function. We tested whether such non-linear responses to low and ultra-low dose N-methyl-D-aspartate (NMDA), 1-methyl-4-phenylpyridinium (MPP+) or cycloheximide moderated toxic glutamate exposure in cultured cerebellar granule cells. Neurons were incubated over 72 h with successive NMDA, MPP+ iodide or cycloheximide additions producing specified low (10(-5), 10(-7), 10(-9), 10(-11), and 10(-13) M) and ultra-low (10(-27),10(-29), 10(-63), and 10(-65) M) concentrations. Subsequently these neuronal cells were exposed to a 50% excitotoxic concentration of glutamate for 24 h. Neuronal viability was significantly reduced in neurons treated with micromolar (10(-5) M) cycloheximide whereas viability was enhanced in neurons treated with an ultra-low dose exposure of 10(-27) M cycloheximide. Neither NMDA nor MPP+ elicited harmful or protective responses. This is the first report demonstrating non-linear dose-response effects of cycloheximide in low and ultra-low concentration ranges. Link to abstract/paper: [http://www.ncbi.nlm.nih.gov/pubmed/12387359](http://www.ncbi.nlm.nih.gov/pubmed/12387359)

**Association of c-myc overexpression and hyperproliferation with arsenite-induced malignant transformation.**
Chen H, Liu J, Zhao CQ, Diwan BA, Merrick BA, Waalkes MP.

Laboratory of Comparative Carcinogenesis, National Cancer Institute (NCI) at National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709, USA.

Abstract
Numerous studies link arsenic exposure to human cancers in a variety of tissues, including the liver. However, inorganic arsenic has never been unequivocally shown to be an animal carcinogen, and its carcinogenic mechanism remains undefined. Our previous studies indicate that chronic (> or =18 weeks), low-level (125 to 500 nM) exposure to arsenite induces malignant transformation in the normally nontumorigenic rat liver epithelial cell line (TRL 1215), and these chronic arsenic-exposed (CAsE) cells produce invasive and metastatic tumors upon inoculation into nude mice. In addition, a prior microarray screening analysis of aberrant gene expression showed several oncogenes were overexpressed in CAsE cells exposed to 500 nM arsenite, including a prominent overexpression of the protooncogene c-myc, as well as genes related to cell proliferation. Thus, to better understand the mechanism of arsenic carcinogenesis, we studied the role of c-myc overexpression in arsenite-induced cell transformation. The upregulation of c-myc was confirmed by RT-PCR at the transcription level and by Western blot analysis for the translation product. Further analysis showed that arsenite produced significant increases in the steady-state expression of c-myc in a time- and concentration-dependent manner during the malignant transformation process. The level of c-myc expression was highly correlated (r = 0.988) with tumor formation after inoculation of CAsE cells into nude mice and was also highly correlated (r = 0.997) with genomic DNA hypomethylation. CAsE cells showed a high cell proliferation rate in a fashion related to the level of arsenic exposure. The expression of c-myc was highly correlated with cellular hyperproliferation (r = 0.961). Consistent with the enhanced proliferation both proliferating cell nuclear antigen and cyclin D1 were overexpressed in CAsE cells. In summary, a prominent overexpression of c-myc, a gene frequently activated during hepatocarcinogenesis, is strongly correlated with several events possibly associated with arsenic-induced malignant transformation, including hyperproliferation, DNA hypomethylation and tumor formation upon inoculation into nude mice. These correlations provide convincing evidence c-myc overexpression is mechanistically important in arsenic-induced malignant transformation in this model system.


Wirkungen homöopathischer Zubereitungen eines Wurzelextraktes von Saussurea lappa auf verschiedene Immunparameter des peripheren Bluts von Ziegen.
[Effects of Saussurea lappa root extract on different immunoparameters of the peripheral blood of goats].
[Article in German]
Sarwar A, Enbergs H.

Link to abstract/paper: http://bibnet.org/vufind/Record/ccmed513487

Neuroprotection from glutamate toxicity with ultra-low dose glutamate.
Jonas W, Lin Y, Tortella F.
Abstract
The protective effects of ultra-low doses (ULD) of glutamate against glutamate toxicity was studied in primary rat spinal, cortical and cerebellar neurons. Neurons were exposed to four subtoxic, ultra-low concentrations of glutamate (10(-18) M, 10(-20) M, 10(-22) M and 10(-30) M) for 72 h and then subsequently challenged with toxic concentrations (25 microM) of glutamate. Neuron viability was consistently 10% higher in spinal and cortical neurons pre-exposed to glutamate concentrations of 10(-18) M and 10(-22) M, and in cerebellar neurons pre-exposed to 10(-20) M and 10(-30) M. Using laser scanning confocal microscopy and the fluorescent calcium probe fluo-3, we found no alterations in intracellular calcium dynamics in the protected cells. This protective effect is consistent with a growing body of evidence for tolerance induced by low-dose toxin exposure but is the first time that such tolerance has been demonstrated with ultra-low glutamate exposure. Our data show that pre-exposure of neuronal cells to ULD glutamate can protect against subsequent exposure to toxic levels of glutamate.

We here show that the increase in AP expression induced by FMS*Calciumfluor is dependent on the activation of G alpha 0/G alpha i proteins, while it is unaffected by the activation stage of the G alpha s protein. Moreover, we show that the expression of endogenous AP during osteogenesis in vitro is regulated independently from G proteins, and unaffected by their activation stage and therefore that treatment with FMS*Calciumfluor activates a new pathway of cellular response.


