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Dr. Saurav Arora

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Evaluation of chemopreventive potentials of ethanolic extract of *Ruta graveolens* against A375 skin melanoma cells *in vitro* and induced skin cancer in mice *in vivo.*

Ghosh S, Sikdar S, Mukherjee A, **Khuda-Bukhsh AR.** J Integr Med. 2015 Jan;13(1):34-44. doi: 10.1016/S2095-4964(15)60156-X.

Abstract

OBJECTIVE: Chemopreventive approach with natural products, particularly plants and plant-derived ones, is receiving increasing attention for their effective role against cancer without any palpable side effects. In this study, efficacy of ethanolic extract of Ruta graveolens (RG) on skin melanoma cells (A375) in vitro and on 7,12-dimethylbenz(a)anthracene (DMBA)-induced skin cancer in vivo has been tested in Swiss albino mice.

METHODS: Studies on cell viability, apoptosis and autophagy induction were conducted in vitro. To check apoptosis, assays like alteration in mitochondrial membrane potential, annexin V-fluorescein isothiocyanate/propidium iodide assay and immunoblot were performed. Fluorescence microscopic and immunoblot assays were performed to confirm autophagy induction. The effects of RG were determined by evaluating body weight, tumor incidence, tumor volume and tumor burden in mice. Enzymatic and non-enzymatic antioxidant status was assessed. The role of some relevant signaling proteins was also analyzed.

RESULTS: RG caused death of A375 cells through induction of caspase 3-mediated apoptosis and Beclin-1-associated autophagy. Moreover, RG administration (75 mg/kg body weight) which showed no acute or chronic toxicity, showed significant reduction in the skin tumor burden of DMBA-painted mice. RG also demonstrated potent anti-lipid peroxidative and antioxidant functions during the course of skin cancer induction by DMBA.

CONCLUSION: Chemopreventive potential of RG was demonstrated from overall results of this study, indicating its possible use in therapeutic formulation of an effective drug to treat skin cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/25609370

Current trends in ultra-high dilution research with particular reference to gene regulatory hypothesis: Review article.

Khuda-Bukhsh AR. The Nucleus (Springer) Nucleus DOI 10.1007/s13237-014-0105-0,

Abstract

In homeopathy, ultra-low doses of drugs at ultra-high dilutions are often used with great benefits to patients although at such dilutions physical existence of even a single molecule of the original drug substance is highly improbable. Despite serious challenges thrown by scientists and rationalists from time to time, homeopathy has managed to survive over 200 years now, and is no more considered a myth. Research activities on homeopathy in recent years, at clinical, physical, chemical, biological and medical levels with acceptable scientific norms and approach have paved the way for more rigorous research, particularly at the molecular level to understand the physico-chemical nature and mechanism of action of ultra-high dilutions. Although major breakthrough has been made in understanding many physical aspects and interactions between the "drug" and "aquatic ethanol" used as vehicle/solvent/diluents, certain aspects in regard to structure of water/aquatic ethanol and the latter's changing structural organization still remain unclear. In recent years, the quest for understanding the mechanism of biological action of the ultra-high dilutions has made homeopathy a hot bed of research. Much progress has been made in understanding the molecular mechanism in the light of the "gene regulatory hypothesis" that can explain the action of the homeopathic high dilutions in all living organisms, both in higher and lower animals as well as in plants. The present review focuses mainly on research in support of the gene regulatory hypothesis, and mention has been made of some relevant physical and biological aspects at cellular and molecular levels.

Article Link: http://link.springer.com/article/10.1007/s13237-014-0105-0

Anticancer potential of Conium maculatum extract against cancer cells in vitro: Drug-DNA interaction and its ability to induce apoptosis through ROS generation

Mondal J, Panigrahi AK, **Khuda-Bukhsh AR**. Pharmacogn Mag. 2014 Aug;10(Suppl 3):S524-33. doi: 10.4103/0973-1296.139792.

Abstract

OBJECTIVE: Conium maculatum extract is used as a traditional medicine for cervix carcinoma including homeopathy. However, no systematic work has so far been carried out to test its anti-cancer potential against cervix cancer cells in vitro. Thus, in this study, we investigated whether ethanolic extract of conium is capable of inducing cytotoxicity in different normal and cancer cell lines including an elaborate study in HeLa cells.

MATERIALS AND METHODS: Conium's effects on cell cycle, reactive oxygen species (ROS) accumulation, mitochondrial membrane potential (MMP) and apoptosis, if any, were analyzed through flow cytometry. Whether Conium could damage DNA and induce morphological changes were also determined microscopically. Expression of different proteins related to cell death and survival was critically studied by western blotting and ELISA methods. If Conium could interact directly with DNA was also determined by circular dichroism (CD) spectroscopy.

RESULTS: Conium treatment reduced cell viability and colony formation at 48 h and inhibited cell proliferation, arresting cell cycle at sub-G stage. Conium treatment lead to increased generation of reactive oxygen species (ROS) at 24 h, increase in MMP depolarization, morphological changes and DNA damage in HeLa cells along with externalization of phosphatidyl serine at 48 hours. While cytochrome c release and caspase-3 activation led HeLa cells toward apoptosis, down-regulation of Akt and NFkB inhibited cellular proliferation, indicating the signaling pathway to be mediated via the mitochondria-mediated caspase-3-dependent pathway. CD-spectroscopy revealed that Conium interacted with DNA molecule.

CONCLUSION: Overall results validate anti-cancer potential of Conium and provide support for its use in traditional systems of medicine.

KEYWORDS: Apoptosis; Conium maculatum; drug-DNA interaction; proliferation; reactive oxygen species

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/25298670

Low doses of ethanolic extract of Boldo (Peumus boldus) can ameliorate toxicity generated by cisplatin in normal liver cells of mice in vivo and in WRL-68 cells in vitro, but not in cancer cells in vivo or in vitro.

Mondal J, Bishayee K, Panigrahi AK, **Khuda-Bukhsh** AR. J Integr Med. 2014 Sep;12(5):425-38. doi: 10.1016/S2095-4964(14)60045-5.

Abstract

OBJECTIVE: Use of cisplatin, a conventional anticancer drug, is restricted because it generates strong hepatotoxicity by accumulating in liver. Therefore its anticancer potential can only be fully exploited if its own toxicity is considerably reduced. Towards this goal, ethanolic extract of the plant, Boldo (Peumus boldus), known for its antihepatotoxic effects, was used simultaneously with cisplatin, to test its ability to reduce cisplatin's cytotoxicity without affecting its anticancer potential.

METHODS: The cytotoxicity of Boldo extract (BE) and cisplatin, administered alone and in combination, was determined in three cancer cell lines (A549, HeLa, and HepG2) and in normal liver cells (WRL-68). Drug-DNA interaction, DNA damage, cell cycle, apoptosis, reactive oxygen species (ROS) and mitochondrial membrane potential (MMP, $\Delta\Psi$) were also studied. Hepatotoxicity and antioxidant activity levels were determined by alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and glutathione assays in mice. The cytotoxicity of related proteins was tested by Western blotting.

RESULTS: Co-administration of BE and cisplatin increased viability of normal cells, but had no effect on the viability of cancer cells. Boldo protected liver from damage and normalized different antioxidant enzyme levels in vivo and also reduced ROS and re-polarized MMP in vitro. Bax and cytochrome c translocation was reduced with caspase 3 down-regulation. Further, a drug-DNA interaction study revealed that BE reduced cisplatin's DNA-binding capacity, resulting in a reduction in DNA damage.

CONCLUSION: Results indicated that a low dose of BE could be used beneficially in combination with cisplatin to reduce its toxicity without hampering cisplatin's anticancer effect. These findings signify a potential future use of BE in cancer therapy.

Ethanolic Extract of *Marsdenia condurango* ameliorates Benzo[a]pyrene-induced lung cancer of rats.

Sikdar, S, Mukherjee A, **Khuda-Bukhsh AR**. Journal of Pharmacopuncture, 2014, Vol. 17: (p7-17).

Abstract

Objectives: Condurango is widely used in various systems of complementary and alternative medicines (CAM) against oesophageal and stomach ailments including certain types of cancer. However, until now no systematic study has been conducted to verify its efficacy and dose with proper experimental support. Therefore, we examined if ethanolic extract of Condurango could ameliorate benzo[a]pyrene (BaP)-induced lung cancer in rats, in vivo to validate its use as traditional medicine.

Methods: Fifteen male and 15 female Sprague-Dawley (SD) rats were treated with 0.28 mg/kg of Sweet Bee Venom (SBV) (high-dosage group) and the same numbers of male and female SD rats were treated with 0.2 mL/kg of normal saline (control group) for 13 weeks. We selected five male and five female SD rats from the high-dosage group and the same numbers of male and female SD rats from the control group, and we observed these rats for four weeks. We conducted body-weight measurements, ophthalmic examinations, urinalyses and hematology, biochemistry, histology tests.

Results: A histological study revealed gradual progress in lung tissue-repair activity in Condurango-fed cancer-bearing rats, showing gradual tissue recovery after three months of drug administration. Condurango has the capacity to generate reactive oxygen species (ROS), which may contribute to a reduction in anti-oxidative activity and to an induction of oxidative stress-mediated cancer cell-death. Condurango-activated pro-apoptotic genes (Bax, caspase-3, caspase-9, p53, cytochrome-c, apaf-1, ICAD and PARP) and down-regulated antiapoptotic-Bcl-2 expression were noted both at mRNA and protein levels. Studies on caspase-3 activation and PARP cleavage by western blot analysis revealed that Condurango induced apoptosis through a caspase-3-dependent pathway.

Conclusion: The anticancer efficacy of an ethanolic extract of Condurango for treating BaP-induced lung cancer in rats lends support for its use in various traditional systems of medicine.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/25780694

Condurango 30C induces epigenetic modification of lung cancer specific tumour suppressor genes via demethylation

Sikdar S and **Khuda-Bukhsh AR**. Forschende Komplementarmetizin/Research in Complementary Medicine. (In Press).

Abstract

Background: DNA hypermethylation induces cancer progression involving CpG island of DNA and causes inactivation of tumour suppressor genes. In this study, DNA hypermethylation status of lung cancer and ability of ultra-highly diluted Condurango 30C to modulate DNA methylation were ascertained by analysis of lung cancer-specific tumour suppressor genes in respect to placebo.

Materials And Methods: DNA methylation status, if any, was determined by PCR-SSCP analyses in lung cancer-specific tumour suppressor genes (p15, p16 and p53) using H460-NSCLC cell and BaP-induced lung cancer of rats. The ability of Condurango 30C to modulate DNA methylation, if any, was verified against placebo control in blinded manner.

Results: Condurango 30C-treated DNA showed significant decrease in band intensity of p15 and p53 genes especially in methylated condition in vitro, at IC50 dose (2.43µl/100µl). SSCP analysis of p15 and p53 genes in Condurango 30C-treated DNA also suggests that Condurango 30C can decrease methylation, in vitro. Inhibition of p15 hypermethylation was observed in post-cancer treatment of rats with Condurango 30C. SSCP results gave a better indication of differences in band position of p15 and p53 in Condurango 30C-treated lung samples.

Conclusion: Condurango 30C could trigger epigenetic modification in lung cancer via modulation of DNA hypermethylation. © 2015 S. Karger GmbH, Freiburg.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/26088552

Berberine alters epigenetic modifications, disrupts microtubule network, and modulates HPV-18E6-E7 oncoproteins by targeting p53 in cervical cancer cell HeLa: A mechanistic study including molecular docking

Saha S and **Khuda-Bukhsh AR.** *European Journal of Pharmacology*, 2014; 244:132-146.

Abstract

Increased evidence of chemo-resistance, toxicity and carcinogenicity necessitates search for alternative approaches for determining next generation cancer therapeutics and targets. We therefore tested the efficacy of plant alkaloid berberine on human papilloma virus (HPV) -18 positive cervical cancer cell HeLa systematically-involving certain cellular, viral and epigenetic factors. We observed disruptions of microtubule network and changes in membrane topology due to berberine influx through confocal and atomic force microscopies (AFM). We examined nuclear uptake, internucleosomal DNA damages, mitochondrial membrane potential (MMP) alterations and cell migration assays to validate possible mode of cell death events. Analytical data on interactions of berberine with pBR322 through fourier transform infrared (FTIR) and gel migration assay strengthen berberine's biologically significant DNA binding abilities. We measured cellular uptake, DNA ploidy and DNA strand-breaks through fluorescence activated cell sorting (FACS). To elucidate epigenetic modifications, in support of DNA binding associated processes, if any, we conducted methylation-specific restriction enzyme (RE) assay, methylation specific-PCR (MSP) and expression studies of histone proteins. We also analyzed differential interactions and localization of cellular tumor suppressor p53 and viral oncoproteins HPV-18 E6–E7 through siRNA approach. We further made in-silico approaches to determine possible binding sites of berberine on histone proteins. Overall results indicated cellular uptake of berberine through cell membrane depolarization causing disruption of microtubule networks and its biological DNA binding abilities that probably contributed to epigenetic modifications. Results of modulation in p53 and viral oncoproteins HPV-18 E6–E7 by berberine further proved its potential as a promising chemotherapeutic agent in cervical cancer.

Article Link: http://www.sciencedirect.com/science/article/pii/S0014299914007055

Flavonol isolated from ethanolic leaf extract of Thuja occidentalis arrests the cell cycle at G2-M and induces ROS-independent apoptosis in A549 cells, targeting nuclear DNA.

Mukherjee A, Sikdar S, Bishayee K, Boujedaini N, **Khuda-Bukhsh AR.** Cell Prolif. 2014;47:56-71. doi: 10.1111/cpr.12079.

Abstract

Objectives: The K-ras gene mutation commonly found in lung adenocarcinomas contributes to their non-invasive expansion. Our main objective here was to develop a chemopreventive agent against K-ras-mutated lung adenocarcinoma cell line like-A549.

Materials and Methods: We isolated flavonol from ethanolic leaf extract of Thuja occidentalis, and evaluated its apoptotic potentials on A549 cells. They were treated with 1-10 µg/ml of flavonol and viability was tested retaining normal lung cells L-132 as control. We performed assays such as TUNEL, annexin V, cell-cycle and mitochondrial membrane potentials, by FACS analysis. ROS-mediated oxidative stress and drug-DNA interactions were analysed along with gene expression studies for p53, Bax-Bcl2, cytochrome c, the caspase cascade genes and PARP.

Results: Flavonol reduced A549 cell viability in a dose- and time-dependent manner (IC50 value = 7.6 ± 0.05 µg/ml following 48 h incubation) sparing normal L-132 cells. It effected G2-M phase cell cycle arrest and apoptosis, as indicated by progressive increase in the sub-G1, annexin V and TUNEL-positive cell populations. Apoptotic effects appeared to be mitochondria-dependent, caspase-3-mediated, but ROS-independent. Analysis of circular dichroism data revealed that flavonol intercalated with nuclear DNA. In vivo studies on non small cell lung carcinoma (NSCLC)-induced mice confirmed anti-cancer potential of flavonol.

Conclusion: Flavonol-induced apoptosis apparently resulted from intercalation of cells' nuclear DNA. Flavonol inhibited growth of induced lung tumours in the mice, indicating its potential as an effective agent against NSCLC.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24267912

Relative Apoptosis-inducing Potential of Homeopathic Condurango 6C and 30C in H460 Lung Cancer Cells In vitro: - Apoptosis-induction by homeopathic Condurango in H460 cells.

Sikdar S, Saha SK, **Khuda-Bukhsh AR**. Journal of Pharmacopuncture, 2014, 17: (p59-69), 17.

Abstract

Objectives: In homeopathy, it is claimed that more homeopathically-diluted potencies render more protective/curative effects against any disease condition. Potentized forms of Condurango are used successfully to treat digestive problems, as well as esophageal and stomach cancers. However, the comparative efficacies of Condurango 6C and 30C, one diluted below and one above Avogadro's limit (lacking original drug molecule), respectively, have not been critically analyzed for their cell-killing (apoptosis) efficacy against lung cancer cells in vitro, and signalling cascades have not been studied. Hence, the present study was undertaken.

Methods: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT) assays were conducted on H460-non-small-cell lung cancer (NSCLC) cells by using a succussed ethyl alcohol vehicle (placebo) as a control. Studies on cellular morphology, cell cycle regulation, generation of reactive oxygen species (ROS), changes in mitochondrial membrane potential (MMP), and DNA-damage were made, and expressions of related signaling markers were studied. The observations were done in a "blinded" manner.

Results: Both Condurango 6C and 30C induced apoptosis via cell cycle arrest at subG0/G1 and altered expressions of certain apoptotic markers significantly in H460 cells. The drugs induced oxidative stress through ROS elevation and MMP depolarization at 18-24 hours. These events presumably activated a caspase-3-mediated signalling cascade, as evidenced by reverse transcriptase- polymerase chain reaction (RT-PCR), western blot and immunofluorescence studies at a late phase (48 hours) in which cells were pushed towards apoptosis.

Conclusion: Condurango 30C had greater apoptotic effect than Condurango 6C as claimed in the homeopathic doctrine.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/25780691

Post-cancer treatment of Condurango 30C, traditionally used in homeopathy, ameliorates tissue damage and stimulates reactive oxygen species in benzo[a]pyrene-induced lung cancer of rat

Sikdar S, **Khuda-Bukhsh AR.** Humanitas Traditional Medicine (TANG), 2013, 3, e25.

Homoeopathically prepared Condurango 30C is traditionally used in amelioration of certain types of cancer by homeopathic practitioners. In this study, ability of Condurango 30C in amelioration of the conventional benzo[a]pyrene (BaP)-induced lung cancer in rat has been tested. After one month of scheduled oral feeding of BaP, lung cancer is routinely developed after four months in rats. Tumorbearing rats were then treated with Condurango 30C for the next one (5th), two (6th) and three (7th) months, respectively, and sacrificed. Efficacy of post-cancer treatment by Condurango 30C was evaluated against controls (placebo) by different study parameters like: body and lung weights, number and diameter of lung tumour nodules, lung architecture, DNA damage, anti-oxidant activity and reactive oxygen species (ROS) accumulation. Administration of this homeopathic remedy caused increase of body weight and decrease of lung weight, decrease in number and diameter of lung tumour nodules, particularly after one and two months of drug treatment. BaP intoxication significantly increased lipid peroxidase (LPO) with concomitant decrease in activities of different antioxidants, while Condurango 30C administration certainly reduce their levels than normal and cancerous groups, notably after one and two months' of drug treatment. Condurango 30C showed capability to induce ROS-mediated cell death evidenced from the study of ROS activities at different time-points. Further, the remedy possibly achieved its anticancer goal through mediation of DNA-nicks that possibly led cancer cells to the apoptotic pathway. Thus, Condurango 30C has anticancer potential in BaP-induced lung cancer of rats via tissue damage recovery and ROS-mediated programmed cell death.