Effects of Homoeopathic Potencies: Growth of *Saccharomyces cerevisiae* in Potentised Copper Sulphate Dilutions.
Fleisbach A, Fejfar V, Spranger J.

Physical, chemical and biological assay of *Tylophora indica* mother tincture--a comparative study.
Nandi M.
Division of Pharmacology, National Institute of Homoeopathy, Calcutta, West Bengal, India.

Abstract
Successful use of homeopathic medicines is related to the purity and quality of crude and finished products. To maintain the quality of *Tylophora indica* mother tincture, a comparative study on physical, chemical and biological assay of five samples (reference laboratory and market) of *Tylophora indica* was carried out. The market sample showed different chromatographic characteristics and may have been prepared from a different species. *T. indica* has antispasmodic and hypotensive properties.


Stimulation of survival capacity in heat shocked cells by subsequent exposure to minute amounts of chemical stressors; role of similarity in hsp-inducing effects.
Wiegant FA, Souren JE, van Wijk R.
Department of Molecular Cell Biology, Utrecht University, The Netherlands.

Abstract
A brief and moderate heat shock to Reuber H35 hepatoma cells causes a rapid increase in the synthesis of heat shock proteins (hsp) and initiates the development of thermotolerance, which results in an increased ability to survive exposure to otherwise lethal temperatures. We now demonstrate that low doses of various
chemical stressors (arsenite, cadmium, mercury, lead, copper, menadione and diethyldithiocarbamate (ddtc)), at concentrations that do not exert any effect in control cultures, are able to enhance the synthesis of hsps and to stimulate the development of thermotolerance when applied to cultures which were pretreated with a mild heat shock. The degree of stimulation appears to be stressor-specific, which is not only observed in the ensuing development of thermotolerance but also in the enhancement of the heat shock-induced synthesis of stress proteins. The different hsps that show an enhanced induction when heat shocked cultures are exposed to the various secondary applied low doses of chemical stressors, were found to resemble the hsp pattern that is characteristic for the secondary stressor and not for the initial heat shock. In other words, the nature of the post-treatment determines the observed pattern of enhanced synthesis of hsps. In order to analyze the origin of the stimulation of survival capacity by low doses of the mentioned stressors, we studied whether the degree of stimulation is determined by the degree of similarity between the overall stress response to heat shock and to the second stress condition when applied singly. The degree in which low doses of chemical stressors stimulate tolerance development and enhance the synthesis of hsps in cells that were previously heat shocked, appears to be related to the degree of similarity in the hsp pattern induced by both stressors. Our results support the notion that low doses of toxic compounds may, under certain conditions, have beneficial effects related to a stimulation of endogenous cytoprotective mechanisms.


**Mechanisms of behavioral effects of poteniated morphine forms.**
Epshtein OI, Zapara TA, Pavlov IF, Simonova OG.

Abstract
Effects of morphine and its poteniated (homeopathic) form on rat behavior in an elevated plus-maze were studied. Combined application of poteniated and non-poteniated morphine enhanced the anxiolytic and sedative effects. Patch-clamp experiments on isolated *Helix pomatia* giant neurons revealed a blocking effect of poteniated morphine on μ-receptors.


**Effects of homeopathic doses of antibodies to S100 antigen on electric characteristics of neuronal membranes.**
Epshtein OI, Gainutdinov KL, Shtark MB.

Abstract
Various dilutions of antibodies to brain-specific protein S100, including those prepared by multiple consecutive dilutions up to $10^{-12}$ and $10^{-400}$ of total weight, produced similar effects on the membranes of *Helix pomatia* giant neurons, which varied only quantitatively. They induced membrane depolarization, reduced the amplitude or completely blocked the action potential, accelerated the maximal rise of
the action potential, reduced maximal conductance, and facilitated the steady-state inactivation of ionic channels.

Link to abstract/paper: 
http://link.springer.com/content/pdf/10.1007%2FBF02433399.pdf


**Osteogenesis in vitro in rat tibia-derived osteoblasts is promoted by the homeopathic preparation, FMS*Calciumfluor.*

Palermo C, Filanti C, Poggi S, Manduca P.

Genetics, Department of Oncology, University of Genova, 26 C. Europa, Genova, 16132, Italy.

Abstract

We studied the effects of in vitro treatment of differentiating osteogenic cells with FMS*Calciumfluor,* to determine whether it caused changes in proliferative or differentiation potential of osteoblasts. FMS*Calciumfluor* was developed for the therapy of post-menopausal and age-related osteoporosis on the basis of the principles of resonance homeopathy and VTR Vega test. Its daily prescribed therapeutical usage is about 30,000-fold less in fluoride concentration than that recommended for NaF associated with calcium monophosphate. Rat tibial osteoblast (ROB) primary cultures represent populations of early osteoblasts and their derivative cultures of more than 60 cumulative population doubling (CPD) represent more mature osteogenic cells. Both these populations were shown to undergo in vitro differentiation, as monitored by the sequential expression of markers that define the stages of the osteogenic progression. Here we report that continual treatment of ROB during osteogenesis with FMS*Calciumfluor* modulated the expression of critical osteogenic markers: alkaline phosphatase (AP), an indicator of osteoblast maturation, and (45)Ca incorporation into the matrix and nodule formation, events of the last phase of osteogenesis and a measure of osteoid mineralization. Treatment did not affect proliferation, or expression and activation of metalloproteinases (MMP). AP activity and levels of AP mRNA were increased by treatment with FMS*Calciumfluor,* the incorporation of radiolabelled Ca into the matrix was also increased and the formation of nodules occurred in a shorter time and with higher frequency than in untreated control cultures. The effects of FMS*Calciumfluor* were concentration dependent and specific for its modalities of preparation, and were observed at a concentration about three orders of magnitude lower than similar effects reported in the literature by treatment of osteoblast cultures in vitro with NaF.


*Biologische Medizin.* 1999;28(3):142-146.

**Die antivirale Wirkung von Euphorbium compositum S.**

[The antiviral effect of Euphorbium compositum S.]

[Article in German]

Metelmann H, Glatthaar-Saalmüller B.
Abstract
INTRODUCTION: The oxidative degradation of urate to allantoin and CO2 is catalyzed by the enzyme uricase. Its activity was determined in the presence of two potassium cyanatum preparations in the dilution step D8, which differed by the method of preparation. While variant 1 (homeopathic D8) was prepared homoeopathically, variant 2 (electronic D8) was produced electronically.

OBJECTIVE: The target of these studies was to investigate the impact of homoeopathic and electronic D8 on the catalytic activity of uricase.

METHODS: In the presence of these two D8 variants, the enzymic degradation of urate was determined by a spectrophotometric assay over a period of 10 minutes.

RESULTS: 1. In the presence of homoeopathic D8 a stimulation of enzyme activity was detected. 2. In the case of electronic D8 neither a stimulation nor an inhibition of enzyme-catalyzed urate degradation was observed. 3. The differences in the effect of homoeopathic and electronic D8 on uricase were found to be statistically relevant.

CONCLUSIONS: With the help of a cell-free system, such as uricase, it is possible to detect differing effects of homoeopathically and electronically prepared D8. In contrast to the electronic D8, the homoeopathic D8 is capable of modulating the enzyme activity. This observation leads to the assumption that homoeopathically prepared drugs are superior in their therapeutical efficiency to electronically produced drugs. However, the interpretation would be allowed, too, that the cell-free system used in this study, which has been isolated from an organism, is no longer in a position to react to an electronically prepared potency.

Unterschiedlicher Einfluß von cAMP-Potenzen und cAMP-Verdünnungen am Beispiel verschiedener Enzymsysteme.
[Different influence of potencies and dilutions of cAMP exemplified on several enzyme systems]
[Article in German]
Harisch G, Dittmann J.

Toxicology. 1998 May 15;127(1-3):107-19.
Stressor-specific enhancement of hsp induction by low doses of stressors in conditions of self- and cross-sensitization.
Wiegant FA, Spiker N, van Wijk R.

Department of Molecular Cell Biology, Utrecht University, The Netherlands.
f.a.c.wiegant@bio.uu.nl

Abstract
In this paper, the pattern of induction of heat shock proteins (hsp) was studied in cultured Reuber H35 rat hepatoma cells by sequential application of different stressors. We analyzed whether a specific stress condition is able to induce an enhanced sensitivity to a subsequent application of a low dose of either the same or another stressor (self-sensitization and cross-sensitization, respectively). As a measure of sensitization, the stimulation of hsp induction was employed. Three different stressor conditions (heat shock, sodium arsenite and cadmium chloride) were used in doses which exerted a similar impact on overall protein synthesis. A synergistic effect in induction of the synthesis of various hsp was observed when a high stressor dose was followed by an 8-h incubation in a lower stressor dose in both self- and cross-sensitization experiments. The low-dose conditions used as second treatments did not induce any responses in non-pretreated cells. Studies in cultured cells have demonstrated stressor-specific hsp induction patterns. In this study we analyzed whether the pattern of hsp induced by the low-dose condition is characteristic for the first sensitizing stressor or for the secondary stressor applied in a low dose. The pattern of hsp which was induced above the level of the high-dose effect, due to the incubation with the secondary applied low-dose condition, was found to be characteristic for the secondary stressor and not for the sensitizing primary treatment. These results are of importance for an improved understanding of the regulation of heat shock protein synthesis in conditions of self- and cross-sensitization, as well as for a proper use of hsp as biomarkers of exposure to environmental stress.

Untersuchungen zur Wirkung von Ubichinon Injeel und Injee forte mit zellfreien Systemen.
[Studies of the effects of ubiquinol and Injeel forte with cell-free systems].
[Article in German]
Harisch G, Dittmann J.

Enhancement of the stress response by minute amounts of cadmium in sensitized Reuber H35 hepatoma cells.
Wiegant FA, van Rijn J, van Wijk R.