Article Link: http://www.dbpia.co.kr/Journal/ArticleDetail/3234928

Post-cancer Treatment with Condurango 30C Shows Amelioration of Benzo[a]pyrene-induced Lung Cancer in Rats Through the Molecular Pathway of Caspa- se-3-mediated Apoptosis Induction

Sikdar S, Mukherjee A, Bishayee K, Paul A, Saha S, Ghosh S, Chakraborty D, Boujedaini N, **Khuda-Bukhsh A.R**. Journal of Pharmacopuncture, 2013; 16:011-022.

Abstract

Objectives: The present investigation aimed at examining if post-cancer treatment with a potentized homeopathic drug, Condurango 30C, which is generally used to treat oesophageal cancer, could also show an ameliorating effect through apoptosis induction on lung cancer induced by benzo[a]pyrene (BaP) in white rats (Rattus norvegicus).

Methods: Lung cancer was induced after four months by chronic feeding of BaP to rats through gavage at a dose of 50 mg/kg body weight for one month. After four months, the lung-cancer-bearing rats were treated with Condurango 30C for the next one (5th), two (5th-6th) and three (5th-7th) months, respectively, and were sacrificed at the corresponding time- points. The ameliorating effect, if any, after Condurango 30C treatment for the various periods was evaluated by using protocols such as histology, scanning electron microscopy (SEM), annexinV-FITC/PI assay, flow cytometry of the apoptosis marker, DNA fragmentation, reverse transcriptase-polymerase chain reaction (RT-PCR), immunohistochemistry, and western blot analyses of lung tissue samples.

Results: Striking recovery of lung tissue to a near normal status was noticed after post-cancerous drug treatment, as evidenced by SEM and histology, especially after one and two months of drug treatment. Data from the annexinV-FITC/PI and DNA fragmentation assays revealed that Condurango 30C could induce apoptosis in cancer cells after post-cancer treatment. A critical analysis of signalling cascade, evidenced through a RT-PCR study, demonstrated up-regulation and down-regulation of different pro- and anti-apoptotic genes, respectively, related to a caspase-3-mediated apoptotic pathway, which was especially discernible after one-month and two- month drug treatments. Correspondingly, Western blot and immunohistochemistry studies confirmed the ameliorative potential of Condurango 30C by its ability to down-regulate the elevated epidermal growth factor receptor (EGFR) expression, a hallmark of lung cancer.

Conclusion: The overall result validated a positive effect of Condurango 30C in ameliorating lung cancer through caspase-3-mediated apoptosis induction and EGFR down-regulation.

Keywords: apoptosis, benzo[a]pyrene (BaP), caspase-3, Condurango 30C, homeopathy, lung cancer

Article Link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4331970/

Evidence in support of gene regulatory hypothesis: Gene expression profiling manifests homeopathy effect as more than placebo

Saha SK, Roy S, **Khuda-Bukhsh AR**. Int J High Dilution Res 2013;12:162-7.

Abstract

Background: Use of ultra-high diluted remedies in homeopathy and their claimed efficacy in curing diseases has been challenged time and again by non-believers despite many evidence-based positive results published in favor of their efficacy in curing/ameliorating disease symptoms.

Aims: To test the ability of ultra-high diluted homeopathic remedies beyond Avogadro's limit, if any, in manifesting gene modulating effects in controlled in vitro experimental model.

Methods: Since cancer cells manifest aberrant epigenetic gene expressions, we conducted global microarray gene expression profiling of HeLa cells (an established epigenetic model of HPV18 positive cell line) treated with two different potentized homeopathic remedies, namely, Condurango 30c and Hydrastis canadensis 30C (used in the treatment of cancer), as compared to that of placebo (succussed alcohol 30c).

Results: Data revealed distinctly different expression patterns of over 100 genes as a consequence of treatment with both homeopathc remedies compared to placebo.

Conclusion: Results indicate that action of the potentized drugs was "more than placebo" and these ultra-highly diluted drugs acted primarily through modulation of gene expression.

Article Link: http://www.feg.unesp.br/~ojs/index.php/ijhdr/article/viewArticle/635

Ethanolic extract of the Goldenseal, Hydrastis canadensis, has demonstrable chemopreventive effects on HeLa cells in vitro: Drug-DNA interaction with calf thymus DNA as target

Saha SK, Sikdar S, Mukherjee A, Bhadra K, Boujedaini N, **Khuda-Bukhsh AR.** Environ Toxicol Pharmacol. 2013;36:202-14. doi: 10.1016/j.etap.2013.03.023.

Abstract

This study tested chemotherapeutic potential of Hydrastis canadensis (HC) extract in HeLa cells in vitro, with emphasis on its drug-DNA interaction and apoptosis induction ability. Nuclear uptake of HC by DAPI, Ao/Eb staining and internucleosomal DNA damage by comet assay was studied through fluorescence microscopy. Possible changes in MMP and apoptotic signalling events were critically analyzed. Cell cycle progression studied through FACS and fragmented DNA through "TUNEL" assay were critically analyzed. RT-PCR studies were conducted for analyzing Cyt-C and Bax translocation in mitochondrial and cytosolic extracts, and Caspase 3 in whole cell lysate. Role of p53-mediated regulation of NF-κβ and TNF-α was elucidated by Western blot analysis. Data of CD and Tm profile of CT-DNA were analyzed. Overall results indicated anti-cancer potential of HC through its ability to induce apoptosis, and interaction with CT-DNA that changed structural conformation of DNA, proving HC to be a promising candidate for chemoprevention.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23628949

Condurango glycoside-rich components stimulate DNA damage-induced cell cycle arrest and ROS-mediated caspase-3 dependent apoptosis through inhibition of cell-proliferation in lung cancer, in vitro and in vivo

Sikdar S, Mukherjee A, Ghosh S, **Khuda-Bukhsh AR**. Environ Toxicol Pharmacol. 2013;37:300-314.

Abstract

Chemotherapeutic potential of Condurango glycoside-rich components (CGS) was evaluated in NSCLC, in vitro and in BaP-intoxicated rats, in vivo. NSCLC cells were treated with different concentrations of CGS to test their effect on cell viability. Cellular morphology, DNA-damage, AnnexinV-FITC/PI, cell cycle regulation, ROS-accumulation, MMP, and expressions of related signalling genes were critically analysed. 0.22 μg/μl CGS

(IC_{50} dose at 24 h) was selected for the study. CGS-induced apoptosis via DNA damage was evidenced by DNA-ladder formation, increase of AnnexinV-positive cells, cell cycle arrest at subG0/G1 and differential expressions of apoptotic genes. ROS-elevation and MMP-depolarization with significant caspase-3 activation might lead to apoptotic cell death. Anti-proliferative activity was confirmed by EGFR-expression modulation. ROS accumulation and DNA-nick formation with tissue damage-repair activity after post-cancerous CGS treatment, in vivo, supported the in vitro findings. Overall results advocate considerable apoptosis-inducing potential of CGS against NSCLC, validating its use against lung cancer by CAM practitioners.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24384279

Efficacy of PLGA-loaded apigenin nanoparticles in Benzo[a]pyrene and ultraviolet-B induced skin cancer of mice: Mitochondria mediated apoptotic signalling cascades

Das S, Das J, Samadder A, Paul A, **Khuda-Bukhsh AR**. **Food Chem Toxicol**. 2013 Oct 9. doi:pii: S0278-6915(13)00675-3. 10.1016/j.fct.2013.09.037.

Abstract

Skin cancer is increasing at an alarming rate and becoming resistant to conventional chemotherapy necessitating improved drug delivery system. We loaded apigenin (Ap), a dietary flavonoid having anti-cancer property, with poly (lactic-co-glycolide) (PLGA) nanoparticles (NAp) to explore if nano-encapsulation could enhance anti-carcinogenic effect against ultra-violet B (UVB) and Benzo(a)pyrene (BaP) induced skin tumor and mitochondrial dysfunction in mice. Particle size, morphology and zeta potential of NAp were determined using dynamic light scattering and atomic force microscopy. Tumor incidence and multiplicity in UVB-BaP induced mice with/without NAp treatment were ascertained and their histolopathological sections and chromosomal aberrations were studied. ROS accumulation and mitochondrial functioning through relevant markers like mitochondrial transmembrane potential were analyzed. Mitochondrial volume changes/swelling, cytochrome c (cyt c) release, mRNA and protein expressions of Apaf-1, bax, bcl-2, cyt c, cleaved caspase-9 and 3 were studied. Results showed that NAp produced better effects than Ap, due to their smaller size, and faster mobility. NAp reduced tissue damage and frequency of chromosomal aberrations, increased ROS accumulation to mediate mitochondrial-apoptosis through modulation of several apoptotic markers and mitochondrial matrix swelling. NAp showed ameliorative potentials in combating skin cancer and therefore has greater prospect of use in therapeutic management of skin cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24120900

Strategic formulation of apigenin-loaded PLGA nanoparticles for intracellular trafficking, DNA targeting and improved therapeutic effects in skin melanoma in vitro

Das S, Das J, Samadder A, Paul A, **Khuda-Bukhsh AR**. **Toxicol Lett.** 2013 Sep 23. doi:pii: S0378-4274(13)01326-X. 10.1016/j.toxlet.2013.09.012

Abstract

The aim of the present study was the evaluation of anti-proliferative potentials of apigenin (Ap), (a dietary flavonoid) loaded in poly (lactic-co-glycolide) nanoparticles (NAp) in A375 cells in vitro. NAp was characterized for particle size, morphology, zeta potential, drug release and encapsulation. Cellular entry and intracellular localization of NAp were assessed by transmission electron and confocal microscopies. Circular dichroic spectral analysis and stability curve for Gibb's free energy of dsDNA of A375 cells were also analyzed. DNA fragmentation, intracellular ROS accumulation, superoxide-dismutase activity, intracellular glutathione-reductase content and mitochondrial functioning through relevant markers like mitochondrial transmembrane potential, ATPase activity, ATP/ADP ratio, volume changes/swelling, cytochrome-c release, expressions of Apaf-1, bax, bcl-2, caspase-9, 3, and PARP cleavage were analyzed. NAp produced better effects due to their smaller size, faster mobility and site-specific action. Photostability studies revealed that PLGA encapsulations were efficient at preserving apigenin ultraviolet-light mediated photodegradation. NAp readily entered cancer cells, could intercalate with dsDNA, inducing conformational change. Corresponding increase in ROS accumulation and depletion of the antioxidant enzyme activities exacerbated DNA damage, mediating apoptosis through mitochondrial dysfunction. Overall results indicate that therapeutic efficacy of NAp may be enhanced by PLGA nanoparticle formulations to have better ameliorative potentials in combating skin melanoma.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24070738

Condurango-glycoside-A fraction of Gonolobus condurango induces DNA damage associated senescence and apoptosis via ROS-dependent p53 signalling pathway in HeLa cells

Bishayee K, Paul A, Ghosh S, Sikdar S, Mukherjee A, Biswas R, Boujedaini N, **Khuda-Bukhsh AR**. Mol Cell Biochem. 2013; 382:173-83.

Abstract

Gonolobus condurango plant extract is used as an anticancer drug in some traditional systems of medicine including homeopathy, but it apparently lacks any scientific validation. Further, no detailed study is available to suggest whether condurango-glycoside-A (CGA), a major ingredient of condurango serves as a potent anticancer compound. Therefore, we investigated apoptosis-inducing ability of CGA against cervix carcinoma cells (HeLa). β-galactosidase-activity and DNA damage were critically studied at different time points; while induced DNA-damage was observed at 9-12th hours, senescence of cells appeared at a later stage (18th hour after CGA treatment), implicating thereby a possible role of DNA damage in inducing pre-mature cell senescence. Concurrently, the number of cells undergoing apoptosis increased along with increase in reactive oxygen species (ROS) generation. Expression of p53 was also up-regulated, indicating that apoptosis could have been mediated through p53 pathway. DCHFDA (4',6-Diamidino-2-phenylindole dihydrochloride) assay, acridine orange/ethidium bromide staining and annexin V/PI assay results collectively confirmed that apoptosis was induced by increased ROS generation. Reduction in proliferation of cells was further evidenced by the cell cycle arrest at G0/G1 stage. Expression profiles of certain relevant genes and proteins like p53, Akt, Bcl-2, Bax, cytochrome c and caspase 3 also provided evidence of ROS mediated p53 up-regulation and further boost in Bax expression and followed by cytochrome c release and activation of caspase 3. Overall results suggest that CGA initiates ROS generation, promoting up-regulation of p53 expression, thus resulting in apoptosis and pre-mature senescence associated with DNA damage.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23807740

Ultra-Highly Diluted Gonolobus Condurango Extract Inhibits Histone De-Acetylase2 Activity in Cervix Cancer Cells in Vitro: Evidence of Epigenetic Modification in Cell Cycle Arrest

Bishayee K, Sikdar S, **Khuda-Bukhsh AR**.. J Pharmacopunctue. 2013; 16:7-13.

Abstract

Objectives: Whether the ultra-highly-diluted remedies used in homeopathy can effectively bring about modulations of gene expressions through acetylation/deacetylation of histones has not been explored. Therefore, in this study, we pointedly checked if the homeopathically-diluted anti-cancer remedy Condurango 30C (ethanolic extract of Gonolobus condurango diluted 10-60 times) was capable of arresting the cell cycles in cervical cancer cells HeLa by triggering an epigenetic modification through modulation of the activity of the key enzyme histone deacetylase 2 vis-a-vis the succussed alcohol (placebo) control.

Methods: We checked the activity of different signal proteins (like p21WAF, p53, Akt, STAT3) related to deacetylation, cell growth and differentiation by western blotting and analyzed cell-cycle arrest, if any, by fluorescence activated cell sorting. After viability assays had been performed with Condurango 30C and with a placebo, the activities of histone de-acetylase (HDAC) enzymes 1 and 2 were measured colorimetrically.

Results: While Condurango 30C induced cytotoxicity in HeLa cells in vitro and reduced HDAC2 activity quite strikingly, it apparently did not alter the HDAC1 enzyme; the placebo had no or negligible cytotoxicity against HeLa cells and could not alter either the HDAC 1 or 2 activity. Data on p21WAF, p53, Akt, and STAT3 activities and a cell-cycle analysis revealed a reduction in DNA synthesis and G1-phase cell-cycle arrest when Condurango 30C was used at a 2% dose.

Conclusion: Condurango 30C appeared to trigger key epigenetic events of gene modulation in effectively combating cancer cells, which the placebo was unable to do.

Keywords: ultra-highly-diluted remedy, Condurango 30C, histone deacetylase activity, HDAC2, cell cycle

Article Link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4331975/

Lycopodine triggers apoptosis by modulating 5-lipoxygenase, and depolarizing mitochondrial membrane potential in androgen sensitive and refractory prostate cancer cells without modulating p53 activity: Signalling cascade and drug-DNA interaction

Bishayee K, Chakraborty D, Ghosh S, Boujedaini N, **Khuda-Bukhsh AR**. Eur J Pharmacol. 2013; 698:110–21.

Abstract

When the prostate cancer cells become unresponsive to androgen therapy, resistance to chemotherapy becomes imminent, resulting in high mortality. To combat this situation, lycopodine, a pharmacologically important bioactive component derived from Lycopodium clavatum spores, was tested against hormone sensitive (LnCaP) and refractory (PC3) prostate cancer cells in vitro. This study aims to check if lycopodine has demonstrable anti-cancer effects and if it has, to find out the possible mechanism of its action. The MTT assay was performed to evaluate the cytotoxic effect. Depolarization of mitochondrial membrane potential, cell cycle, EGF receptor activity and apoptosis were recorded by FACS; profiles of different anti- and pro-apoptotic genes and their products were studied by semi-quantitative RT-PCR, indirect-ELISA, western blotting. Drug-DNA interaction was determined by CD spectroscopy. Administration of lycopodine down-regulated the expression of 5-lipoxygenase and the 5-oxo-ETE receptor (OXE receptor1) and EGF receptor, and caused up-regulation of cytochrome c with depolarization of mitochondrial inner membrane potential, without palpable change in p53 activity, resulting in apoptosis, cell arrest at G0/G1 stage and ultimately reduced proliferation of cancer cells; concomitantly, there was externalization of phosphotidyl serine residues. CD spectroscopic analysis revealed intercalating property of lycopodine with DNA molecule, implicating its ability to block cellular DNA synthesis. The overall results suggest that lycopodine is a promising candidate suitable for therapeutic use as an anti-cancer drug.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23142370

Anti-hyperglycemic drug *Gymnema sylvestre* also shows anti-cancer potentials in human melanoma A375 cells via ROS generation and mitochondria-dependent caspase pathway

Chakraborty, D, Ghosh, S, Bishayee, K, Mukherjee A, Sikdar S, **Khuda-Bukhsh, AR.** Integrative Cancer Therapies, 2013;12:433-41. doi: 10.1177/1534735413485419.

Abstract

Objective: Ethanolic extract of Gymnema sylvestre (GS) leaves is used as a potent antidiabetic drug in various systems of alternative medicine, including homeopathy. The present study was aimed at examining if GS also had anticancer potentials, and if it had, to elucidate its possible mechanism of action.

Methods: We initially tested possible anticancer potential of GS on A375 cells (human skin melanoma) through MTT assay and determined cytotoxicity levels in A375 and normal liver cells; we then thoroughly studied its apoptotic effects on A375 cells through protocols such as Hoechst 33258, H2DCFDA, and rhodamine 123 staining and conducted ELISA for cytochrome c, caspase 3, and PARP activity levels; we determined the mRNA level expression of cytochrome c, caspase 3, Bcl2, Bax, PARP, ICAD, and EGFR signaling genes through semiquantitative reverse transcriptase polymerase chain reaction and conducted Western blot analysis of caspase 3 and PARP. We also analyzed cell cycle events, determined reactive oxygen species accumulation, measured annexin V-FITC/PI and rhodamine 123 intensity by flow cytometry.

Results: Compared with both normal liver cells and drug-untreated A375, the mortality of GS-treated A375 cells increased in a dose-dependent manner. Additionally, GS induced nuclear DNA fragmentation and showed an increased level of mRNA expression of apoptotic signal related genes cytochrome c, caspase 3, PARP, Bax, and reduced expression level of ICAD, EGFR, and the anti-apoptotic gene Bcl2.

Conclusion: Overall results indicate GS to have significant anticancer effect on A375 cells apart from its reported antidiabetic effect, indicating possibility of its palliative use in patients with symptoms of both the diseases.

Keywords: A375 melanoma cells; DNA damage; Gymnema sylvestre; anticancer potential; apoptosis; reactive oxygen species

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23615751

Apigenin, a bioactive flavonoid from Lycopodium clavatum, stimulates nucleotide excision repair genes to protect skin keratinocytes from ultraviolet B-induced reactive oxygen species and DNA damage.

Das S, Das J, Paul A, Samadder A, **Khuda-Bukhsh AR**. J Acupunct Meridian Stud. 2013; 6:252-62.