Department of Molecular Cell Biology, Utrecht University, The Netherlands.
f.a.c.wiegant@biol.ruu.nl

Abstract
The aim of this study was to determine whether the cadmium-induced cellular stress response can be modulated by the subsequent application of low concentrations of the same ion. It is shown that exposure of Reuber H35 rat hepatoma cells to cadmium concentrations of 10 or 30 microM for 1 h leads to a biphasic change in their sensitivity towards a second exposure to cadmium, an initial sensitization is followed by development of tolerance towards the secondary treatment with cadmium. Furthermore, incubations for 1 h in the presence of 10 microM of cadmium induce the synthesis of the major heat shock proteins except for hsp60. A step-down cadmium regime, i.e. a pretreatment of 1 h with 10 or 30 microM immediately followed by incubations with lower concentrations of cadmium (ranging from 0.03 to 1 microM), leads to additional increases in hsp synthesis. Since no effect of these low concentrations was observed on hsp synthesis in non-pretreated cells, the effect of a step-down treatment thus results in a higher effect on hsp synthesis than could be expected based on their summation. The sensitized cells also develop a higher level of tolerance in the presence of the above mentioned low concentrations of cadmium. It can be concluded that during the transient period of enhanced sensitivity, low concentrations of the original stressor enhance the synthesis of hsp and thus induce higher levels of tolerance in comparison with cells which only received the primary cadmium treatment.


[Effects of Nux vomica, D4, D6, D10, Nux vomica-Homaccord ad us. vet. and Atropinum compositum ad us. vet. on intestinal motility in vitro].
[Article in German]
Kanui TI, Enbergs H.

Characterization of differing effects caused by homeopathically prepared and conventional dilutions using cytochrome P450 2E1 and other enzymes as detection systems.
Dittmann J, Harisch G.

Institut für Physiologische Chemie, Tierärztliche Hochschule Hannover, Germany.

Abstract
Determination of cytochrome P450 2E1 activity was carried out via hydroxylation of the synthetic substrate p-nitrophenol to p-nitrocatechol. Crude microsomal preparation isolated from rat liver served as source for cytochrome P450 2E1. Under assay conditions guaranteeing a linear course of the reaction the cytochrome P450 2E1 was stimulated in the presence of a 10(-6) dilution of As2O3 corresponding to 0.915 microM final concentration compared with control. All other concentrations of As2O3 used inhibited the enzyme activity more or less drastically. Furthermore, we used this enzyme system to study the influence of arsenicum album (As2O3) and potassium cyanatum (KCN) in homeopathically prepared (i.e., by consecutive 1:10 steps) and conventional dilutions. We found significant differences between the effects caused by homeopathic potencies (D) and equally concentrated dilutions on catalytic activity of cytochrome P450 2E1. Such differing effects were observed in the case of arsenicum album (As2O3) between D4/10(-4) and D6/10(-6) and in the case of potassium cyanatum (KCN) between D6/10(-6) and D12/10(-12). When we used glutathione-S-transferases and uricase we also found different effects mediated by potencies and conventional dilutions. The results obtained suggest that these three enzyme systems are appropriate detection systems to hunt out differing effects of differently prepared dilutions of specific test substances.

Zytosolische Glutathion-S-Transferasen und Xanthin-Oxidase/-Dehydrogenase als Indikatoren für die unterschiedliche Wirkung von Potenz und konzentrationsgleicher Verdünnung.
[Cytosolic glutathione S-transferases and Xanthin-Oxidase/-Dehydrogenase as indicators of the effect of different concentrations of the same potency and dilution].
[Article in German]
Dittmann J, Harisch G.

Institut für Physiologische Chemie, Tierärztliche Hochschule Hannover

Abstract

Link to abstract/paper: http://www.karger.com/Article/Abstract/210222

Forsch Komplemetärmed. 1996;3(2):64–70.
Etablierung eines Modellsystems zur Detektierung unterschiedlicher Wirkungen von Potenz und konzentrationsgleicher Verdünnung dargestellt am Beispiel der Uricase aus Schweineleber.
[Establishment of a model system to detect different effects of potency and concentration the same dilution as exemplified by the uricase from porcine liver].
[Article in German]
Dittmann J, Harisch G.

Abstract
Einleitung: Die Uricase aus Schweineleber ist ein Enzym, das die Umwandlung von Urat in Allantoin katalysiert. Basierend auf dieser Reaktionssequenz wurde ein Detektionssystem für die Uricase etabliert und dessen Anwendbarkeit am Beispiel zweier Prüfsubstanzen untersucht. Fragestellung: Hauptzielsetzung war es, ein Modell zu etablieren und mit dessen Hilfe zu untersuchen, ob Potenz und konzentrationsgleiche Verdünnung die katalytische Aktivität des ausgewählten Enzyms unterschiedlich beeinflussen. Methoden: Eingesetzt wurden als Prüfsubstanzen Arsenicum album (AS2O3) und Kalium cyanatum (KCN) in Potenz- bzw. Verdünnungsstufen D4 bzw. 10^{-4}, D6 bzw. 10^{-6}, D8 bzw. 10^{-8} und D12 bzw. 10^{-12}. In Gegenwart dieser Präparationen wurde die Aktivität der Uricase spektralphotometrisch bestimmt. Ergebnisse: 1. Bei Anwendung von Kalium cyanatum zeigte sich in Gegenwart von D4 bzw. 10 eine fast vollständige Enzyminhibierung. 2. Bei D6 bzw. 10^{-6} wurde eine hochsignifikant unterschiedliche Beeinflussung (p< 0,001) der Enzymaktivität gefunden, die sich auch nach Modifizierung des Versuchsansatzes (Präinkubation des Enzyms mit der Prüfsubstanz) zeigte. 3. Kinetikuntersuchungen in Gegenwart von Kalium cyanatum in der Potenzstufe D6 erbrachten für die Uricase einen K_{m}-Wert von 0,114 ± 0,021 mM und einen V_{max}-Wert von 0,718 ± 0,070 mmol × min^{-1} × mg^{-1}. Im Falle der Verdünnungsstufe 10^{-6} ergaben sich ein K_{m}-Wert von 0,070 ± 0,011 mM und ein V_{max}-Wert von 0,421 ± 0,026 mmol × min^{-1} × mg^{-1}. 4. Die eingesetzten Potenz- und

Link to abstract/paper: http://www.karger.com/Article/Abstract/210205

An automated microtitre plate assay for acid phosphatase as a model system for studying the influence of small amounts of Hg2-ions on enzyme activity.
Dittmann J, Harisch G.