Abstract

In this study, we examined the antioxidative and the DNA protective potentials of apigenin, a flavonoid polyphenol isolated from Lycopodium clavatum, in both in-vitro (HaCaT skin keratinocytes) and in-vivo (mice) models against UV-B radiation. We used DAPI staining in UV-B-irradiated HaCaT skin keratinocytes pre-treated with and without apigenin to assess DNA damage. We also used a flow-cytometric analysis in mice exposed to UV-B radiation with or without topical application of apigenin to assess, through a comet assay, chromosomal aberrations and quanta from reactive oxygen species (ROS) generation. Data from the stability curves for the Gibb's free energy determined from a melting-temperature profile study indicated that apigenin increased the stability of calf thymus DNA. Immunofluorescence studies revealed that apigenin caused a reduction in the number of cyclobutane pyrimidine dimers (CPDs) after 24 h, the time at which the nucleotide excision repair (NER) genes were activated. Thus, apigenin accelerated reversal of UV-B-induced CPDs through up-regulation of NER genes, removal of cyclobutane rings, inhibition of ROS generation, and down-regulation of NF-κB and MAPK, thereby revealing the precise mechanism of DNA repair.

Keywords: DNA damage; apigenin; cyclobutane pyrimidine dimers; reactive oxygen species; ultraviolet B

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24139463

Biosynthesized silver nanoparticles by ethanolic extracts of Phytolacca decandra, Gelsemium sempervirens, Hydrastis canadensis and Thuja occidentalis induce differential cytotoxicity through G2/M arrest in A375 cells.

Das S, Das J, Samadder A, Bhattacharyya SS, Das D, **Khuda-Bukhsh AR.** Colloids Surf B Biointerfaces. 2013; 101: 325-336.

Abstract

The capability of crude ethanolic extracts of certain medicinal plants like Phytolacca decandra, Gelsemium sempervirens, Hydrastis canadensis and Thuja occidentalis used as homeopathic mother tinctures in precipitating silver nanoparticles from aqueous solution of silver nitrate has been explored. Nanoparticles thus precipitated were characterized by spectroscopic, dynamic light scattering, X-ray diffraction, atomic force and transmission electron microscopic analyses. The drug-DNA interactions of silver nanoparticles were analyzed from data of circular dichroism spectroscopy and melting temperature profiles using calf thymus DNA (CT-DNA) as target. Biological activities of silver nanoparticles of different origin were then tested to evaluate their effective anti-proliferative and anti-bacterial properties, if any, by exposing them to A375 skin melanoma cells and to Escherichia coli C, respectively. Silver nanoparticles showed differences in their level of anti-cancer and anti-bacterial potentials. The nanoparticles of different origin interacted differently with CT-DNA, showing differences in their binding capacities. Particle size differences of the nanoparticles could be attributed for causing differences in their cellular entry and biological action. The ethanolic extracts of these plants had not been tested earlier for their possible efficacies in synthesizing nanoparticles from silver nitrate solution that had beneficial biological action, opening up a possibility of having therapeutic values in the management of diseases including cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23010037

Homeopathic Thuja 30C ameliorates benzo(a)pyrene-induced DNA damage, stress and viability of perfused lung cells of mice in vitro.

Mukherjee A, Boujedaini N, **Khuda-Bukhsh AR**. J Integr Med. 2013;11:397-404. doi: 10.3736/jintegrmed2013054.

Abstract

Objective: To examine if the ultra-highly diluted homeopathic remedy Thuja 30C can ameliorate benzo(a)pyrene (BaP)-induced DNA damage, stress and viability of perfused lung cells of Swiss albino mice in vitro.

Methods: Perfused normal lung cells from mice were cultured in 5% Roswell Park Memorial Institute medium and exposed to BaP, a potent carcinogen, at the half maximal inhibitory concentration dose (2.2 µmol/L) for 24 h. Thereafter, the intoxicated cells were either treated with Thuja 30C (used against tumor or cancer) or its vehicle media, succussed alcohol 30C. Relevant parameters of study involving reactive oxygen species (ROS) accumulation, total glutathione (GSH) content, and generations of heat shock protein (hsp)-90 were measured; the cell viability and other test parameters were measured after treatment with either Thuja 30C or its vehicle media. Circular dichroism spectroscopy was performed to examine if Thuja 30C directly interacted with calf thymus DNA as target. For ascertaining if DNA damaged by BaP could be partially repaired and restituted by the remedy, 4',6-diamidino-2-phenylindole staining was performed.

Results: Thuja 30C increased cell viability of BaP-intoxicated cells significantly, as compared to drug-untreated or drug-vehicle control. A minimal dose of Thuja 30C significantly inhibited BaP-induced stress level, by down-regulating ROS and hsp-90, and increasing GSH content. Thuja 30C itself had no DNA-damaging effect, and no direct drug-DNA interaction. However, it showed quite striking ability to repair DNA damage caused by BaP.

Conclusion: Thuja 30C ameliorates BaP-induced toxicity, stress and DNA damage in perfused lung cells of mice and it apparently has no effect on normal lung cells.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24299603

Graveoline isolated from ethanolic extract of Ruta graveolens triggers apoptosis and autophagy in skin melanoma cells: A novel apoptosis-independent autophagic signaling pathway.

Ghosh S, Bishayee K, **Khuda-Bukhsh AR.** Phytother Res. 2013 Dec 17. doi: 10.1002/ptr.5107.

Abstract

Anti-cancer drugs generally kill cancer cells by apoptosis but fail to do so when they become resistant and escape apoptosis signals. But these resistant cells can still be killed by autophagy. Therefore, drugs having both apoptotic and autophagic abilities are solicited in effective cancer management. In search of such a drug, we examined the efficacy of graveoline, a bioactive compound isolated from Ruta graveolens on skin melanoma A375 cells through the use of specific signaling cascades and their inhibitors. Cytotoxicity of graveoline was tested by conducting MTT assay. Induction of autophagy and apoptosis was checked. Expression of related proteins and their localization were studied by conducting immunoblot assay and through confocal microscopy, respectively. We found graveoline-induced Beclin-1 associated autophagy in A375 cells and 3-methyladenine, an inhibitor of autophagy did not affect apoptosis. Conversely, caspase inhibitor that blocked apoptosis did not affect autophagic cell death, suggesting thereby that these two were independent events. Use of reactive oxygen species (ROS) scavengers inhibited cell death, but blocking autophagy did not affect graveoline-induced ROS generation, suggesting that ROS generation ensued autophagy. Thus, graveoline-induced both apoptotic and autophagic cell death in skin melanoma cells, a desirable quality in effective anti-cancer drug design.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24343999

Homeopathic mother tincture of Phytolacca decandra induces apoptosis in skin melanoma cells by activating caspase mediated signaling via Reactive Oxygen Species elevation.

Ghosh S, Bishayee K, Paul A, Mukherjee A, Sikdar S, Chakraborty D, Boujedaini N, **Khuda-Bukhsh AR**. J Integr Med. 2013;11:116-24. doi: 10.3736/jintegrmed2013014.

Abstract

Objective: Preventive measures against skin melanoma like chemotherapy are useful but suffer from chronic side effects and drug resistance. Ethanolic extract of Phytolacca decandra (PD), used in homeopathy for the treatment of various ailments like chronic

rheumatism, regular conjunctivitis, psoriasis, and in some skin diseases was tested for its possible anticancer potential.

Methods: Cytotoxicity of the drug was tested by conducting 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay on both normal (peripheral blood mononuclear cells) and A375 cells. Fluorescence microscopic study of 4',6-diamidino-2-phenylindole dihydrochloride-stained cells was conducted for DNA fragmentation assay, and changes in cellular morphology, if any, were also recorded. Lactate dehydrogenase activity assay was done to evaluate the percentages of apoptosis and necrosis. Reactive oxygen species (ROS) accumulation, if any, and expression study of apoptotic genes also were evaluated to pin-point the actual events of apoptosis.

Results: Results showed that PD administration caused a remarkable reduction in proliferation of A375 cells, without showing much cytotoxicity on peripheral blood mononuclear cells. Generation of ROS and DNA damage, which made the cancer cells prone to apoptosis, were found to be enhanced in PD-treated cells. These results were duly supported by the analytical data on expression of different cellular and nuclear proteins, as for example, by down-regulation of Akt and Bcl-2, up-regulation of p53, Bax and caspase 3, and an increase in number of cell deaths by apoptosis in A375 cells.

Conclusion: Overall results demonstrate anticancer potentials of PD on A375 cells through activation of caspase-mediated signaling and ROS generation.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23506692

Anticancer potential of myricanone, a major bioactive component of Myrica cerifera: novel signaling cascade for accomplishing apoptosis.

Paul A, Das J, Das S, Samadder A, **Khuda-Bukhsh AR**. J Acupunct Meridian Stud. 2013;6: 188-98.

Abstract

Extract of Myrica cerifera bark has long been fruitfully used as a hepato-protective and anti-cancer drug in various complementary and alternative systems of medicine. Myricanone, its principal bioactive compound, had also been reported to have apoptosis-promoting ability. We evaluated its anti-cancer potential in vitro in HepG2 liver cancer cells and tried to understand the signal cascades involved in accomplishing apoptosis. Further, we ascertained by using a (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay (MTT) assay if it had cytotoxic effects on normal noncancerous liver cells (WRL-68).

We deployed various tools and protocols, like phase contrast, scanning electron and fluorescence microscopies, performed an annexinV-FITC/PI assay and cell cycle analysis, and estimated the reactive oxygen species (ROS) generation and mitochondrial membrane depolarization through flow cytometry. Further, analyses of cytochrome-c translocation and of HSP70 and caspase expressions were also done by using immunoblota and Enzyme linked immunosorbent assay (ELISA). Results revealed that myricanone induced apoptosis in HepG2 cells through generation of ROS, depolarization of the mitochondrial membrane, early release of cytochrome-c, down-regulation of HSP70 and activation of a caspase cascade; it had no, or insignificant, cytotoxic effects in WRL-68 cells in vitro and in mice in vivo. Thus, myricanone has great potential for use in formulating an effective drug against both hepatotoxicity and hepatocellular cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23972241

PLGA nano-encapsulation of chelidonine enhances the ameliorative potential against cadmium induced oxidative damage and hepatic injury in mice.

Paul A, Das J, Das S, Samadder A, **Khuda-Bukhsh AR**. Environ Toxicol Pharmacol.. 2013; 36:937-47.

Abstract

This study evaluates the possible protective potentials of chelidonine and its poly lactide-co-glycolide (PLGA) encapsulated nano-form against cadmium chloride (CdCl2) induced oxidative stress and hepatotoxicity in mice, ex vivo and in vivo. Acute exposure to CdCl2 (1.0mg/kg b.w; i.p., twice a week for 30 days) generated oxidative stress in mice through accumulation of reactive oxygen species and increased lipid peroxidation, and levels of certain liver marker enzymes (ALT, AST, ALP) with decrease in levels of GSH and certain other antioxidant enzymes (SOD, CAT, GR) in liver. Treatment with nano-chelidonine for 30 days after CdCl2 intoxication significantly reduced oxidative stress and lipid peroxidation and restored levels of GSH, cholesterol, triglyceride and antioxidant enzymes, showing ameliorative changes in histopathology of liver. Expression pattern of certain inflammatory and apoptotic signal proteins also indicated better hepato-protective abilities of nano-chelidonine, making it a more suitable protective drug than chelidonine against cadmium toxicity in mice.

Article Link: http://bit.ly/1HKlZ0C

Diarylheptanoid-myricanone isolated from ethanolic extract of Myrica cerifera shows anticancer effects on HeLa and PC3 cell lines: signalling pathway and drug-DNA interaction.

Paul A, Das S, Das J, Samadder A, Bishayee K, Sadhukhan R, **Khuda-Bukhsh AR**. J Integr Med. 2013;11:405-15.

Abstract

Objective: To test if myricanone (C21H24O5), a cyclic diarylheptanoid, has anticancer effects on two different cancer cell lines HeLa and PC3. The present study was conducted with a note on the drug-DNA interaction and apoptotic signalling pathway.

Methods: Several studies like cytotoxicity, nuclear damage, annexin-V-fluorescein isothiocyanate (FITC)/propidium iodide (PI)-labelled apoptotic assay and cell cycle arrest, immunoblot and reverse transcriptase-polymerase chain reaction (RT-PCR) were used following standard protocols. Circular dichroism (CD) spectroscopy was also done to evaluate whether myricanone effectively interacted with DNA to bring about conformational changes that could strongly inhibit the cancer cell proliferation.

Results: Myricanone showed a greater cytotoxic effect on PC3 cells than on HeLa cells. Myricanone promoted G0/G1 arrest in HeLa cells and S phase arrest in PC3 cells. Nuclear condensation and annexin V-FITC/PI studies revealed that myricanone promoted apoptotic cell death. CD spectroscopic data indicated that myricanone had an interaction with calf thymus DNA that changed DNA structural conformation. RT-PCR and immunoblot studies revealed that myricanone activated the apoptotic signalling cascades through down-regulation of transcription factors like nuclear factor-κB (NF-κB) (p65), and signal transducers and activators of transcription 3 (STAT3); cell cycle regulators like cyclin D1, and survivin and other signal proteins like Bcl-2 and up-regulation of Bax, caspase-9 and caspase-3.

Conclusion: Myricanone induced apoptosis in both types of cancer cells by triggering caspase activation, and suppression of cell proliferation by down-regulation of NF-κB and STAT3 signalling cascades, which makes it a suitable candidate for possible use in the formulation of therapeutic agent for combating cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24299604

Cytotoxicity and apoptotic signalling cascade induced by chelidonine-loaded PLGA nanoparticles in HepG2 cells in vitro and bioavailability of nano-chelidonine in mice in vivo.

Paul A, Das S, Das J, Samadder A, **Khuda-Bukhsh AR**. Toxicol Lett. 2013; 222:10-22.

Abstract

Poor oral bioavailability of chelidonine, a bio-active ingredient of Chelidonium majus, showing anti-cancer potentials against cancer cells with multidrug resistance, makes its optimal use rather limited. To address this problem, we encapsulated chelidonine in biodegradable poly(lactide-co-glycolide) (PLGA) polymers and evaluated nano-chelidonine's (NCs) anti-cancer efficacy vis-à-vis free chelidonine (FC) against HepG2 cells and also evaluated its bioavailability in mice. Physicochemical characteristics indicated that stable spherical NC were formed in nanometer size range (123±1.15 nm) with good yield (86.34±1.91%), better encapsulation efficiency (82.6±0.574%), negative surface charge (-19.6±2.48 mV) and ability of prolonged and sustained release of chelidonine. Fourier transform infrared analysis revealed that NC resembled similar peaks as that of FC suggesting effective encapsulation in PLGA. NC exhibited rapid cellular uptake and stronger apoptotic effect (~46.6% reduced IC_{50} value) than FC, blocking HepG2 cells at G2/M phase. p53, cyclin-D1, Bax, Bcl-2, cytochrome c, Apaf-1, caspase-9 and caspase-3 expressions also corroborated well to suggest greater anticancer potentials of NC. Our in vivo studies demonstrated NC to be more bio-available than FC and showed a better tissue distribution profile without inducing any toxicity (100 mg/kg bw) in mice. Unlike FC, NC could permeate into brain tissue, indicating thereby NC's better potentials for use in therapeutic oncology.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23850776

Dihydroxy-Isosteviol Methyl Ester from Pulsatilla nigricans Induces Apoptosis in HeLa Cells: Its Cytoxicity and Interaction with Calf Thymus DNA.

Das S, Das J, Samadder A**, Khuda-Bukhsh AR**. Phytother Res. 2013; 27:664-71. doi: 10.1002/ptr.4768.

Abstract

Dihydroxy-isosteviol methyl ester (DIME), the principal biological compound isolated from the medicinal plant Pulsatilla nigricans (Fam: Ranunculaceae) having the molecular formula of C 21 H 34 O 3 (molecular weight 334.25), was administered to cervical cancer cells (HeLa) in vitro to evaluate its possible apoptotic (anti-cancer) potentials. We analyzed the expression of p53, Bax, Bcl2, Apaf, and caspase 3 signal proteins and analyzed the early apoptotic events in HeLa cells induced by DIME using protocols like Annexin V-FITC and PI staining. DIME caused a significant decrease in cell viability, induced nuclear condensation and inter-nucleosomal DNA fragmentation. We further studied the interaction of DIME with calf thymus DNA as target through circular-dichroism spectra. Results showed that DIME interacted with DNA, bringing indiscernible changes in structure and conformation. Thus, DIME showed its capability to induce apoptosis in cancer cells, signifying its utility in drug design as a possible candidate for chemoprevention.

Article Link: http://bit.ly/1eQECZG

Efficacy of PLGA-loaded apigenin nanoparticles in Benzo[a]pyrene and ultraviolet-B induced skin cancer of mice: Mitochondria mediated apoptotic signalling cascades.

Das S, Das J, Samadder A, Paul A, **Khuda-Bukhsh AR.** Food Chem Toxicol. 2013; 62:670-80. doi: 10.1016/j.fct.2013.09.037.

Abstract

Skin cancer is increasing at an alarming rate and becoming resistant to conventional chemotherapy necessitating improved drug delivery system. We loaded apigenin (Ap), a dietary flavonoid having anti-cancer property, with poly (lactic-co-glycolide) (PLGA) nanoparticles (NAp) to explore if nano-encapsulation could enhance anti-carcinogenic effect against ultra-violet B (UVB) and Benzo(a)pyrene (BaP) induced skin tumor and mitochondrial dysfunction in mice. Particle size, morphology and zeta potential of NAp

were determined using dynamic light scattering and atomic force microscopy. Tumor incidence and multiplicity in UVB-BaP induced mice with/without NAp treatment were ascertained and their histolopathological sections and chromosomal aberrations were studied. ROS accumulation and mitochondrial functioning through relevant markers like mitochondrial transmembrane potential were analyzed. Mitochondrial volume changes/swelling, cytochrome c (cyt c) release, mRNA and protein expressions of Apaf-1, bax, bcl-2, cyt c, cleaved caspase-9 and 3 were studied. Results showed that NAp produced better effects than Ap, due to their smaller size, and faster mobility. NAp reduced tissue damage and frequency of chromosomal aberrations, increased ROS accumulation to mediate mitochondrial-apoptosis through modulation of several apoptotic markers and mitochondrial matrix swelling. NAp showed ameliorative potentials in combating skin cancer and therefore has greater prospect of use in therapeutic management of skin cancer.

Keywords: Apigenin; Apoptosis; Benzo[a]pyrene; PLGA-nano-encapsulation; Ultra-violet-B

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24120900

Strategic formulation of apigenin-loaded PLGA nanoparticles for intracellular trafficking, DNA targeting and improved therapeutic effects in skin melanoma in vitro.

Das S, Das J, Samadder A, Paul A, **Khuda-Bukhsh AR.** Toxicol Lett. 2013 Nov 25;223:124-38. doi: 10.1016/j.toxlet.2013.09.012.

Abstract

The aim of the present study was the evaluation of anti-proliferative potentials of apigenin (Ap), (a dietary flavonoid) loaded in poly (lactic-co-glycolide) nanoparticles (NAp) in A375 cells in vitro. NAp was characterized for particle size, morphology, zeta potential, drug release and encapsulation. Cellular entry and intracellular localization of NAp were assessed by transmission electron and confocal microscopies. Circular dichroic spectral analysis and stability curve for Gibb's free energy of dsDNA of A375 cells were also analyzed. DNA fragmentation, intracellular ROS accumulation, superoxide-dismutase activity, intracellular glutathione-reductase content and mitochondrial functioning through relevant markers like mitochondrial transmembrane potential, ATPase activity, ATP/ADP ratio, volume changes/swelling, cytochrome-c release, expressions of Apaf-1, bax, bcl-2, caspase-9, 3, and PARP cleavage were analyzed. NAp produced better effects due to their smaller size, faster mobility and site-specific action. Photostability studies revealed that PLGA encapsulations were efficient at preserving apigenin ultraviolet-light mediated

photodegradation. NAp readily entered cancer cells, could intercalate with dsDNA, inducing conformational change. Corresponding increase in ROS accumulation and depletion of the antioxidant enzyme activities exacerbated DNA damage, mediating apoptosis through mitochondrial dysfunction. Overall results indicate that therapeutic efficacy of NAp may be enhanced by PLGA nanoparticle formulations to have better ameliorative potentials in combating skin melanoma.

Keywords: Apigenin; Apoptosis; Drug–DNA interaction; Mitochondrial dysfunction; PLGA-nano-encapsulation; Skin melanoma

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/24070738

The potentized homeopathic drug, Lycopodium clavatum (5C and 15C) has anti-cancer effect on HeLa cells in vitro.

Samadder A, Das S, Das J, Paul A, Boujedaini N, **Khuda-Bukhsh AR**. J Acupunct Meridian Stud. 2013; 6:180-7.

Abstract

Cancer is a disease that needs a multi-faceted approach from different systems of medicine. The purpose of this study was to evaluate whether homeopathically-potentized ultra-high dilutions of Lycopodium Clavatum (LC-5C and LC-15C, respectively) have any anti-cancer effects on HeLa cells. Cells were exposed to either LC-5C (diluted below Avogadro's limit, i.e., 10(-10)) or LC-15C (diluted beyond Avogadro's limit, i.e., 10(-30)) (drug-treated) or to 30% succussed ethanol ("vehicle" of the drug). The drug-induced modulation in the percent cell viability, the onset of apoptosis, and changes in the expressions of Bax, Bcl2, caspase 3, and Apaf proteins in inter-nucleosomal DNA, in mitochondrial membrane potentials and in the release of cytochrome-c were analyzed by utilizing different experimental protocols. Results revealed that administration of LC-5C and LC-15C had little or no cytotoxic effect in normal peripheral blood mononuclear cells, but caused considerable cell death through apoptosis in cancer (HeLa) cells, which was evident from the induction of DNA fragmentation, the increases in the expressions of protein and mRNA of caspase 3 and Bax, and the decreases in the expressions of Bcl2 and Apaf and in the release of cytochrome-c. Thus, the highly-diluted, dynamized homeopathic remedies LC-5C and LC-15C demonstrated their capabilities to induce apoptosis in cancer cells, signifying their possible use as supportive medicines in cancer therapy.

Keywords: HeLa; Lycopodium (5C and 15C); apoptosis; cancer; homeopathy; signal proteins

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23972240

Potential of the homeopathic remedy, Arnica Montana 30C, to reduce DNA damage in Escherichia coli exposed to ultraviolet irradiation through up-regulation of nucleotide excision repair genes.

Das S, Saha SK, De A, Das D, **Khuda-Bukhsh AR**. Jour of Chinese Integrative Medicine 2012; 10:337-46.

Abstract

Objective: To examine to what degree an ultra-highly diluted homeopathic remedy, Arnica Montana 30C (AM-30C), used in the treatment of shock and injury, can modulate the expression of nucleotide excision repair genes in Escherichia coli exposed to ultraviolet (UV) irradiation.

Methods: E. coli were cultured to their log phase in a standard Luria-Bertani medium and then exposed to sublethal doses of UV irradiation at 25 and 50 J/m(2) for 22.5 and 45 s, respectively. The UV-exposed bacteria were then supplemented with either AM-30C (drug) or placebo (P-30C). The drug-treated and placebo-treated bacteria were subjected to assay for DNA damage and oxidative stress 90 min after UV exposure. Several protocols like comet assay, gel electrophoresis for DNA ladder and intracellular reactive oxygen species (ROS) generation, and biomarker measurement like superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were conducted. The mRNA expressions of the excision repair genes like ultraviolet repair uvrA, B and C genes (or also known as excision repair genes) were estimated by reverse transcription-polymerase chain reaction method.

Results: The UV-exposed bacteria showed DNA damage and oxidative stress, as revealed by an increase in ROS generation, and a decrease in SOD, CAT and GSH activities. As compared to placebo, the AM-30C-treated bacteria showed less DNA damage and oxidative stress as manifested by a decrease in ROS generation, and an increase in SOD, CAT and GSH activities. AM-30C also up-regulated the expression of repair genes as compared to the control.

Conclusion: AM-30C helped repair the DNA damage through up-regulation of repair genes and also ameliorated the oxidative stress through the reduction of ROS generation and suitable modulation of anti-oxidative stress enzymes.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/22409925

Dihydroxy-isosteviol-methyl-ester, an active biological component of Pulsatilla nigricans, reduces arsenic induced cellular dysfunction in testis of male mice.

Samadder A, Das J, Das S, **Khuda-Bukhsh AR**. Environ Toxicol Pharmacol. 2012; 34:743-52. doi: 10.1016/j.etap.2012.09.013.

Abstract

Arsenic contamination has become a menacing health concern, warranting search for new drugs capable of ameliorating its toxicity. Extract of Pulsatilla nigricans is occasionally used as traditional medicine including homeopathy to combat/alleviate toxicity-related symptoms of known or unknown cause. Mice were intoxicated with a sub-lethal dose of sodium arsenite (20mg/kg b.w./day, determined through a range-finding trial) and the effect on testicular toxicity after 30, 60, and 90 days was examined. We observed an increased level of reactive oxygen species, cellular damage in testes of SA-intoxicated mice and further analysed expressions of apoptotic signal proteins and mRNA like Bax, Bcl2 and caspase3. Treatment with EEPN showed significant inhibition/reversal of the arsenic-induced toxic effect in testis and reduced oxidative stress through modulating expressions of signal proteins, thereby inhibiting the progression of events of apoptosis in testis cells and sperm. Therefore, EEPN has potentials for therapeutic use in arsenic- induced reproductive toxicity.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23117066

Potentized homeopathic drug Arsenicum Album 30C inhibits intracellular reactive species generation and up-regulates expression of arsenic resistance gene in arsenic exposed bacteria Escherichia coli

De A, Das D, Dutta S, Chakraborty D, Boujedaini N, **Khuda-Bukhsh A.R.**. Zhong Xi Yi Jie He XueBao. 2012; 10:210-27.

Abstract

Objective: To examine if potentiated homeopathic drug Arsenicum Album 30C (Ars Alb 30C) can reduce sodium arsenite-induced toxicity in Escherichia coli.

Methods: E. coli were exposed to low arsenite insult after they grew up to log phase in standard Luria-Bertani medium. E. coli were treated with 1 or 2 mmol/L sodium arsenite alone (control), or Ars Alb 30C was added to the medium of a subset of sodium arsenite-treated bacteria (drug-treated), or homeopathically agitated alcohol was added to the medium containing a subset of sodium arsenite-treated bacteria (placebo-treated). A sub-set of untreated E. coli served as the negative control. Glucose uptake, specific activities of hexokinase, lipid peroxidase (LPO), superoxide dismutase (SOD) and catalase, intra- and extra-cellular sodium arsenite content, cell growth, cell membrane potential, DNA damage, intracellular reactive oxygen species (ROS), adenosine triphosphate (ATP) and free glutathione content and expressions of arsB and ptsG gene in normal control, sodium arsenite-treated, drug-treated and placebo-treated E. coli were analyzed. Treatments were blinded and randomized.

Results: In sodium arsenite-treated E. coli, glucose uptake, intracellular ROS, LPO and DNA damage increased along with decrease in the specific activities of hexokinase, SOD and catalase, intracellular ATP and free glutathione contents and cell membrane potential and growth, and there were increases in expression levels of arsB gene and ptsG gene. Ars Alb 30C administration reduced arsenic toxicity in E. coli by inhibiting generation of ROS and increasing tolerance to arsenite toxicity and cell growth.

Conclusion: Ars Alb 30C ameliorated arsenic toxicity and DNA damage, validating efficacy of ultra-highly diluted remedies used in homeopathy.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/22313889

Poly(lactic-co-glycolic) acid loaded nano-insulin has greater potentials of combating arsenic induced hyperglycemia in mice: some novel findings

Samadder A, Das J, Das S, De A, Saha SK, Bhattacharyya SS, **Khuda-Bukhsh AR**. Toxicol Appl Pharmacol. 2013 ; 15;267:57-73. doi: 10.1016/j.taap.2012.12.018.

Abstract

Diabetes is a menacing problem, particularly to inhabitants of groundwater arsenic contaminated areas needing new medical approaches. This study examines if PLGA loaded nano-insulin (NIn), administered either intraperitoneally (i.p.) or through oral route, has a greater cost-effective anti-hyperglycemic potential than that of insulin in chronically arsenite-fed hyperglycemic mice. The particle size, morphology and zeta potential of nano-insulin were determined using dynamic light scattering method, scanning electronic and atomic force microscopies. The ability of the nano-insulin (NIn) to cross the blood-brain barrier (BBB) was also checked. Circular dichroic spectroscopic (CD) data of insulin and nano-insulin in presence or absence of arsenic were compared. Several diabetic markers in different groups of experimental and control mice were assessed. The mitochondrial functioning through indices like cytochrome c, pyruvate-kinase, glucokinase, ATP/ADP ratio, mitochondrial membrane potential, cell membrane potential and calcium-ion level was also evaluated. Expressions of the relevant marker proteins and mRNAs like insulin, GLUT2, GLUT4, IRS1, IRS2, UCP2, PI3, PPARγ, CYP1A1, Bcl2, caspase3 and p38 for tracking-down the signaling cascade were also analyzed. Results revealed that i.p.-injected nano-encapsulated-insulin showed better results; NIn, due to its smaller size, faster mobility, site-specific release, could cross BBB and showed positive modulation in mitochondrial signaling cascades and other downstream signaling molecules in reducing arsenic-induced-hyperglycemia. CD data indicated that nano-insulin had less distorted secondary structure as compared with that of insulin in presence of arsenic. Thus, overall analyses revealed that PLGA nano-insulin showed better efficacy in combating arsenite-induced-hyperglycemia than that of insulin and therefore, has greater potentials for use in nano-encapsulated form.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23276653

Homeopathic mother tincture of Conium initiates reactive oxygen species mediated DNA damage and makes HeLa cells prone to apoptosis.

Bishayee K, Mukherjee A, Paul A, **Khuda-Bukhsh AR**. TANG: *International Journal of Genuine Traditional Medicine*, 2012, 2: e-26. DOI: 10.5667/tang.2012.0018

Adverse side-effects and lack of scientific validation of some chemotherapeutic agents prevent the use of many traditional medicines claimed to have anti-cancer effects. Ethanolic extract of Conium maculatum has long been used in traditional and alternative systems of medicine including homeopathy for the treatment of glandular enlargements, cancerous tumours or hard lumps of testicles, prostate, ovaries, breasts and/ or uterus, particularly in the breast. However, if and how it acts still remains scientifically unknown. This study aims to test if Conium extract (CE), used as mother tincture of Conium in homeopathy, has demonstrable anti-cancer potentials without having much cytotoxicity in normal cells. Cytotoxicity of the drug was tested by conducting MTT assay on both normal (peripheral blood mononuclear cells) and HeLa cells. We also evaluated DNA fragmentation and DNA damage by DAPI and diphenylamine assay. The LDH activity assay was done to evaluate the percentages of apoptosis and necrosis. ROS accumulation also was evaluated to pin-point the actual events of apoptosis. Administration of drug clearly demonstrated its anti-cancer potentials as evidenced by the DNA damage analysis. The ROS activity also increased in case of the CE treated cells. LDH data revealed that the mode of cell death was mainly apoptotic and not necrotic. CE appears to induce apoptosis of cancer cells through ROS mediated pathway, and has negligible cytotoxicity against normal cells.

Article Link:
www.koreascience.or.kr/article/ArticleFullRecord.jsp?cn=TJHOBI_2012_v2n3_26.1

Ameliorative effects of Syzygium jambolanum extract and its poly(lactic-co-glycolic) acid nano-encapsulated form on arsenic induced hypoglycemic stress: A multiparametric evaluation

Samadder A, Das S, Das J, Paul A, **Khuda-Bukhsh AR**. *Journal of Acupncture and Meridian Studies* 2012; 5:310-8.

Abstract

In South East Asia, groundwater arsenic contamination has become a great menace. Chronic arsenic intoxication leads to a hyperglycemic condition in animals and man. Because of undesirable side-effects and affordability, orthodox medicine, like insulin, is not

preferred by many who like natural products instead. Unfortunately, such natural products mostly lack scientific validation. Therefore, we became interested in assessing the efficacy of the ethanolic seed extract of Syzygium jambolanum (SJ), traditionally used against diabetic conditions. We also formulated poly (lactic-co-glycolic) acid (PLGA)-encapsulated nano-SJ (NSJ) and tested whether the ameliorative potentials of SJ could be enhanced by nano-encapsulation. In this study, we conducted both in vitro (in L6 cells) and in vivo (in mice) experiments to assess the relative efficacy of SJ and NSJ. We characterized the physico-chemical features of NSJ by atomic force microscopy and critically analyzed several bio-markers and signal proteins associated with arsenic-induced stress and hyperglycemia. We also determined the relative ameliorative potentials of SJ and NSJ by using standard protocols. NSJ could cross the blood brain barrier in mice. Overall results suggested that NSJ had a greater potential than that of SJ, indicating the possibility of using NSJ in the future drug design and management of arsenic-induced hyperglycemia and stress.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23265083

Rapid green synthesis of silver nanoparticles from silver nitrate by a homeopathic mother tincture Phytolacca Decandra

Bhattacharyya SS, Das J, Das S, Samadder A, Das D, De A, Paul S, Khuda-Bukhsh AR. *Jour of Chinese Integrative Medicine*. 2012; 10:546-54.

Abstract

Objective: To examine if a homeopathic mother tincture (Phytolacca Decandra) is capable of precipitating silver nanoparticles from silver nitrate (AgNO(3)) and to characterize the biosynthesized nanoparticles for evaluating their biological activities.

Methods: A total of 100 mg of AgNO(3) was added to 20mL of Milli-Q water and stirred vigorously. Then 5mL of the homeopathic mother tincture of Phytolacca Decandra (ethanolic root extract of Phytolacca decandra) was added and stirred continuously. Reduction took place rapidly at 300K and completed in 10 min as shown by stable light greenish-yellow color of the solution which gave colloid of silver nanoparticles. The colloid solution was then centrifuged at 5000×g to separate the nanoparticles for further use. The nanoparticles were characterized by spectroscopic analysis, particle size analysis and zeta potential measurements, and morphology was analyzed by atomic force microscopy. The drug-DNA interaction was determined by circular dichroism spectrophotometry and melting temperature profiles by using calf thymus DNA as the target. The biological activities were determined using a cancer cell line A549 in vitro and using bacteria Escherichia coli and fungus Saccharomyces cerevisiae as test models.

Results: Phytolacca Decandra precipitated silver nanoparticles in ambient conditions. The nanoparticles had 91 nm particle size, with polydispersity index of 0.119 and zeta potential of -15.6 mV. The silver nanoparticles showed anticancer and antibacterial properties, but no clear antifungal properties.

Conclusion: This could be a novel environment-friendly method to biosynthesize silver nanoparticles using a cost-effective, nontoxic manner. The homeopathic mother tincture may utilize this property of nano-precipitation in curing diseases or disease symptoms.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/22587977

Two homeopathic remedies used intermittently provide additional protective effects against hepatotoxicity induced by carcinogens in mice

Bhattacharjee N, **Khuda-Bukhsh AR**. *Journal of Acupuncture and Meridian Studies*, 2012; 5:166-75. Doi:10.1016/j.jams.2012.05.004

Abstract

The purpose of the study was to evaluate whether potentized cholesterinum (Chol) intermittently used with another homeopathic remedy, Natrum Sulphuricum (Nat Sulph) can provide additional benefits in combating hepatotoxicity generated by chronic feeding of carcinogens, p-dimethylaminoazobenzene (p-DAB), and phenobarbital (PB). Mice were categorized into subgroups: normal untreated (Gr-1); normal + alcohol "vehicle" (Alc) (Gr-2), 0.06% p-DAB +0.05% PB (Gr-3), p-DAB+PB+Alc (Gr-4), p-DAB+PB+Nat Sulph-30 (Gr-5), p-DAB+PB+Chol-200 (Gr-6), p-DAB+PB+Nat Sulph-30+Chol-200 (Gr-7), p-DAB+PB+Nat Sulph-200 (Gr-8), and DAB+PB+Nat Sulph-200+Chol-200 (Gr-9). Hepatotoxicity was assessed through biomarkers like aspartate and alanine aminotransferases (AST and ALT), acid and alkaline phosphatases (AcP and AlkP), reduced glutathione content (GSH), glucose 6-phosphate dehydrogenase (G6PD), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), and analysis of lipid peroxidation (LPO) at 30, 60, 90, and 120 days and antioxidant biomarkers like superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) were assayed. Electron microscopic studies (scanning and transmission) and gelatin zymography for matrix metalloproteinases were conducted in liver. The feeding of the homeopathic drugs showed intervention in regard to the increased activities of AST, ALT, AcP, AlkP, GGT, LDH, and LPO and decreased activities of G6PD, SOD, CAT, GR, and GSH noted in the intoxicated mice, more appreciable in Groups 7 and 9. Thus, combined therapy provided additional antihepatotoxic and anticancer effects.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/22898065

Poly(lactide-co-glycolide) encapsulated extract of Phytolacca decandra demonstrates better intervention against induced lung adenocarcinoma in mice and on A549 cells

Das J, Das S, Samadder A, Bhadra K, **Khuda-Bukhsh AR.** Eur J Pharm Sci. 2012 29; 47:313-24. doi: 10.1016/j.ejps.2012.06.018

Abstract

We tested relative efficacy of the extract of Phytolacca decandra (PD) and its PLGA nano-encapsulated form (NPD) in mice intoxicated with benzo[a]pyrene (BaP) (25 mg/kg b.w.) plus sodium-arsenite (SA) (10 mg/kg b.w.) and on A549 lung cancer cells in vitro. We characterized nanoparticles by physico-chemical and morphological studies using dynamic light scattering, scanning electron and atomic force microscopies. We also conducted FTIR and (1)H NMR studies to determine if NPD had a co-polymeric nature and analyzed drug-DNA interaction through circular dichroism spectra (CD) and melting temperature profiles (T(m)) taking calf thymus DNA as target. An oral dose of 0.3mg/kg b.w. for NPD and 30 mg/kg b.w. for PD in mice showed chemopreventive effects in regard to DNA fragmentation, comet tail length and toxicity biomarkers like ROS generation, NFκβ, p53, PARP, CYP1A1 and caspase 3. NPD showed greater effects than that by PD. Results of in vivo studies showed similar effects on A549 in regard to cell viability, DAPI and PI staining, Comet tail length, DNA fragmentation. To further confirm the biological molecule present in PD we analyzed its chromatographic fraction through mass spectroscopy, NMR and FT-IR studies and characterized it to be a tri-terpenoid, a derivative of betulinic acid with a molecular formula C(30)H(46)O(2.) Thus, overall results suggest that nano-encapsulation of PD (NPD) increases drug bioavailability and thereby has a better chemo-preventive action against lung cancer in vivo and on A549 cells in vitro than that of PD.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/22771545

Dihydroxy-isosteviol methyl ester of Pulsatilla nigricans extract reduces arsenic-induced DNA damage in testis cells of male mice: its toxicity, drug-DNA interaction and signaling cascades

Samadder A, Das J, Das S, Das D, De A, Bhadra K, **Khuda-Bukhsh AR**. *Jou of Chinese Intgr Medicine*, 2012;10:1433-42. doi: 10.3736/jcim20121216.