Enhancement of the stress response by low concentrations of arsenite in arsenite-pretreated Reuber H35 hepatoma cells.
Ovelgönne HH, Wiegant FA, Souren JE, Van Rijn H, Van Wijk R.

Department of Molecular Cell Biology, Utrecht University, The Netherlands.

Abstract
The present study is aimed at determining whether the induction of heat-shock protein (hsp) synthesis, heat-shock mRNAs, and tolerance development after arsenite application has been sensitized to low concentrations of arsenite in Reuber H35 rat hepatoma cells. Using a step-down arsenite treatment, consisting of a 1-hr pretreatment with 100 or 300 microM followed by an incubation with a lower concentration (1-10 microM), H35 cells were shown to exhibit increased sensitivity to low concentrations of sodium arsenite shortly after exposure to the high arsenite concentration, but not any longer when the low concentration was applied 4 hr after pretreatment. In this paper it is shown that exposure of H35 cells to sodium arsenite concentrations of 100 or 300 microM for 1 hr rapidly changes the sensitivity toward a second arsenite treatment with respect to the induction of the heat-shock response. It was observed that under conditions of enhanced sensitivity, an additional increase occurred in hsp synthesis as well as in hsp mRNA (as exemplified by hsp68 mRNA behavior) when low concentrations of arsenite were applied to arsenite pretreated cells. Since no effect of these low concentrations was observed in nonpretreated cells, the effect of step-down treatment results in a higher effect than could be expected based on summation. Furthermore, in sensitized cultures, cells are able to develop a higher level of tolerance in the presence of low concentrations of arsenite. It can be concluded that during a transient period of enhanced sensitivity, low concentrations of the original stressor are able to enhance hsp synthesis and to induce a higher level of tolerance in comparison with control cultures that are sensitized but not incubated in the presence of low concentrations of the original stressor.

Nachweis der Schutzwirkung sehr hoher Verdünnungen von Metallen auf mit Cadmium vergiftete Nierenzellkulturen.
[Proof of protective effect of very high dilutions of metals on cadmium intoxicated renal tubular cell cultures].
[Article in German]
Delbancut A, Merlet D, Mellado M, Dorfman P, Cambar J.

Thrombogenic properties of ultra-low-dose of acetylsalicylic acid in a vessel model of laser-induced thrombus formation.
Doutremepuich C, Aguejouf O, Pintigny D, Sertillanges MN, De Seze O.

Temperature dependent influence of As2O3, HgHPO4 and KCl on lysosomal acid phosphatase isolated from rat liver.
Dittmann J, Harisch G.
Institut für Physiologische Chemie, Tierärztliche Hochschule Hannover, Germany.
Abstract
Studies were carried out to investigate acid phosphatase activity in the presence of As2O3, HgHPO4 and KCl at 25 degrees C and 37 degrees C. In all cases examined enzyme activities measured at 25 degrees differ from those detected at 37 degrees C. When activity was measured in the presence of As2O3 at 25 degrees C a stimulation was found while at 37 degrees C activities remained within the control range. Similar results were obtained, when As2O3 was replaced by HgHPO4. In contrast to that, added amounts of KCl cause an increase of activity at both incubation temperatures, but the increment being greater at 37 degrees C. Furthermore in most cases correlation between increasing amounts of substances added and enzyme activity measured was non-linear.

Use of urate oxidase as a test system to characterize the effect of homeopathic potencies and of equally concentrated conventional dilutions.
Dittmann J, Selbach AC, Hentges A, Harisch G.
Mechanistic Approach to the Effect of High Dilutions of Cadmium to Protect from Cytotoxic Cadmium doses in Renal Tubular Cell Cultures. Delbancut A, Barrouillet MA, Maury-Brachet R, Boudou A, Dorfman P, Cambar J.

Link to abstract/paper: http://www.journals.elsevierhealth.com/periodicals/brihj/article/S0007-0785%2894%2980009-X/fulltext

Etude de l'effet protecteur de très hautes dilutions de cadmium vis-à-vis de concentrations cytotoxiques de ce même métal sur des cultures cellulaires rénales. Delbancut A, Merlet D, Mellado M, Dorfman P, Cambar J.

Zur Wirkung von Lachesis in verschiedenen homöopathischen Potenzen auf Lymphozytenkulturen. Enbergs H, Arndt G.

Effect of High Dilutions of Epidermal Growth Factor on in-vitro Proliferation of Keratinocyte and Fibroblast Cell Lines. Fougeray S, Moubry K, Vallot N, Bastide M.