Abstract

Objective: To evaluate the ameliorative efficacy of dihydroxy-isosteviol methyl ester (DIME) of Pulsatilla nigricans extract in arsenic-induced DNA damage in testis cells of mice.

Methods: The mice were treated with sodium arsenite (SA) solution intragastrically at a dose of 20 mg/kg per day and examined at 30, 60, and 90 d after treatment. We divided SA-intoxicated mice into two sub-groups: one fed with DIME at a dose of 35 mg/kg and the other with 85% alcohol. We analyzed the expressions of apoptotic signal proteins like CYP1A1, p53 and caspase 3, ascertained the level of cellular and DNA damage and estimated the level of testicular-toxicity biomarkers. We studied the interaction of DIME with calf thymus DNA as target through circular dichroism spectra and melting temperature profiles.

Results: We observed an elevation in all apoptotic and toxicity biomarkers leading to cellular and DNA damage in the SA-intoxicated mice which showed significant inhibition or reversal on administration of DIME. Results also showed that DIME interacted with DNA, bringing in discernible changes in structure and conformation.

Conclusion: DIME has potentials for therapeutic use in amelioration of arsenic-induced reproductive toxicity.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23257138

Chelidonine isolated from ethanolic extract of Chelidonium majus promotes apoptosis in HeLa cells through p38-p53 and PI3K/AKT signalling pathways

Paul A, Bishayee K, Ghosh S, Mukherjee A, Sikdar S, Chakraborty D, Boujedaini N, **Khuda-Bukhsh AR**. *Zhong Xi Yi Jie He Xue Bao.* 2012 Sep;10(9):1025-38.

Abstract

Objective: To evaluate the role of chelidonine isolated from ethanolic extract of Chelidonium majus in inducing apoptosis in HeLa cells and to assess the main signalling pathways involved.

Methods: Cells were initially treated with different concentrations of chelidonine for 48 h and the median lethal dose (LD50) value was selected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Morphological analysis of nuclear condensation and DNA damage and fragmentation were measured by 4',6-diamidino-2-phenylindole staining and comet assay. Further, reactive oxygen species (ROS) generation, cell cycle arrest and change in mitochondrial membrane potential were also examined and analyzed by flow cytometry. Evaluation of interaction of drug with CT DNA was investigated by circular dichroism (CD) spectral analysis to find any possible drug-CT DNA interaction. The mRNA and protein expressions of major signal proteins like p38, p53, protein kinase B (AKT), phosphatidylinositol 3-kinases (PI3K), Janus kinase 3 (JAK3), signal transducer and activator of transcription 3 (STAT3) and E6 and E7 oncoproteins as well as the pro-apoptotic genes and antiapoptotic genes were also estimated by reverse transcriptase-polymerase chain reaction and Western blotting.

Results: Based on LD(50) value (30 μg/mL) of chelidonine, three doses were selected, namely, 22.5 μg/mL (D1), 30.0 μg/mL (D2) and 37.5 μg/mL (D3). Results showed that chelidonine inhibited proliferation and induced apoptosis in HeLa cells through generation of ROS, cell cycle arrest at sub-G1 and G0/G1 stage, change in mitochondrial membrane potential and fragmentation of DNA. Results of CD spectra showed effective interaction between chelidonine and calf thymus DNA. Studies of signalling pathway revealed that chelidonine could efficiently induce apoptosis through up-regulation of expressions of p38, p53 and other pro-apoptotic genes and down-regulation of expressions of AKT, PI3K, JAK3, STAT3, E6, E7 and other antiapoptotic genes.

Conclusion: Chelidonine isolated from Chelidonium majus efficiently induced apoptosis in HeLa cells through possible alteration of p38-p53 and AKT/PI3 kinase signalling pathways.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/22979935

Phenotypic evidence of ultra-highly diluted homeopathic remedies to act at gene expression level: a novel probe on experimental phage infectivity in bacteria

Saha SK, Das S, **Khuda-Bukhsh AR**. *Jour of Chinese Integrative Medicine* 2012;10: 462-70

Abstract

Objective: To explore if some ultra-highly diluted homeopathic remedies claimed to have antiviral effects can demonstrate any discernible action in the bacteria Escherichia coli through modulating infectivity potentials of the bacteriophage ΦX174 DNA.

Methods: ΦX174 was selected because of its known host specificity to E. coli and its constitutive expression of lytic gene E when inside the bacterial host. We deployed the "bacteriophage assay system" by "top layer agar plating" method of plaque-counting for evaluation of efficacy of the homeopathic remedies in rendering the bacteria's protective ability against the attack of ΦX174. The plaque number in the agar-plated Petri dishes, either containing the phage-bacteria mixture subjected to one of the diluted homeopathic drugs under test (1% volume ratio; Belladonna 30C, Rhus Tox 30C, Arnica 30C) or the succussed 1% "alcoholic vehicle" of the drug was recorded. The plaques represented the bacterial colony actually infected and lysed by ΦX174. Conversely, we subjected ΦX174 to the homeopathic drug treatment before allowing them to interact with the bacteria to ascertain if the drug itself had any direct effect on the infective potential of the phage DNA entering into the bacterial cell.

Results: Each homeopathic remedy showed a significant decrease in plaque number on pretreated bacteria (1 h prior to infection) with respect to untreated and placebo-treated controls; there was only an insignificant change in the plaque number when ΦX174 was pretreated with the drugs. As ΦX174 starts lytic cycle when inside the bacterial cell, the loss of plaque number would mean that either the lytic gene E in many was repressed or the entire phage DNA was annihilated by the bacterial gene product (restriction enzymes) known to be regulated by a cluster of genes.

Conclusion: This provides phenotypic evidence for the ability of ultra-highly diluted homeopathic remedies to regulate expression of certain gene(s) depending on need of the organism.

Article Link:
http://www.jcimjournal.com/jim/showAbstrPage.aspx?articleID=jcim20120416

Possible signaling cascades involved in attenuation of alloxan-induced oxidative stress and hyperglycemia in mice by ethanolic extract of Syzygium jambolanum: drug-DNA interaction with calf thymus DNA as target

Samadder A, Chakraborty D, De A, Bhattacharyya SS, Bhadra K, **Khuda-Bukhsh AR.** Eur J Pharm Sci. 2011; 9;44:207-217. doi: 10.1016/j.ejps.2011.07.012.

Abstract

We injected alloxan (100 mg/kg b.w.) in mice (Mus musculus) intra-peritoneally to induce hyperglycemia and divided the hyperglycemic mice into two sub-groups: one was fed ethanolic extract of Syzygium jambolanum (EESJ) (20 mg/kg b.w. for 8 weeks) and the other 85% ethyl alcohol ("vehicle"-control). Chromatographic and mass spectroscopic studies of EESJ revealed two principal components, one corresponding to an iridoid glycoside. We estimated blood glucose, glycosylated hemoglobin, glucokinase, and fructosamine and analyzed the expression of marker proteins like insulin, GLUT2, and GLUT4. We also studied anti-oxidant biomarkers like lipid peroxidase, superoxide dismutase, total thiole and catalase. We assayed generation of reactive oxygen species (ROS) and several inflammatory and apoptotic signal proteins like NFkB, IFNγ, iNOS, Bcl(2,) Bax, STAT1 and Caspase3. We further evaluated the effects of hyperglycemia on DNA through comet assay and DNA fragmentation study and assessed drug-DNA interaction by comparative analysis of circular dichroism (CD) spectral data and melting temperature profiles (T(m)) of calf thymus DNA treated with or without EESJ. We observed an elevation of all biomarkers for oxidative stress, generation of ROS and activation of NFkB and down regulation in expression of insulin, GLUT2 and glucokinase in hyperglycemic mice. Administration of EESJ reversed these changes. Histo-pathological observations of pancreas, liver and kidney also revealed relevant changes. Data of CD and (T(m)) indicated an interaction of EESJ with calf thymus DNA, indicating change in structure and conformation. Thus, EESJ has anti-oxidant as well as anti-hyperglycemic activities in diabetic mice, and potentially useful in management of hyperglycemia.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/21839831

Anticancer Potentials of Root Extract of Polygala senega and Its PLGA Nanoparticles-Encapsulated Form

Paul S, Bhattacharyya SS, Boujedaini N, **Khuda-Bukhsh AR**. *Evid Based Complement Alternat Med.* 2011; 2011. pii: 517204. doi: 10.1155/2011/517204

Abstract

Ethanolic extract of Polygala senega (EEPS) had little or no cytotoxic effects on normal lung cells, but caused cell death and apoptosis to lung cancer cell line A549. In the present paper, ethanolic root extract of P. senega (EEPS) was nanoencapsulated (size: 147.7 nm) by deploying a biodegradable poly-(lactic-co-glycolic) acid (PLGA). The small size of the NEEPS resulted in an enhanced cellular entry and greater bioavailability. The growth of cancer cells was inhibited better by NEEPS than EEPS. Both EEPS and NEEPS induced apoptosis of A549 cells, which was associated with decreased expression of survivin, PCNA mRNA, and increased expression of caspase-3, p53 mRNAs of A549 cells. The results show that the anticancer potential of the formulation of EEPS-loaded PLGA nanoparticles was more effective than EEPS per se, probably due to more aqueous dispersion after nanoencapsulation. Therefore, nanoencapsulated ethanolic root extract of P. senega may serve as a potential chemopreventive agent against lung cancer.

Article Link: http://www.hindawi.com/journals/ecam/2011/517204/

An initial report on the efficacy of a millesmal potency Arsenicum Album LM 0/3 in ameliorating arsenic toxicity in humans living in a high risk arsenic village.

Khuda-Bukhsh AR, Banerjee A, Biswas SJ, Roy Karmakar S, Banerjee P, Pathak S, Guha B, NaoualB. Zhong Xi Yi Jie He Xue Bao. 2011;9:596-604

Abstract

Background: Millions of people are at risk of groundwater arsenic contamination, and there is no known remedy that can effectively remove the symptoms of prolonged arsenic poisoning. A potentized homeopathic drug, Arsenicum Album LM 0/3 (Ars Alb LM 0/3), is claimed in homeopathic literature to have the ability to treat symptoms similar to that of arsenic poisoning.

Objective: This study examines whether Ars Alb LM 0/3 could provide some degree of amelioration for the victims living in an arsenic-affected village where no arsenic-free drinking water is available.

Design, setting, participants and interventions: This study was carried out on volunteers living in an arsenic-affected village where no arsenic-free drinking water is available. Twenty-eight volunteers from the village of Dasdiya, in Haringhata block under Nadia District, West Bengal, India, an arsenic-contaminated village where wells contain 55 to 95 µg/L arsenic, were selected to undertake a double-blind and placebo-controlled trial. The subjects provided samples of blood and urine before and after 2 months of taking either "verum" or "placebo". Another 18 subjects living in an arsenic-free village, served as the negative controls.

Main Outcome Measures: Samples of blood and urine from the subjects were assayed for arsenic content, according to various toxicity biomarkers and pathophysiological parameters.

Results: Out of the original 28 subjects, only 14 subjects provided samples while the other 14 dropped out. There were elevated levels of arsenic in the blood and urine, alkaline and acid phosphatases, lipid peroxidation, and glutathione activities and increased blood glucose, triacylglycerol, cholesterol, and low-density lipoprotein cholesterol contents, whereas there were decreased levels of aspartate and alanine aminotransferases, gamma glutamyl transferase, glucose-6-phosphate dehydrogenase contents, high-density lipoprotein cholesterol and packed cell volume in the subjects. After 2 months of homeopathic remedy administration, the verum-fed subjects showed positive modulations within these parameters with slight lowering of matrix metalloproteinase activity as compared with the placebo group.

Conclusion: Ars Alb LM 0/3 shows potential for use in high-risk arsenic villages as an interim treatment for amelioration of arsenic toxicity until more extensive medical treatment and facilities can be provided to the numerous victims of arsenic poisoning.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/21669162

Poly (lactide-co-glycolide) acid nanoencapsulation of a synthetic coumarin: Cytotoxicity and bio-distribution in mice, in cancer cell line and interaction with calf thymus DNA as target

Bhattacharyya SS, Paul S, De A, Das D. Samadder A, Boujedai N, **Khuda-Bukhsh AR.** *Toxicol Appl Pharmacol.* 2011; 253:270-81. doi: 10.1016/j.taap.2011.04.010.

Abstract

Several naturally occurring coumarin compounds, including scopoletin (7 hydroxy-6 methoxycoumarin), of plant origin have been reported to have anti-cancer potentials. A related but chemically synthesized coumarin, 4-methyl-7-hydroxy coumarin (SC), was also shown to have similar anti-cancer potentials. In the present study, to test if nano-encapsulated SC could be a more potent anti-cancer agent, we encapsulated SC with poly lactide-co-glycolide acid (PLGA) nanoparticles (Nano Coumarin; NC) and tested its potentials with a variety of protocols. NC demonstrated greater efficiency of drug uptake and showed anti-cancer potentials in melanoma cell line A375, as revealed from scanning electronic and atomic force microscopies. To test its possible interaction with target DNA, the combined data of circular dichroism spectra (CD) and melting temperature profile (T(m)) of calf thymus DNA treated with NC were analyzed. Results indicated a concentration dependent interaction of NC with calf thymus DNA, bringing in effective change in structure and conformation, and forming a new complex that increased its stability. Particle size and morphology of NC determined through polydispersity index and zeta potential using dynamic light scattering qualified NC to be a more potent anti-cancer agent than SC. Further, SC and NC showed negligible cytotoxic effects on normal skin cells and peripheral blood mononuclear cells of mice. Distribution assay of PLGA nanoparticles in different tissues like brain, heart, kidneys, liver, lungs, and spleen in mice revealed the presence of nanoparticles in different tissues including brain, indicating that the particles could cross the blood brain barrier, significant information for drug design.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/21549736

Modulation of signal proteins: a plausible mechanism to explain how a potentized drug Secale Cor 30C diluted beyond Avogadro's limit combats skin papilloma in mice

Khuda-Bukhsh AR, Bhattacharyya SS, Paul S, Dutta S, Boujedaini N, Belon P. *eCAM*, 2011; 2011:286320. doi: 10.1093/ecam/nep084

Abstract

In homeopathy, ability of ultra-high diluted drugs at or above potency 12C (diluted beyond Avogadro's limit) in ameliorating/curing various diseases is often questioned, particularly because the mechanism of action is not precisely known. We tested the hypothesis if suitable modulations of signal proteins could be one of the possible pathways of action of a highly diluted homeopathic drug, Secale cornutum 30C (diluted 1060 times; Sec cor 30). It could successfully combat DMBA + croton oil-induced skin papilloma in mice as evidenced by histological, cytogenetical, immunofluorescence, ELISA and immunoblot findings. Critical analysis of several signal proteins like AhR, PCNA, Akt, Bcl-2, Bcl-xL, NF-B and IL-6 and of pro-apoptotic proteins like cytochrome c, Bax, Bad, Apaf, caspase-3 and -9 revealed that Sec cor 30 suitably modulated their expression levels along with amelioration of skin papilloma. FACS data also suggested an increase of cell population at S and G2 phases and decrease in sub-G1 and G1 phages in carcinogen-treated drug-unfed mice, but these were found to be near normal in the Sec cor 30-fed mice. There was reduction in genotoxic and DNA damages in bone marrow cells of Sec Cor 30-fed mice, as revealed from cytogenetic and Comet assays. Changes in histological features of skin papilloma were noted. Immunofluorescence studies of AhR and PCNA also suggested reduced expression of these proteins in Sec cor 30-fed mice, thereby showing its anti-cancer potentials against skin papilloma. Furthermore, this study also supports the hypothesis that potentized homeopathic drugs act at gene regulatory level.

Article Link: http://www.hindawi.com/journals/ecam/2011/286320/

Analysis of the capability of ultra-highly diluted glucose to increase glucose uptake in arsenite-stressed bacteria Escherichia coli

Khuda-Bukhsh AR, De A, Das D, Dutta S, Boujedaini N. *Zhong Xi Yi Jie He Xue Bao.* 2011 Aug;9(8):901-12.

Abstract

Objective: Whether ultra-highly diluted homeopathic remedies can affect living systems is questionable. Therefore, this study sees value in the analysis of whether homeopathically diluted glucose 30C has any effect on Escherichia coli exposed to arsenite stress.

Methods: E. coli were cultured to their log phase in standard Luria-Bertani medium and then treated with either 1 mmol/L or 2 mmol/L sodium arsenite, with or without supplementation of either 1% or 3% glucose, an ultra-highly diluted and agitated ethanolic solution (70%) of glucose (diluted 10(60) times), glucose 30C or 70% ethanol (placebo) in the medium. Glucose uptake, specific activities of hexokinase and glucokinase, membrane potential, intracellular adenosine triphosphate (ATP) and expression of glucose permease in E. coli were analyzed at two different time intervals. Arsenic content in E. coli (intracellular) and in the spent medium (extracellular) was also determined.

Results: In arsenite-exposed E. coli, the glucose uptake increased along with decreases in the specific activities of hexokinase and glucokinase, intracellular ATP and membrane potential and an increase in the gene expression level of glucose permease. Glucose uptake increased further by addition of 1%, 3% or ultra-highly diluted glucose in the medium, but not by the placebo.