Link to abstract/paper: http://www.journals.elsevierhealth.com/periodicals/brihj/article/S0007-0785%2805%2981043-9/fulltext

Effects of highly diluted beta2-adrenergic agonists on isolated guinea pig trachea. Callens E, Debiane H, Santais MC, Ruff F.
Molecular signaling at high dilution or by means of electronic circuitry.
Aissa J, Litime MH, Attias E, Benveniste J.

Transfer of molecular signals via electronic circuitry.
Aissa J, Litime MH, Attias E, Allal A, Benveniste J.

Antigen signalling at high dilution.
Litime MH, Aissa J, Benveniste J.

Highly dilute antigen increases coronary flow of isolated heart from immunized guinea-pigs.
Benveniste J, Arnoux B, Hadji L.

Mechanical agitation, the main factor in increasing the efficacy of Agaricus muscarius, a homoeopathic drug.
Sukul, N.C.

Effect of Viscum album preparations on gastric chief cells and DNA methylation by N-methyl-N-nitro-N-nitrosoguanidine.
Defize J, Zwiers T, Pals G, Kipp JBA, Eriksson AW.

Abstract
The protective effect of extracts of mistletoe Viscum album (Iscador) with reference to carcinogenesis was tested on in vitro cultured porcine gastric chief cells. Putative protection against in vitro methylation of lambda DNA by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) was studied with restriction endonucleases. Preparations of Iscador from mistletoe grown on oak as host tree had a high cytotoxic effect on gastric chief cells, with an LC50 after 24 hrs that ranged between 0.01 and 0.03 mg/ml. At 0.01 mg/ml, Iscador was also found to protect lambda DNA from methylation or destruction by MNNG. During the course of this study a high variability was found both in cytotoxicity and protection rate against methylation which we attribute to batch to batch differences. Thus the LC50 for Iscador MH 86 L12 was 0.005 mg/ml, while for MH87 D24 it was 0.05 mg/ml after 24 hrs. The Iscador batch W frf 50 mg/ml, which was made from a fresh plant extract at the
Hisicia Institute, showed less variability. Results are therefore given for this batch of Iscador only.

Link to abstract/paper: http://www.journals.elsevierhealth.com/periodicals/brihj/article/PIIS0007078505805599/abstract

Effect of dilute histamine on coronary flow of isolated guinea-pig heart.
L. Hadji, B. Arnoux, H. Benveniste.


Recherches pharmacodynamiques expérimentales concernant des dilutions homéopathiques de Belladone.
[Pharmacodynamic Experimental research on homeopathic dilutions of belladonna].
[Article in French]
Cristea A.

Abstract
Nous publions cette étude qui illustre bien les recherches menées en Roumanie. L'absence de précisions scientifiques nécessaires (fabrication des dilutions, étude statistique) confère à ces intéressants travaux le caractère d'étude préliminaire.

Link to abstract/paper: http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=HomeoIndex&lang=p&nextAction=lnk&exprSearch=1327&indexSearch=ID

Pretreatment with low dose of cadmium (Cd) protects rat renal mesangial cells against the direct toxic effect of cadmium.
Bascands JL, Cabos-Boutot G, Manuel Y, Girolami JP.

Abstract
We have compared response of renal mesangial cells to different doses of cadmium. The effect of cadmium was assessed by measuring the growth rate of an homogeneous rat renal cell line. The cells grown in the presence of 10-6 Mcd exhibited a 37+/-6% mortality rate within 24 h. When the cells were first placed in the presence of 10-15 or 10-20 McdCl2 for 24, 48, 72 and 98 hours respectively and then placed in the presence of 10-6 McdCl2, the toxic effect initially observed with this concentration was significantly reduced. This decrease in Cd cytotoxicity was also dependent on the pretreatment time. The maximum resistance effect was obtained after 72 hours of pretreatment, a 45=-6% increase in survival rate was
observed. However, 96 hours pretreatment did not increase the protective effects. In conclusion renal mesangial cells alone may exhibit a transient protective mechanism against a toxic dose of cadmium itself at doses as low as 10-15 and 10-20 M. The induction of metallothionein is likely to be involved.

Link to abstract/paper: http://ccrhindia.org/renal_disorders/pretreatment.htm

Porcine granulocyte functions: evaluation and modulation.
Stahl M, Reifenberg K, Okpanyi S, Lösch U.

Institut für Physiologie, Physiologische Chemie und Ernährungsphysiologie, Tierärztlichen Fakultät, Universität München, FRG.

Abstract
Functions of porcine polymorphonuclear neutrophils are evaluated with in vitro test systems. Results are compared with those from human PMN and the relevance for in vivo conditions is discussed. Ethanol was inhibitory to all porcine PMN functions investigated here. Influenx, a combination product, containing extracts of Echinacea, Aconitum, Apis and Lachesis stimulated adherence, chemotaxis, and phagocytosis, but inhibited chemiluminescence. These results suggest an effect of the product in the generation of reactive oxygen species.


Influence of mouse age on PMA-induced chemiluminescence of peritoneal cells incubated with alpha/beta interferon at very low and moderate doses.
Carriere V, Bastide M.

Effect of two homoeopathic drugs, Agaricus muscarius and Nux vomica on the isolated ileum of rats.
Sukul, N.C.; Zaghlool, H.A.

Effect of homeopathic dilutions on subcellular enzymatic activity.
Petit C, Belon P, Got R.

Laboratoire de Biochimie des Membranes, C.N.R.S., Villeurbanne, France.

Abstract
The activity of various inhibitors on several subcellular enzymes was studied. First we determined the inhibitory concentration required to reduce maximum enzymatic
activity by 50%, then the effect of various hahnemannian dilutions of the same inhibitory agent was tested. Seven inhibitory agents were tested in this way on seven different enzymatic systems. No effects of these hahnemannian dilutions were shown.


Only the smile is left.
Metzger H, Dreskin SC.

A Pharmacological Study of Thujone in Mice.
Karouby Y, Doucet M, Boudard F, Dorfman P, Bastide M.

Homéopathie Francaise. 1987;75:151-156.
De l'utilisation de silicea en homéopathie à l'effet de hautes dilutions de silice sur les macrophages.
[The use of silica in homeopathy to the effect of high dilutions of silica on macrophages].
[Article in French]
Poitevin B.

Link to abstract/paper: http://cat.inist.fr/?aModele=afficheN&cpsidt=7508851

Effect of mouse peritoneal macrophages of orally administered very high dilutions of silica.
Davenas E, Poitevin B, Benveniste J.

Abstract
The activity of very high dilutions of silica, a substance cytotoxic for macrophages, was tested on the synthesis by mouse peritoneal macrophages of the inflammatory ether-lipid paf-acether and its precursor lyso paf-acether. C57Bl6 female mice received for 25 days either 1.66 X 10(-11) M silica (11 sil) or 1.66 X 10(-19) M (19 sil) (final concentration) in the tap-water they were given to drink while control mice remained untreated. Isolated macrophages from mice treated with 11 sil produced 44.2 and 30.8% more paf-acether than cells from untreated mice in the presence of 50 and 200 micrograms zymosan (Z)/ml respectively. When 19 sil was given to the mice, the respective increases were 67.5 and 38%. In an experiment with a blind design, the mice were either untreated or received 19 sil or saline submitted to the same dilution procedure (19 sal). After administration of 19 sil, paf-acether synthesis was 55.5 and 33.5% higher upon stimulation with 50 and 200 micrograms Z/ml,
respectively, than in the 19 sal group. In a third blind experiment, macrophages from mice that received 19 sil formed 61.3 and 28.6% more paf-acether upon stimulation with 50 and 200 micrograms Z/ml respectively, as compared to mice receiving 19 sal or lactose submitted to the same dilution procedure (19 lac). There was no difference between the 19 sal and the 19 lac groups. The differences between control and silica-treated mice were highly statistically significant in all experiments. There was no effect on the synthesis of lyso paf-acether. These results demonstrate clear ex vivo cellular effect of high dilutions of silica that cannot be explained in our present state of knowledge. 


**Antiviral efficacy of homeopathic drugs against animal viruses**

L.M. Singh, Girish Gupta

Division of Virology, Central Drug Research Institute, Lucknow-226001 (U.P.), India

Abstract

The antiviral effect of homœopathic drugs against two animal viruses, Chicken Embryo Virus (CEV) of fowls and Simliki Forest Virus (SFV), causing encephalitis and death in mice were investigated. In all 10 drugs in 33 potencies were tested against CEV and 8 drugs in 26 potencies showed varying degree of virus inhibition. The drugs that caused 100% inhibition of CEV were *Typhoidinum* 200, *Hydrophobinum* 1000, *Tuberculinum* 1000, *Nux vomica* 200, and *Malandrinum* 1000. Of the drugs tested in 11 potencies against SFV, none were found effective in either preventing disease or death of mice infected with this virus.


*Brit Hom Res Group Communications. 1985;14:30-35.*

**Virus Chemotherapy through Homoeopathic Drugs.**

Gupta G, Singh LM.


**Action of Very Low Doses of Biological Immunmodulators on the Humoral Immune Response in Mice.**

Doucet-Jaboeuf M, Pelegrin A, Sizes M, Guillemain J, Bastide M.


**Homeo Drugs Show Nematicial Properties against Tomato Root-Knot Nematode in Vitro.**

Ray S, Pradhan AK.
**Effects of Logarithmic Serial Dilutions of Arsenic Album (As2O3) on the Excitability of the Sciatic nerve of Frog.**
Jussal RL, Roy D, Singh R, Dua RD, Mishra RK.

**Effect of ultradilutions on neurotransmitter/enzyme.**
Jussal RL, Dua RD, Mishra RK, Meera S, Agarwal A.

**Effects of aconitine and veratrine on the isolated perfused heart of the common eel (Anguilla anguilla L.).**
Pennec JP, Aubin M.

Abstract
The effects of two alkaloids: aconitine and veratrine have been investigated in isolated perfused heart of the common eel (Anguilla anguilla L.). Low concentrations (less than 10(-6)M) of aconitine induced a decrease of the heart rate where high concentrations (greater than 10(-6)M) produced a tachycardia and finally led to a depolarization of cardiac cells. Veratrine induced a decrease of the heart rate depending on the concentration. A cardiac arrest was observed with high concentrations (greater than 10(-4)M). Removal of aconitine or veratrine from perfusion medium did not reverse the effects of high concentrations. The tachycardia and arrhythmias were triggered or enhanced.


**Action de différentes dilutions de vératrine sur le coeur isolé perfusé de rat.**
[Article in French]
Pennec JP, Aubin M, Manlhiot JL, Payrau B, Scaliger D.