Conclusion: The results demonstrated the efficacy of the ultra-highly diluted and agitated glucose in mimicking the action of actual glucose supplementation and its ability to modulate expressions of hexokinase and glucokinase enzymes and glucose permease genes, thereby validating the efficacy of ultra-high dilutions used in homeopathy.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/21849152

Potentized homeopathic drug Arsenicum Album 30C positively modulates protein biomarkers and gene expressions in Saccharomyces cerevisae exposed to arsenate

Das D, De A, Dutta S, Biswas R, Boujedaini N, **Khuda-Bukhsh AR**. *Jour of Chinese Integrative Medicine.* 2011; 9:752-60.

Abstract

Objective: This study examines if homeopathic drug Arsenicum Album 30C (Ars Alb 30C) can elicit ameliorative responses in yeast (Saccharomyces cerevisiae) exposed to arsenate.

Methods: The yeast S. cerevisiae 699 was cultured in a standard yeast extract peptone dextrose broth medium. It was exposed to the final concentration of 0.15 mmol/L arsenate for two intervals, 1 h and 2 h, respectively. The cell viability was determined along with the assessment of several toxicity biomarkers such as catalase (CAT), superoxide dismutase (SOD), total thiol (GSH) and glucose-6-phosphate dehydrogenase (G6PDH), lipid peroxidation, protein carbonylation and DNA damage. Reactive oxygen species (ROS) accumulation, expressions of relevant stress transcription activators like Yap-1 and Msn 2, and mRNA expression of yeast caspase-1 (Yca-1) were also measured.

Results: Treatment of arsenate increased lipid peroxidation, protein carbonylation, DNA damage, ROS accumulation and expressions of Yap-1, Msn 2 and Yca-1 and decreased GSH, G6PDH, CAT and SOD. Ars Alb 30C administration decreased lipid peroxidation, protein carbonylation, DNA damage, ROS formation and Msn 2 and Yca-1 expressions and increased cell viability, GSH, G6PDH, CAT and SOD significantly ($P<0.05$), except for a slight increase in Yap-1 expression.

Conclusion: Ars Alb 30C triggers ameliorative responses in S. cerevisiae exposed to arsenate.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/21749826

Thujone rich fraction of Thuja occidentalis demonstrates major anti-cancer potentials: Evidences from in vitro studies on A375 cells

Biswas R, Mandal SK, Dutta S, Bhattacharyya SS, Boujedani N, **Khuda-Bukhsh AR.** eCAM. 2011; DOI: 10.1093/ecam/neq042.

Abstract

CRUDE ETHANOLIC EXTRACT OF THUJA OCCIDENTALIS (FAM: Cupressaceae) is used as homeopathic mother tincture (TOΦ) to treat various ailments, particularly moles and tumors, and also used in various other systems of traditional medicine. Anti-proliferative and apoptosis-inducing properties of TOΦ and the thujone-rich fraction (TRF) separated from it have been evaluated for their possible anti-cancer potentials in the malignant melanoma cell line A375. On initial trial by S-diphenyltetrazolium bromide assay, both TOΦ and TRF showed maximum cytotoxic effect on A375 cell line while the other three principal fractions separated by chromatography had negligible or no such effect, because of which only TRF was further characterized and subjected to certain other assays for determining its precise anti-proliferative and apoptotic potentials. TRF was reported to have a molecular formula of C(10)H(16)O with a molecular weight of 152. Exposure of TRF of Thuja occidentalis to A375 cells in vitro showed more cytotoxic, anti-proliferative and apoptotic effects as compared with TOΦ, but had minimal growth inhibitory responses when exposed to normal cells (peripheral blood mononuclear cell). Furthermore, both TOΦ and TRF also caused a significant decrease in cell viability, induced inter-nucleosomal DNA fragmentation, mitochondrial transmembrane potential collapse, increase in ROS generation, and release of cytochrome c and caspase-3 activation, all of which are closely related to the induction of apoptosis in A375 cells. Thus, TRF showed and matched all the anti-cancer responses of TOΦ and could be the main bio-active fraction. The use of TOΦ in traditional medicines against tumors has, therefore, a scientific basis.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/21647317

Can Homeopathy Bring Additional Benefits to Thalassemic Patients on Hydroxyurea Therapy? Encouraging Results of a Preliminary Study

Banerjee A, Chakrabarty SB, Karmakar SR, Chakrabarty A, Biswas SJ, Haque S, Das D, Paul S, Mandal B, Naoual B, Belon P, **Khuda-Bukhsh AR**. *Evidence-Based Complementary and Alternative Medicine*. 2010; 7:129-36. doi: 10.1093/ecam/nem161

Abstract

Several homeopathic remedies, namely, Pulsatilla Nigricans (30th potency), Ceanothus Americanus (both mother tincture and 6th potency) and Ferrum Metallicum (30th potency) selected as per similia principles were administered to 38 thalassemic patients receiving Hydroxyurea (HU) therapy for a varying period of time. Levels of serum ferritin (SF), fetal hemoglobin (HbF), hemoglobin (Hb), platelet count (PC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), white blood cell (WBC) count, bilirubin content, alanine amino transferase (ALT), aspartate amino transferase (AST) and serum total protein content of patients were determined before and 3 months after administration of the homeopathic remedies in combination with HU to evaluate additional benefits, if any, derived by the homeopathic remedies, by comparing the data with those of 38 subjects receiving only HU therapy. Preliminary results indicated that there was a significant decrease in the SF and increase in HbF levels in the combined, treated subjects. Although the changes in other parameters were not so significant, there was a significant decrease in size of spleen in most patients with spleenomegaly and improvement in general health conditions along with an increased gap between transfusions in most patients receiving the combined homeopathic treatment. The homeopathic remedies being inexpensive and without any known side-effects seem to have great potentials in bringing additional benefits to thalassemic patients; particularly in the developing world where blood transfusions suffer from inadequate screening and fall short of the stringent safety standards followed in the developed countries. Further independent studies are encouraged.

Keywords: Ceanothus Americanus, ferritin, Ferrum Metallicum, fetal hemoglobin, homeopathic remedy, Hydroxyurea, Pulsatilla Nigricans, Thalassemia

Article Link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2816384/

Chelidonium majus 30C and 200C in induced hepato-toxicity in rats

Banerjee A, Pathak S, Biswas SJ, Roy-Karmakar S, Boujedaini N, Belon P, **Khuda-Bukhsh AR**. *Homeopathy* 2010; 99:167-76. doi: 10.1016/j.homp.2010.05.008.

Abstract

Introduction: Homeopathy is a popular form of complementary and alternative medicine and is used to treat for certain liver ailments.

Aim: To analyze the efficacy of homeopathic Chelidonium majus (Chel) 30C and 200C in amelioration of experimentally induced hepato-toxicity in rats.

Methods: Rats were randomized into six sub-groups: negative control; negative control+EtOH; positive control; positive control+EtOH group; Chel 30; Chel 200. Rats were sacrificed at day 30, 60, 90 and 120; various toxicity biomarkers and pathological parameters were evaluated. Gelatin zymography for determination of metalloproteinases activity and Western blot of p53 and Bcl-2 proteins were also employed. All analyses were observer blind.

Results: Chronic feeding of p-dimethyl amino azo benzene (p-DAB) and phenobarbital (PB) elevated the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), triglyceride, cholesterol, creatinine and bilirubin and lowered the levels of glutathione (GSH), glucose-6-phosphate dehydrogenase (G-6-PD), catalase and HDL-cholesterol. There were statistically significant modulations of these parameters in the treated animals, compared to positive controls. In both treated groups, there was downregulation of metalloproteinases, p53 and Bcl-2 proteins compared to over-expression in the positive control groups.

Conclusion: Both the potencies of Chel exhibited anti-tumor and anti-oxidative stress potential against artificially induced hepatic tumors and hepato-toxicity in rats. More studies are warranted.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/20674840

Anti-oncogenic potentials of a plant coumarin (7-hydroxy coumarin) against DMBA induced skin papilloma in mice: The possible role of several key signal proteins

Bhattacharyya SS, Paul S, Dutta S, Boujedani N, **Khuda-Bukhsh AR.** Jour of Chinese Integrative Medicine 2010; 8:645-54.

Abstract

Objective: Anti-cancer potentials of scopoletin (7-hydroxy-6-methoxy coumarin) separated from plant extract (Gelsemium sempervirens) were demonstrated earlier from our in vitro studies. In the present study, its in vivo effects have been evaluated in mice.

Methods: Mice were chronically administered 7,12-dimethylbenz [a] anthracene (DMBA) once a week and croton oil twice a week on their back, which resulted in the development of fully grown finger-like projections (papilloma) after 24 weeks. Two subgroups of mice (drug-treated) were treated with two doses of scopoletin (50 mg and 100 mg/kg body weight) respectively while control received 2% ethyl alcohol (the "vehicle" of scopoletin). After the 24-week drug administration, expressions of several key receptors such as aryl hydrocarbon receptor (AhR) and signal proteins like p53, cytochrome P450 1A1 (CYP1A1), proliferating cell nuclear antigen (PCNA), signal transducer and activator of transcription-3 (Stat-3), survivin, matrix metalloproteinase-2 (MMP-2), cyclin D1, c-myc, tissue inhibitor of matrix metalloproteinase-2 (TIMP-2) and caspase-3, and some anti-oxidant markers were studied. Lipid peroxidation, superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase in supernatant were also detected.

Results: Carcinogens induced toxicity, and over-expression of AhR, CYP1A1, PCNA, Stat-3, survivin, MMP-2, cyclin D1 and c-myc and down-regulation of p53, caspase-3 and TIMP-2. In mice treated with scopoletin, the expressions of these proteins and toxicity biomarkers were reverted.

Conclusion: Since AhR is known to be ligand-activated by DMBA to release signals for several downstream proteins initiating reactive oxygen species generation, the down-regulation of AhR by scopoletin appeared to play a significant role in subsequent down-regulation of some key signal proteins. One possible mechanism of down-regulation of AhR may be through competitive inhibition by scopoletin. Mitogen-activated protein kinases may also have some critical role. This compound can be considered as a possible candidate for chemoprevention.

Polymeric nanoparticle encapsulation of a naturally occurring plant scopoletin and its effects on human melanoma cell A375

Khuda-Bukhsh AR, Bhattacharyya SS, Paul S, Boujedaini N. Jour Chinese Intgr Med. 2010; 8:853-62.

Abstract

Objective: We formulated nano-encapsulation of a naturally occurring coumarin-scopoletin (7-hydroxy-6-methoxy coumarin, HMC, C(10)H(8)O(4)), isolated from plant Gelsemium sempervirens having anticancer potentials, with a bio-adhesive agent - polylactic-co-glycolic acid (PLGA) and tested if its cellular uptake, bioavailability and apoptotic (anticancer) potentials could thus be increased vis-a-vis unencapsulated HMC.

Methods: A375 melanoma cancer cells were used for testing cellular entry and anticancer potentials of HMC and nano-7-hydroxy-6-methoxy coumarin (NHMC) through several standard protocols. Characterization of NHMC was done by dynamic light scattering for determination of particle size, polydispersity index (PDI), and zeta potential. Surface morphology of nanoparticles was determined by scanning electron microscopy and atomic force microscopy.

Results: HMC was encapsulated with more than 85% entrapment efficiency, the average particle size of NHMC being less than 110 nm and a PDI 0.237, which resulted in enhanced cellular entry and greater bioavailability. NHMC showed a faster cellular uptake (15 min) than its unencapsulated counterpart (30 min). Study of signal molecules through mRNA expressions revealed that NHMC caused down-regulation of cyclin-D1, proliferating cell nuclear antigen (PCNA), survivin and Stat-3, and up-regulation of p53 and caspase-3, that in turn induced a greater number of apoptosis vis-a-vis unencapsulated HMC.

Conclusion: The formulation yielded small-sized NHMC by biodegradable PLGA that took less time for cellular entry, and caused more apoptosis to cancer cells, but apparently had negligible cytotoxicity against normal skin cells. Nano-encapsulation of bioactive plant ingredients can be a strategy worth trying for designing effective chemopreventive drug products.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/20836976

Lycopodine from Lycopodium clavatum extract inhibits proliferation of HeLa cells through induction of apoptosis via caspase-3 activation

Mandal SK, Biswas R, Bhattacharyya SS, Paul S, Dutta S, Pathak S, **Khuda-Bukhsh AR**.. Eur J Pharmacol. 2010; 626:115-22.

Abstract

Crude ethanolic extract of the plant Lycopodium clavatum has long been used in complementary and alternative medicine for treating various liver ailments and Alzheimer's disease. It has also been claimed to have potential anti-cancer properties in vivo in mice chronically fed liver carcinogens, p-dimethylamino azobenzene (initiator) and phenobarbital (promoter). Incidentally, crude ethanolic extract of Lycopodium clavatum is a mixture of some 201 alkaloids. In order to ascertain if any major fraction can be attributed to have pronounced anti-cancer effect, we examined this major fraction by eluting the crude extract in petroleum ether:ethyl aetate (17:3 vol/vol;) solvent and tried to understand its underlying mechanism. Studies on morphological changes, cell viability and cytotoxicity by microscopy and FACS, Western blot and immunofluorescence of Bcl-2, Bax, cytochrome c, caspase-3 were conducted. Lycopodine was found to induce chromatin condensation, inter-nucleosomal DNA fragmentation and enhanced cell population in sub-G1 region along with increase in reactive oxygen species generation and mitochondrial membrane potential depolarization, release of cytochrome c and activation of caspase-3 which are the events closely involved in apoptosis. An overall analysis of results showed that Lycopodine considerably inhibited growth of HeLa cells which indicates its potential use in chemotherapy.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/19786013

Encapsulated plant extract (Gelsemium semipervirens) poly (lactide-co-glycolide) nanoparticles enhance cellular uptake and increases bioactivity in vitro

Bhattacharyya SS, Paul S, **Khuda-Bukhsh AR**. *Exp Biol Med (Maywood)* 2010; 235:678-88. doi: 10.1258/ebm.2010.009338.

Abstract

Ethanolic extract of Gelsemium sempervirens (family: Loganiaceae), henceforth to be called EEGS, is used in various traditional systems of medicine. In homeopathy, EEGS is known as mother tincture of G. sempervirens, which is generally used to treat pain and respiratory ailments. We demonstrated earlier anticancer activity of crude EEGS by in vitro studies on human HeLa cells. To test the hypothesis if nanoparticle-encapsulated extract (now onwards to be called NEEGS) could enhance cellular uptake and thereby improve bioactivity, we formulated nanoparticle encapsulation based on poly (lactide-co-glycolide) (PLGA) and confirmed encapsulation by scanning electron microscopy (SEM) and atomic force microscopy. EEGS was encapsulated with 81.6% efficiency in PLGA biodegradable nanoparticle formulation and F68 (polyoxyethylene-polyoxypropylene) was used as a stabilizer. Dynamic laser light scattering and SEM indicated a particle diameter of 122.6 nm. The zeta potential of the drug-loaded nanoparticles was -14.8 mV. NEEGS was characterized for their biological activities in a skin cancer cell line A375 in vitro. NEEGS exhibited relatively rapid (30 min) and more efficient cellular uptake than their un-encapsulated counterpart (45 min). Analysis of data of apoptosis study using Annexin V-FITC, terminal transferase dUTP nick end labeling assay and DNA ladder revealed that encapsulated EEGS was more potent than their un-encapsulated counterpart in inducing apoptosis of A375 cells. Reverse transcriptase-polymerase chain reaction data of survivin, cyclin-D1, caspase-3, PCNA and p53 also corroborated well to suggest greater potentials of NEEGS as anticancer agents.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/20511672

Efficacy of ethanolic spore extract of Lycopodium clavatum in reducing induced hepatotoxicity and genotoxicity in mice

Pathak S, Banerjee A, **Khuda-Bukhsh AR.** *Intl. Jour. of Biol. Chem. Sciences* 2010, 4: 770-781.

Abstract

Ethanolic extract of spores of Lycopodium clavatum L., reportedly has profound effect against liver disorders, but lacks adequate experimental validation. To test this claim, healthy inbred Swiss albino mice, Mus musculus, were divided into different groups: Gr.I mice were fed normal diet (negative control); Gr.II - fed normal diet plus ethanol; Gr.III - fed two carcinogens of liver, [0.06% p-dimethyl aminoazobenzene (initiator) and 0.05% phenobarbital (promoter)] known to induce hepatotoxicity and genotoxicity; Gr.IV- mice fed ethanol plus both the carcinogens, and Gr.V- fed carcinogens plus spore extract of Lycopodium clavatum. They were sacrificed at day 90 and 120 for histological studies of liver, assay of cytotoxicity markers and assessment of genotoxicity using endpoints such as

chromosome aberrations, micronuclei, mitotic index in bone marrow cells and sperm head anomaly. Additionally, western blot for p53 protein expression and matrix metalloproteinase (MMP) activity in liver was compared among different groups of treated and control mice to evaluate its therapeutic potentials. Compared to Gr.III and IV, less number of mice developed tumors in Gr.V along with significant reduction in hepatotoxicity and genotoxicity, thereby validating its potential use against liver ailments as a herbal remedy.

Keywords: p-dimethyl aminoazobenzene, phenobarbital, plant extract, amelioration.

Article Link: http://www.ajol.info/index.php/ijbcs/article/view/60510

Anti-carcinogenic potentials of a plant extract (Hydrastis canadensis): I. Evidence from in vivo studies in mice (Mus musculus)

Roy Karmakar S, Biswas SJ, **Khuda-Bukhsh AR.** Asian Pac J Cancer Prev. 2010; 11:545-51.

Abstract

Ethanolic extract of Hydrastis canadensis has been tested for its possible anti-cancer potentials against p-dimethylaminoazobenzene (p-DAB) induced hepatocarcinogenesis in mice. Mice were chronically fed p-dimethylaminoazobenzene (p-DAB) and phenobarbital (PB), two hepato-carcinogens for 1, 2, 3 and 4 months, respectively, and were divided into sub-groups: i) fed normal low protein diet (Gr. I, normal control); ii) fed diet mixed with 0.06% p-DAB at a daily dose of 165 mg/kg b.w. per mouse plus 0.05% PB plus 0.06 ml 90% alcohol (vehicle of the crude extract) (Gr. II, carcinogen treated); iii) fed diet mixed with p-DAB and PB at the same daily dose plus crude extract of Hydrastis canadensis (Gr. III, drug treated). Several biochemical parameters like acid and alkaline phosphatases, alanine amino-, aspartate amino-, and gamma glutamyl-transferases, lipid peroxidation, reduced glutathione content, lactate dehydrogenase, catalase and glucose-6-phosphate dehydrogenase activities and electron microscopy of liver in different groups of treated and control mice were studied. A critical analysis of results of these studies suggested anti-cancer potentials of the drug suitable for use as a supportive complementary medicine in liver cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/20843149

Anti-carcinogenic potentials of a plant extract (Hydrastis canadensis): I. Evidence from in vivo studies in mice (Mus musculus)

Roy Karmakar S, Biswas SJ, **Khuda-Bukhsh AR.** *Asian Pac J Cancer Prev*. 2010; 11:545-51.