**Action de différentes dilutions de vératrine sur le coeur isolé et perfusé d'anguille.**
[Article in French]
Pennec JP, Aubin M, Manlhiot JL, Payrau B, Scaliger D.
Inhibition du test de transformation Lymphoblastique (TTL) la phytohémagglutinine (PHA) par Phytolacca americana en dilutions homéopathiques.
[Inhibition Lymphoblastic transformation test (TTL) phytohemagglutinin (PHA) by Phytolacca americana in homeopathic dilutions].
[Article in French]
Colas H, Aubin M, Picard P, Lebecq JC, Bastide JM.

The action of 'low potency' homoeopathic remedies on the movement of guinea-pig macrophages and human leucocytes.
Moss VA, Roberts JA, Simpson KL.

Action de différentes hauteurs de dilution de Phosphorus blanc 'Phosphorus' sur la cinétique d'une réaction enzymatique in vitro, impliquant le transfert d'un groupement phosphate.
[The action of different dilutions of Phosphorus in the kinetics of an enzyme reaction in vitro, involving the transfer of a phosphate group].
[Article in French]
Kraus JL, Aubin M, Baronnet S, Manlhiot JH, Yaouanc JJ.

Abstract
On a enregistre des modifications de l'activite enzymatique de la pyruvate kinase, lorsque cette enzyme est incubee dans une milieu contenant outre les cofacteurs ADP et ion Mg++, des dilutions infinitesimales de phosphore blanc "phosphorus"
Link to abstract/paper: [http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=HomeoIndex&lang=p&nextAction=ink&exprSearch=3932&indexSearch=ID](http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=HomeoIndex&lang=p&nextAction=ink&exprSearch=3932&indexSearch=ID)

Study of the action of Hahnemannian dilutions of mercury chloride on the mitotic index in animal cell cultures.
Boiron, J., Abecassis, J., Cotte, J., Bernard, A.M.

Liasons in vitro de dilutions d'ignatia et strychninum aux recepteurs glycineriques, demonstration de leur specifite.
[Links in vitro of dilutions of Ignatia and Strychninum to glycineriques receptors, demonstration of their specificity].
[Article in French]
Guillemain J, Huguet F, Seguin G, Bakri-Logeais F, Tétau M, Narcisse G.

Action de l'Aconitine sur le coeur perfusé d'anguille.
[Action of Aconitine on the perfused eel heart].
[Article in French]
Pennec JP, Aubin M, Baronnet S, Manlhiot JL, Payrau B, Scaliger D.

Abstract
La sensibilite du coeur d'anguille perfuse a l'Aconitine presente une dualite d'aspects, avec d'une part les doses fortes entrainant une intoxication difficilement reversible, et d'autre part les doses faibles produisant l'effet inverse, avec l'optimum situe vers 10⁻⁷ M. Par ailleurs, les dilutions superieures a 5CH, qui ne presentent pratiquement aucune activite sur un coeur normal, possedent vis-a-vis d'un coeur intoxique des proprietes curatives; la dilution la plus haute parmi celles testees (9CH) apparaissant comme la plus efficace en ce sens.
Link to abstract/paper: http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=HomeIndex&lang=p&nextAction=lnk&exprSearch=3930&indexSearch=ID

Etude de l'activite hépatoprotectrice de Phosphorus sur des fragments de foies de rats adultes placees en culture organotypique sur milieu artificiel apres intoxication par le CCL4.
[Study of hepatoprotective activity of Phosphorus on fragments of livers of adult rats placed in organ culture on artificial medium after CCL4 intoxication].
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Etude de l'action de la teinture de Gelsemium sur la capture de neurotransmetteurs par des preparations synaptosomales de differentes fractions du cerveau du rat.
[Study of the action of the Gelsemium tincture on the capture of neurotransmetteurs by synaptosomal preparations of different fractions of rat brain].
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**Estimation of Low and Higher Homoeopathic Potencies by Means of Biochemical and Pharmacological Methods.**
Khan MT, Saify Z.

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[Studies on the effect of dilute sublimate on lymphoblasts in vitro].
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Amons F, van Mansvelt JD.

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**Effets de différentes dilutions de Physostigma venosum sur l'activité de la cholinesterase étudiée 'in vitro'.**
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[A homeopathic pharmacology trial].
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**Action des faibles doses de butelline sur l'intestin isolé de rat.**
[Action of low doses of butelline on isolated rat intestine].
[Article in French]
Wurmser L.

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**Die Einwirkung verschiedener Stoffe in kleinen Dosen auf das Diastatische Ferment der Muskelgewebe.**
[The influence of various substances in small doses on the Diastatic ferment of muscle tissue].
[Article in German]
Perssson WM.
Einwirkung von Mikrodosen homöopathischer Arzneimittel, Chemikalien und Hormonen auf das diastatische Ferment des Froschmuskels.
[Action of micro-doses of homeopathic medicines, chemicals and hormones on the diastic ferment of the frog muscle].
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Die Einwirkung von Mikrodosen homöopathischer Arzneimittel auf Trypsin.
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Persson WM, Ginsberg AS.

Beobachtungen über den Einfluß von Bedingungen auf Arzneiwirkung im besonderen im Hinblick auf 'kleine Dosen'.
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[Experiments for the similarity problem].
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[For scientific and experimental justification of homeopathy].
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Kötschau K.

Über die Grenzen der Empfindlichkeit des lebenden Protoplasmas.
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Krawkow NP.