Abstract

Ethanolic extract of Hydrastis canadensis has been tested for its possible anti-cancer potentials against p-dimethylaminoazobenzene (p-DAB) induced hepatocarcinogenesis in mice. Mice were chronically fed p-dimethylaminoazobenzene (p-DAB) and phenobarbital (PB), two hepato-carcinogens for 1, 2, 3 and 4 months, respectively, and were divided into sub-groups: i) fed normal low protein diet (Gr. I, normal control); ii) fed diet mixed with 0.06% p-DAB at a daily dose of 165 mg/kg b.w. per mouse plus 0.05% PB plus 0.06 ml 90% alcohol (vehicle of the crude extract) (Gr. II, carcinogen treated); iii) fed diet mixed with p-DAB and PB at the same daily dose plus crude extract of Hydrastis canadensis (Gr. III, drug treated). Several biochemical parameters like acid and alkaline phosphatases, alanine amino-, aspartate amino-, and gamma glutamyl-transferases, lipid peroxidation, reduced glutathione content, lactate dehydrogenase, catalase and glucose-6-phosphate dehydrogenase activities and electron microscopy of liver in different groups of treated and control mice were studied. A critical analysis of results of these studies suggested anti-cancer potentials of the drug suitable for use as a supportive complementary medicine in liver cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/20843149

Protective potentials of a plant extract (*lycopodium clavatum*) on mice chronically fed hepato-carcinogens

Pathak S, Banerjee A, Paul S, **Khuda-Bukhsh AR**. *Indian J* Exp Biol. 2009:47:602-607.

Abstract

Chronic feeding of carcinogens p-dimethylamino azobenzene (initiator) and phenobarbital (promoter) for 90 and 120 days elevated activities of acid and alkaline phosphatases, levels of blood glucose and cortisol and decreased the activities of glutathione reductase, succinate dehydrogenase, and blood cholesterol and hemoglobin contents, and levels of serum estradiol and testosterone in mice. Levels of these biomarkers in both liver and spleen tissues were positively altered along with a significant reduction of tumor incidence in liver of carcinogen intoxicated mice treated with spore extract of Lycopodium clavatum.

The results validate the use of this plant extract in complementary and alternative medicines against hepato-toxicity.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/19761046

Homeopathic drugs Natrum Sulphuricum and carcinosin prevent azo dye-induced hepatocarcinogenesis in mice

Bhattacharjee N, Banerjee P, **Khuda-Bukhsh AR**. *Indian J BichemBiophy*. 2009; 46:307-18.

Abstract

The study was undertaken to examine whether Carcinosin-200 (Car-200) could provide additional ameliorative effect, if used intermittently with Natrum sulphuricum-30 (Nat Sulph-30) against hepatocarcinogenesis induced by chronic feeding of p-dimethylaminoazobenzene (p-DAB) and phenobarbital (PB) in mice (Mus musculus). Mice were randomly divided into seven sub-groups: (i) normal untreated; (ii) normal + succussed alcohol; (iii) p-DAB (0.06%) + PB (0.05%); (iv) p-DAB + PB + succussed alcohol, (v) p-DAB + PB + Nat Sulph-30, (vi) p-DAB + PB + Car-200, and (vii) p-DAB + PB + Nat Sulph-30 + Car-200. They were sacrificed at 30, 60, 90 and 120 days for assessment of genotoxicity through cytogenetical end-points like chromosome aberrations, micronuclei, mitotic index and sperm head anomaly and cytotoxicity through assay of widely accepted biomarkers and pathophysiological parameters. Additionally, electron microscopic studies and gelatin zymography for matrix metalloproteinases (MMPs) were conducted in liver at 90 and 120 days. Results showed that administration of Nat Sulph-30 alone and in combination with Car-200 reduced the liver tumors with positive ultra-structural changes and in MMPs expression, genotoxic parameters, lipid peroxidation, -glutamyl transferase, lactate dehydrogenase, blood glucose, bilirubin, creatinine, urea and increased GSH, glucose-6-phosphate dehydrogenase, superoxide dismutase, catalase, glutathione reductase activities and hemoglobin, cholesterol, and albumin levels. Thus, intermittent use of Car-200 along with Nat Sulph-30 yielded additional benefit against genotoxicity, cytotoxicity, hepatotoxicity and oxidative stress induced by the carcinogens during hepatocarcinogenesis.

Article Link: http://nopr.niscair.res.in/handle/123456789/5799

Mice as a model for homeopathy research

Khuda-Bukhsh AR. *Homeopathy* 2009, 98:267-79. doi: 10.1016/j.homp.2009.09.007

Abstract

Mice (Mus musculus) have been used as a model for homeopathy research in relation to cytotoxicity, genotoxicity and carcinogenesis in our laboratory for the last three decades. Initially, anti-radiation activities of several potentized homeopathic drugs were tested against suitable controls by taking into consideration several cytogenetic endpoints. Subsequently, anti-cytotoxic, anti-genotoxic and anti-oxidative stress effects of some homeopathic drugs were tested against several chemical toxic metalloids and metal compounds. Modern techniques including Western blot, immunofluorescence, electron microscopy, UV-spectroscopy, HPLC, FTIR, NMR, RT-PCR etc were deployed to understand the possible mechanisms and pathways of action of potentized homeopathic drugs. We hypothesise that one way by which potentized homeopathic drugs act is through regulatory action on gene expression.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/19945679

Amelioration of Carcinogen Induced Toxicity in Mice by Administration of a Potentized Homeopathic Drug, Natrum Sulphuricum 200

Bhattacharjee N, Pathak S, **Khuda-Bukhsh AR.** *Evid Based Complement Alternat Med*. 2009; 6:65-75. doi: 10.1093/ecam/nem067.

Abstract

To examine if a potentized homeopathic drug, Natrum Sulphuricum 200 (Nat Sulph-200) has protective potentials against hepatocarcinogenesis, liver tumors were induced in mice through chronic feeding of P-dimethylaminoazobenzene (p-DAB; initiator of hepatocarcinogenesis) and phenobarbital (PB; promoter). Mice were divided into five sub-groups: fed normal low protein diet (Gr. I, normal control); fed normal low protein plus alcohol-200 (vehicle of the homeopathic remedy) (Gr. II); fed diet mixed with 0.06% p-DAB plus 0.05% PB (Gr. III); fed diet and carcinogens like Gr.III, plus alcohol 200 (positive control for drug fed mice) (Gr. IV) and fed diet and carcinogens like Gr. III, plus Natrum Sulphuiricum-200 (Gr. V; drug fed). Mice were sacrificed at day 7, 15, 30, 60, 90 and day 120 for study of cytogenetical endpoints like chromosome aberrations (CA), micronuclei

(MN), mitotic index (MI) and sperm head anomaly (SHA) and biochemical toxicity parameters like aspartate amino transferase (AST), alanine amino transferase (ALT), acid (AcP) and alkaline (AlkP) phosphatases, lipid peroxidation (LPO) and reduced glutathione (GSH) content. Less number of liver tumors were observed in Gr. V (drug fed) mice. Administration of Nat Sulph 200 reduced genomic damage, activities of AcP, AlkP, AST, ALT, LPO and increased GSH content. Therefore, independent replication of the study by others is encouraged.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/18955221

Comparative efficacy of two microdoses of a potentized homeopathic drug, arsenicum album, to ameliorate toxicity induced by repeated sublethal injections of arsenic trioxide in mice

Banerjee P, Bhattacharyya SS, Pathak S, Naoual B, Belon P, **Khuda-Bukhsh AR**. Pathobiology. 2008; 75:156-70.

Abstract

Objectives: To evaluate the efficacy of 2 potentized homeopathic remedies of Arsenicum Album (Ars Alb)--6C and 30C--in combating chronic arsenic toxicity induced by repeated sublethal injections in mice (Mus musculus).

Methods: Mice were randomized and divided into sets: (1) normal (control 1); (2) normal + succussed alcohol (control 2); (3) As(2)O(3) (0.016%) injected at 1 ml/100 g body weight every 7 days (treated); (4) As(2)O(3) injected + succussed alcohol (positive control); (5) As(2)O(3) injected + Ars Alb 6C (drug-fed); (6) As(2)O(3) injected + Ars Alb 30C (drug-fed). Cytogenetical endpoints like chromosome aberrations, micronuclei, mitotic index, sperm head abnormality and biochemical protocols like acid and alkaline phosphatases, aspartate and alanine aminotransferases, reduced glutathione, lipid peroxidation, catalase and succinate dehydrogenase were studied at 30, 60, 90 and 120 days.

Results: Compared to controls, chromosome aberrations, micronuclei, sperm head abnormality frequencies and activities of acid and alkaline phosphatases, aspartate and alanine aminotransferases and lipid peroxidation were reduced in both drug-fed series, while mitotic index and activities of glutathione, catalase and succinate dehydrogenase were increased. Ars Alb 30C showed marginally better efficacy than Ars Alb 6C.

Conclusion: Both remedies indicated potentials of use against arsenic intoxication.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/18550913

Homeopathic Drug Discovery: Theory update and Methodological aspect

Khuda-Bukhsh AR, Pathak S. *Expert Opinion in Drug Discovery*. 2008; 3:979-990.

Abstract

Background: Homeopathy treats patient 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.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/23484971

Efficacy of a plant extract (Chelidonium majus L.) in combating induced hepatocarcinogenesis in mice

Biswas SJ, Bhattacharjee N, **Khuda-Bukhsh AR.** *Food and Chemical Toxicology 2008*; 46:1474-87.

Abstract

Ethanolic whole plant extract of Chelidonium majus, extensively used in traditional systems of medicine against various liver ailments, has been tested for its possible anti-tumor, hepato-protective and anti-genotoxic effects in p-dimethylaminoazobenzene (p-DAB) induced hepatocarcinogenesis in mice through multiple assays: cytogenetical, biochemical, histological and electron microscopical. Different sets of mice, 5 (for 7, 15 and

30 days' treatment) or 10 (for 60, 90 and 120 days) each, were chronically fed a diet suitably mixed with p-DAB and phenobarbital to develop liver tumors. One sub-group of carcinogen fed mice was also fed C. majus extract; 0.1 ml daily (drug-treated) while the other equal amount of dilute ethyl alcohol ("vehicle" of plant extract) (positive control). A separate group of mice was maintained with normal diet without any carcinogen treatment (negative control). Data of several cytogenetical endpoints and biochemical assay of some toxicity marker enzymes at all fixation intervals and histology of liver sections through ordinary, scanning and transmission electron microscopy at 60 and 120 days and that of spleen and kidney at 90 days were critically analyzed in the treated lots vis-a-vis controls. The results suggest anti-tumor, anti-genotoxic and hepato-protective effects of the plant extract, showing potentials for use in cancer therapy.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/18215450

In vitro studies demonstrate anticancer activity of an alkaloid of the plant Gelsemium sempervirens

Bhattacharyya SS, Mandal SK, Biswas R, Paul S, Pathak S, Boujedaini N, Belon P, **Khuda-Bukhsh AR**. *ExpBiol Med* (Maywood). 2008; 233:1591-601.

Abstract

The chemical structure of the main fluorescenting compound in the ethanolic extract (mother tincture) of the American yellow jasmine, Gelsemium sempervirens, was determined by employing (1)H nuclear magnetic resonance (NMR), (13)C NMR, mass spectroscopy, high-performance liquid chromatography (HPLC), correlation spectroscopy (COSY), and Fourier transform infrared (FTIR) spectroscopy analyses. Spectrofluorometric analysis has been made of the mother tincture and its agitated serial dilutions (up to 12th potency) prepared according to a homeopathic procedure in which serial, agitated dilutions were made separately in glass and polypropylene containers. The succussions were made by employing three different modes: hand jerk, sonication, and vortexing. The chemical formula of scopoletin, the main fluorescent compound, was determined to be C(10)H(8)O(4) having a molecular weight of 192.17. Significant differences were noted between the remedies prepared in the two types of containers. Further, a comparison between any two methods of agitation revealed significant differences in fluorometric data of remedies at certain potency levels. The biological (anticancer) action of the crude extract, the alkaloid scopoletin, and 2C potency of Gelsemium sp were tested in vitro on the HeLa cell line through fluorescence microscopy, the 3(4, 5-dimethylthiazol-2-yl)-2, 5-

diphenyltetrazolium bromide (MTT) assay, and fluorescent activated cell sorting (FACS). The role of nanoparticles presumably derived from the containers, their orientation, and their interaction with the starting substance during the dynamization process initiated by different modes of agitation could possibly be attributed to the differences noted in the fluorometric data of potencies prepared in the two types of containers and among the three different means of succussion tested.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/18997108

A potentized homeopathic drug, Arsenicum Album 200, can ameliorate genotoxicity induced by repeated injections of arsenic trioxide in mice

Banerjee P, Biswas SJ, Belon P, **Khuda-Bukhsh AR**. *J Vet Med A Physiol Pathol Clin Med*. 2007; 54:370-6.

Abstract

Groundwater arsenic contamination has become a menacing global problem. No drug is available until now to combat chronic arsenic poisoning. To examine if a potentized homeopathic remedy, Arsenicum Album-200, can effectively combat chronic arsenic toxicity induced by repeated injections of Arsenic trioxide in mice, the following experimental design was adopted. Mice (Mus musculus) were injected subcutaneously with 0.016% arsenic trioxide at the rate of 1 ml/100 g body weight, at an interval of 7 days until they were killed at day 30, 60, 90 or 120 and were divided into three groups: (i) one receiving a daily dose of Arsenicum Album-200 through oral administration, (ii) one receiving the same dose of diluted succussed alcohol (Alcohol-200) and (iii) another receiving neither drug, nor succussed alcohol. The remedy or the placebo, as the case may be, was fed from the next day onwards after injection until the day before the next injection, and the cycle was repeated until the mice were killed. Two other control groups were also maintained: one receiving only normal diet, and the other receiving normal diet and succussed alcohol. Several toxicity assays, such as cytogenetical (chromosome aberrations, micronuclei, mitotic index, sperm head anomaly) and biochemical (acid and alkaline phosphatases, lipid peroxidation), were periodically made. Compared with controls, the drug fed mice showed reduced toxicity at statistically significant levels in respect of all the parameters studied, thereby indicating protective potentials of the homeopathic drug against chronic arsenic poisoning.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/17718811

Homeopathic remedy for arsenic toxicity?: Evidence-based findings from a randomized placebo-controlled double blind human trial

Belon P, Banerjee A, Karmakar SR, Biswas SJ, Choudhury SC, Banerjee P, Das JK, Pathak S, Guha B, Paul S, Bhattacharjee N, **Khuda-Bukhsh AR**. *Sci Total Environ.* 2007; 384:141-50.

Abstract

Millions of people are at risk of groundwater arsenic contamination, but supply of arsenic-free drinking water is grossly inadequate. The present study was intended to examine if a potentized homeopathic remedy reportedly showing ameliorating potentials in people inhabiting high-risk arsenic-contaminated areas but drinking arsenic-free water, can also ameliorate arsenic toxicity in subjects living in high-risk arsenic-contaminated areas, and drinking arsenic-contaminated water. This pilot study was conducted on 20 males and 19 females of village Dasdiya (arsenic contaminated) who initially agreed to act as volunteers; but as many as 14, mostly placebo-fed subjects, later dropped out. 18 volunteers, 14 males and 4 females, from a distant village, Padumbasan (arsenic-free), served as negative controls. In a double blind placebo-controlled study, a potentized remedy of homeopathic Arsenicum Album-30 and its placebo (Succussed Alcohol-30) were given randomly to volunteers. Arsenic contents in urine and blood and several widely accepted toxicity biomarkers and pathological parameters in blood were analyzed before and after 2 months of administration of either verum or placebo. Elevated levels of ESR, creatinine and eosinophils and increased activities of AST, ALT, LPO and GGT were recorded in arsenic exposed subjects. Decreased levels of hemoglobin, PCV, neutrophil percentages, and GSH content and low G-6-PD activity were also observed in the arsenic exposed people. The administration of "verum" appeared to make positive modulations of these parameters, suggestive of its ameliorative potentials. Most of the subjects reported better appetite and improvement in general health, thereby indicating possibility of its use in remote arsenic-contaminated areas as an interim health support measure to a large population at risk.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/17628642

Supportive evidences for anti-cancerous potential of an alternative medicine in hepatocarcinogenesis of mice

Pathak S, Bhattacharjee N,Das JK, Choudhury SC, Roy-Karmakar S, Banerjee P, Paul S, Banerjee A, **Khuda-Bukhsh AR**. *Research in Complementary Medicine* (Forsch Komplementarmed). 2007; 14:148-156.

Abstract

Introduction: The present study examines if Lycopodium 200 (Lyco-200) has demonstrable anti-cancer activities in mice which are chronically fed carcinogens, p-dimethylaminoazobenzene (p-DAB) and phenobarbital (PB) to induce liver cancer.

Materials And Methods: Mice in 5 different groups were chronically fed for varying periods of time: group I: normal diet; group II: normal diet + alcohol 200); group III: p-DAB + PB; group IV: p-DAB + PB + alcohol 200 (vehicle of Lyco-200 being ethyl alcohol); group V: p-DAB + PB + Lyco-200. They were sacrificed at day 7, 15, 30, 60, 90 or 120, and the following parameters were assessed: cytogenetic endpoints like chromosome aberrations, micronuclei, mitotic index and sperm-head anomaly; toxicity biomarkers like acid and alkaline phosphatases, alanine and aspartate amino transferase, glutathione reductase, succinate dehydrogenase and catalase activities, lipid peroxidation and reduced glutathione content. Additionally, scanning and transmission electron microscopic analyses of liver tissues were made at day 90 and 120, and immunodetection of p53 protein as well as gelatin zymography for matrix metalloproteinases in liver tissue were performed. Furthermore, studies were conducted on blood glucose, hemoglobin and cholesterol, estradiol, testosterone and cortisol, and lymphocyte and hepatic cell viabilities. Physical properties of Lyco-200 and potentized alcohol 200 were analyzed by using methods such as UV, Fourier Transform Infrared Spectroscopy (FTIR), Fluorescence Spectroscopy, 1H-NMR and 13C-NMR (Nuclear Magnetic Resonance Spectroscopy).

Results: Lyco-200 reduced cytogenetic damages yielding positive modulations of all biochemical, pathological and other risk factors, cell viability and expression of p53 protein and matrix metalloproteinases as compared to controls.

Conclusion: Studies on other mammals are recommended to further investigate the potential of Lyco-200 in liver cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/17596695

Protective potentials of a potentized homeopathic drug, Lycopodium-30, in ameliorating azo dye induced hepatocarcinogenesis in mice

Pathak S, Das JK, Biswas SJ, **Khuda-Bukhsh AR**. *Mol Cell Biochem.* 2006; 285:121-31.

Abstract

The protective potentials of a potentized homeopathic drug, Lycopodium-30, prepared from extract of spores of a plant, Lyocopodium clavatum (Fam: Lycopodiaceae) and used as a remedy for various liver ailments, have been tested in mice chronically fed p-dimethyl amino azo benzene (p-DAB) - an initiator, and phenobarbital (PB) - a promoter of hepatic cancer, by using some cytogenetic endpoints like chromosome aberrations (CA), micronuclei (MN), mitotic index (MI) and sperm head abnormality (SHA), and toxicity biomarkers like acid and alkaline phosphatases (AcP and AlkP, respectively), alanine and aspartate amino transferases (ALT and AST, respectively) and lipid peroxidation (LPO) and reduced glutathione (GSH) activities. The effects of chronic treatment of the carcinogens were assessed at different intervals of fixation, namely, at day 7, 15, 30, 60, 90 and day 120, and compared with that of mice fed conjointly with the carcinogens and the homeopathic remedy. Both the assay systems indicated considerable protective potentials of the homeopathic remedy against p-DAB induced hepatocarcinogenesis in mice.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/16538399

Can administration of potentized homeopathic remedy, Arsenicum album, alter antinuclear antibody (ANA) titer in people living in high-risk arsenic contaminated areas? I. A correlation with certain hematological parameters

Belon P, Banerjee P, Choudhury SC, Banerjee A, Biswas SJ, Karmakar SR, Pathak S, Guha B, Chatterjee S, Bhattacharjee N, Das JK, **Khuda-Bukhsh AR.** *Evid Based Complement Alternat Med*. 2006; 3:99-107.

Abstract

To examine whether elevated antinuclear antibody (ANA) titers reported in random human population of arsenic contaminated villages can be reverted to the normal range by administration of a potentized homeopathic drug, Arsenicum album, randomly selected volunteers in two arsenic contaminated villages and one arsenic-free village in West Bengal (India) were periodically tested for their ANA titer as well as various blood parameters in two types of experiments: 'placebo-controlled double blind' experiment for shorter duration and 'uncontrolled verum fed experiment' for longer duration. Positive modulation of ANA titer was observed along with changes in certain relevant hematological parameters, namely total count of red blood cells and white blood cells, packed cell volume, hemoglobin content, erythrocyte sedimentation rate and blood sugar level, mostly within 2 months of drug administration. Thus, Arsenicum album appears to have great potential for ameliorating arsenic induced elevated ANA titer and other hematological toxicities.

Keywords: antinuclear antibody (ANA), Arsenicum album, arsenic toxicity, blood cells, blood sugar, ESR, human, homeopathic remedy

Article Link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1375236/

Laboratory Research in homeopathy: pro

Khuda-Bukhsh A.R. *Integrative Cancer Therapies*. 2006; 5: 320-32.

Abstract

Homeopathy is a holistic method of treatment that uses ultralow doses of highly diluted natural substances originating from plants, minerals, or animals and is based on the principle of "like cures like." Despite being occasionally challenged for its scientific validity

and mechanism of action, homeopathy continues to enjoy the confidence of millions of patients around the world who opt for this mode of treatment. Contrary to skeptics' views, research on home-opathy using modern tools mostly tends to support its efficacy and advocates new ideas toward understanding its mechanism of action. As part of a Point-Counterpoint feature, this review and its companion piece in this issue by Moffett et al (Integr Cancer Ther. 2006;5:333-342) are composed of a thesis section, a response section in reaction to the companion thesis, and a rebuttal section to address issues raised in the companion response.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/17101761

Can homeopathic arsenic remedy combat arsenic poisoning in humans exposed to groundwater arsenic contamination?: a preliminary report on first human trial

Khuda-Bukhsh AR, Pathak S, Guha B, Karmakar SR, Das JK, Banerjee P, Biswas SJ, Mukherjee P, Bhattacharjee N, Choudhury SC, Banerjee A, Bhadra S, Mallick P, Chakrabarti J, Mandal B. Evid Based Complement Alternat Med. 2005; 537-48.

Abstract

Groundwater arsenic (As) has affected millions of people globally distributed over 20 countries. In parts of West Bengal (India) and Bangladesh alone, over 100 million people are at risk, but supply of As-free water is grossly inadequate. Attempts to remove As by using orthodox medicines have mostly been unsuccessful. A potentized homeopathic remedy, Arsenicum Album-30, was administered to a group of As affected people and thereafter the As contents in their urine and blood were periodically determined. The activities of various toxicity marker enzymes and compounds in the blood, namely aspartate amino transferase, alanine amino transferase, acid phosphatase, alkaline phosphatase, lipid peroxidation and reduced glutathione, were also periodically monitored up to 3 months. The results are highly encouraging and suggest that the drug can alleviate As poisoning in humans.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/16322812

Efficacy of a potentized homeopathic drug, Carcinosin-200, fed alone and in combination with another drug, Chelidonium 200, in amelioration of p-DAB induced hepatocarcinogenesis in mice

Biswas SJ, Pathak S, Bhattacharjee N, Das JK, **Khuda-Bukhsh AR**. *J. Altern. Comp Med*. 2005; 11:839-854.

Abstract

Objectives: This study was conducted to examine whether the potentized homeopathic remedy Carcinosin 200, fed alone and in combination with Chelidonium 200, has differential protective effects against p-dimethylaminoazobenzene (p-DAB)-induced hepatocarcinogenesis in mice.

Design: Liver tumors were induced in mice through chronic feeding of p-DAB (initiator) and phenobarbital (PB, promoter). The mice were divided into two subgroups: (1) one was fed potentized Alcohol 200 and served as controls; and (2) the other was fed Carcinosin 200 alone or in combination with Chelidonium 200 and divided into several sets. The relative efficacy of the two potentized remedies, alone or in combination, in combating hepatocarcinogenesis was assessed through several cytogenetical endpoints such as chromosome aberrations, induction of micronuclei, sperm head anomaly, and mitotic index at several intervals of fixation (days 7, 15, 30, 60, 90, and 120). Several toxicity biomarkers such as acid and alkaline phosphatases, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and lipid peroxidation activity were also assayed in three organs of treated and control mice. In addition, recovery by the homeopathic drugs, if any, of tissue damage inflicted because of chronic feeding of p-DAB and PB was also assessed by optical, scanning, and transmission electron microscopies of liver done at days 60 and 120.

Results: Both Carcinosin 200 and Chelidonium 200 when administered alone show considerable ameliorative effect against p-DAB-induced hepatocarcinogenesis in mice; but the conjoint feeding of these two drugs appears to have had a slightly greater protective effect.

Conclusion: These homeopathic remedies have the potential to be used as complementary and alternative medicine in liver cancer therapy, particularly as supporting palliative measures.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/16296917

Comparative Efficacy of Pre-feeding, Post-feeding and Combined Pre- and Post-feeding of Two Microdoses of a Potentized Homeopathic Drug, Mercurius Solubilis, in Ameliorating Genotoxic Effects Produced by Mercuric Chloride in Mice

Datta S, Biswas SJ**, Khuda-Bukhsh AR**. *Evid Based Complement Alternat Med* 2004: 1:291-300.

Abstract

Mercury and its derivatives have become an alarming environmental problem, necessitating the search for effective antagonists, including homeopathic drugs, which are generally used in micro doses and are devoid of any palpable side-effects. On the basis of homeopathic similia principle, two potencies of Mercurius solubilis (Merc Sol-30 and Merc Sol-200) were tested by three administrative modes, i.e. pre-feeding, post-feeding and combined pre- and post-feeding, for their possible efficacy in ameliorating mercuric chloride-induced genotoxicity in mice. Healthy mice, Mus musculus, were intraperitoneally injected with 0.06% solution of mercuric chloride at the rate of 1 ml/100 g of body weight, and assessed for genotoxic effects through conventional endpoints. i.e. chromosome aberrations, micronuclei, mitotic index and sperm head abnormality, keeping suitable controls. Mercuric chloride-treated mice were divided into three sub-groups, which were orally administered with the drug prior to, after and both prior to and after injection of mercuric chloride, and their genotoxic effects were analysed at specific intervals of fixation. Mercuric chloride treatment generally produced more chromosome aberations, micronuclei and sperm head anomaly in mice, but the mitotic index appeared to be slightly reduced. While chromosome aberations, micronuclei and sperm head anomaly were generally reduced in the drug-fed series, the mitotic index showed an apparent increase. In most cases, the combined pre- and post-feeding mode appeared to show the maximum amelioration, followed by post-feeding and pre-feeding, in that order. The amelioration by Merc Sol-200 appeared to be slightly more pronounced. We conclude that potentized homeopathic drugs can serve as possible anti-genotoxic agents against specific environmental mutagens, including toxic heavy metals.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/15864357

Evaluation of protective potentials of a potentized homeopathic drug, Chelidonium majus, during azo dye induced hepatocarcinogenesis in mice

Biswas SJ, **Khuda-Bukhsh AR.** *Indian J Exp Biol.* 2004; 42:698-714.

Abstract

Several cytogenetical and enzymatic protocols were used to test if two microdoses of Chelidonium majus, namely Chelidonium-30 (Ch-30) and Chelidonium-200 (Ch-200), used as homeopathic drugs, showed anti-tumor activity and also favorably modulated genotoxic damages produced by an azo dye in mice at several intervals of fixation. Different sets of healthy mice were fed: (i) hepatocarcinogen, p-dimethylaminoazobenzene (p-DAB, initiator) + phenobarbital (PB, promoter), (ii) only p-DAB, (iii) only PB, and (iv) neither p-DAB nor PB (normal control). Mice fed with p-DAB + PB were divided into different sets that were also fed either Ch-30 (v) or Ch-200 (vi) or diluted alcohol (vii), the "vehicle" of the microdoses of Chelidonium. All mice of group (i), a few of group (ii) and group (vii) and none of groups (iii) and (iv) developed tumors in liver at the longer intervals of fixation. The frequencies of chromosome aberrations (CA), micronucleated erythrocytes (MN), mitotic index (MI) and sperm head abnormality (SHA) were much higher in groups (i) and (vii) mice than in groups (ii), (iii) and (iv) mice at all fixation intervals. However, in mice of both groups (v) and (vi), the frequencies of CA, MN, SHA were strikingly less than those of groups (i) and (vii), and moderately less than those of groups (ii) and (iii). Both Ch-30 and Ch-200 also modulated favourably some toxicity marker enzymes like acid and alkaline phosphatases, peroxidases, glutamate oxaloacetate and glutamate pyruvate transaminases in liver, kidney and spleen tissues of the carcinogen fed mice. The microdoses of Chelidonium having no visible ill effects of their own, may be strong candidates for use in delaying/protecting liver cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/15339035

Towards understanding molecular mechanisms of action of homeopathic drugs: an overview

Khuda-Bukhsh AR. *Mol Cell Biochem.* 2003; 253:339-45.

Abstract

The homeopathic mode of treatment often encourages use of drugs at such ultra-low doses and high dilutions that even the physical existence of a single molecule of the original drug substance becomes theoretically impossible. But homeopathy has sustained for over two hundred years despite periodical challenges thrown by scientists and non-believers regarding its scientificity. There has been a spurt of research activities on homeopathy in recent years, at clinical, physical, chemical, biological and medical levels with acceptable scientific norms and approach. While clinical effects of some homeopathic drugs could be convincingly shown, one of the greatest objections to this science lies in its inability to explain the mechanism of action of the microdoses based on scientific experimentations and proofs. Though many aspects of the mechanism of action still remain unclear, serious efforts have now been made to understand the molecular mechanism(s) of biological responses to the potentized form of homeopathic drugs. In this communication, an overview of some interesting scientific works on homeopathy has been presented with due emphasis on the state of information presently available on several aspects of the molecular mechanism of action of the potentized homeopathic drugs.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/14619985

Ameliorating effect of microdoses of a potentized homeopathic drug, Arsenicum Album, on arsenic-induced toxicity in mice

Mallick P, Mallick JC, Guha B, **Khuda-Bukhsh AR**. *BMC Complement Altern Med*. 2003; 22:3-7.

Abstract

Background: Arsenic in groundwater and its accumulation in plants and animals have assumed a menacing proportion in a large part of West Bengal, India and adjoining areas of Bangladesh. Because of the tremendous magnitude of the problem, there seems to be no way to tackle the problem overnight. Efforts to provide arsenic free water to the millions of people living in these dreaded zones are being made, but are awfully inadequate. In our

quest for finding out an easy, safe and affordable means to combat this problem, a homeopathic drug, Arsenicum Album-30, appears to yield promising results in mice. The relative efficacies of two micro doses of this drug, namely, Arsenicum Album-30 and Arsenicum Album-200, in combating arsenic toxicity have been determined in the present study on the basis of some accepted biochemical protocols.

Methods: Mice were divided into different sets of control (both positive and negative) and treated series (As-intoxicated, As-intoxicated plus drug-fed). Alanine amino transferase (ALT) and aspartate amino transferase (AST) activities and reduced glutathione (GSH) level in liver and blood were analyzed in the different series of mice at six different fixation intervals.

Results: Both Arsenicum Album-30 and Arsenicum Album-200 ameliorated arsenic-induced toxicity to a considerable extent as compared to various controls.

Conclusions: The results lend further support to our earlier views that microdoses of potentized Arsenicum Album are capable of combating arsenic intoxication in mice, and thus are strong candidates for possible use in human subjects in arsenic contaminated areas under medical supervision.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/14570596

Effect of a homeopathic drug, Chelidonium, in amelioration of p-DAB induced hepatocarcinogenesis in mice

Biswas SJ, **Khuda-Bukhsh A.R.** B*MC Complementary & Alternative Medicines.* 2002; 2:1-16.

Abstract

Background: Crude extracts of Chelidonium majus, and also purified compounds derived from crude extracts of this plant, have been reported to exhibit anti-viral, anti-inflammatory, anti-tumor and anti-microbial properties both in vitro and in vivo. Chelidonium is a homeopathic drug routinely used against various liver disorders including cancer in humans. Two potencies of Chelidonium (Ch-30, Ch-200) have been tested for their possible anti-tumor and enzyme modulating activities in liver and anti-clastogenic effects during p-DAB-induced hepatocarcinogenesis in mice compared to suitable controls.

Methods: Several cytogenetic and enzymatic protocols were used at three fixation intervals; at 60 days, 90 days and 120 days of treatment. Different sets of healthy mice

were fed: i) hepatocarcinogen, p-DAB plus phenobarbital (PB), ii) only PB, iii) neither p-DAB nor PB (normal control). One set of mice fed with p-DAB plus PB was also fed Ch-30 (iv) and another set Ch-200 (v). All standard currently used methods were adopted for cytogenetical preparations and for the enzyme assays.

Results: All group (i) mice developed tumors in liver at all fixation intervals, while none of group (ii) and (iii) mice developed any tumors. About 40% mice in group (iv) and group (v) did not show tumor nodules in their liver. Feeding of Chelidonium to group (iv) and (v) mice reduced genotoxic effects to a significant extent ($p < 0.05$ to $p < 0.001$).

Conclusion: The homeopathic drug Chelidonium exhibited anti-tumor and anti-genotoxic activities and also favorably modulated activities of some marker enzymes. Microdoses of Chelidonium may be effectively used in combating liver cancer.

Article Link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC107841/

Cytogenetical effects of sonication in mice and their modulations by actinomycin D and a homeopathic drug Arnica 30

Chakrabarti J, Biswas SJ**, Khuda-Bukhsh AR**. *Indian J Exp Biol.* 2001;39:1235-42.

Abstract

Experiments were designed to examine if Actinomycin D, an antibiotic, and Amica 30, a homeopathic drug used against shock and injury, can ameliorate cytogenetic damage induced by single or multiple exposures to ultrasonication. Separate sets of healthy mice were directly exposed to sonication for two minutes either once or they received multiple exposures at an interval of 20 days. The mice were then assessed at different intervals, against suitable controls, using parameters like chromosome aberrations (CA), mitotic index (MI), sperm head anomaly (SHA) and micronucleated erythrocytes (MNE). Separate groups of sonicated mice were either orally administered with Arnica 30 (alcohol 30 in control) or injected intramuscularly with Actinomycin-D (AMD). Elevated frequencies of CA, MI, MNE and SHA were noted in sonicated series. AMD had genotoxic effects of its own and also had additive effects on sonication induced genotoxicity. Sonicated mice fed with Arnica 30 showed appreciably reduced genotoxicity as against alcohol 30 and distilled water fed controls, thereby showing ameliorating effect which may have human application.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/12018517

Comparative efficacy of two microdoses of a potentized homoeopathic drug, Cadmium Sulphoricum, in reducing genotoxic effects produced by cadmium chloride in mice: A time course study

Datta S, Mallick P, **Khuda-Bukhsh AR**. *BMC Complementary and Alternative Med.* 2001; 1:1-18.

Abstract

Background: Cadmium poisoning in the environment has assumed an alarming problem in recent years. Effective antimutagenic agents which can reverse or combat cadmium induced genotoxicity in mice have not yet been reported. Therefore, in the present study, following the homeopathic principle of "like cures like", we tested the efficacy of two potencies of a homeopathic drug, Cadmium Sulphoricum (Cad Sulph), in reducing the genotoxic effects of Cadmium chloride in mice. Another objective was to determine the relative efficacy of three administrative modes, i.e. pre-, post- and combined pre and post-feeding of the homeopathic drugs. For this, healthy mice, Mus musculus, were intraperitoneally injected with 0.008% solution of CdCl2 @ 1 ml/100 gm of body wt (i.e. 0.8 mcg/gm of bw), and assessed for the genotoxic effects through such studies as chromosome aberrations (CA), micronucleated erythrocytes (MNE), mitotic index (MI) and sperm head anomaly (SHA), keeping suitable successed alcohol fed (positive) and CdCl2 untreated normal (negative) controls. The CdCl2 treated mice were divided into 3 subgroups, which were orally administered with the drug prior to, after and both prior to and after injection of CdCl2 at specific fixation intervals and their genotoxic effects were analyzed.

Results: While the CA, MNE and SHA were reduced in the drug fed series as compared to their respective controls, the MI showed an apparent increase. The combined pre- and post-feeding of Cad Sulph showed maximum reduction of the genotoxic effects.

Conclusions: Both Cad Sulph-30 and 200 were able to combat cadmium induced genotoxic effects in mice and that combined pre- and post-feeding mode of administration was found to be most effective in reducing the genotoxic effect of CdCl2 followed by the post-feeding mode.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/11737881

Efficacy of a potentized homoeopathic drug (Arsenicum Album-30) in reducing cytotoxic effects produced by of arsenic trioxide in mice. III. Tissue damage recovery, and enzymatic changes in liver

Kundu SN, Mitra K, **Khuda-Bukhsh AR.** *Comp Ther Med.* 2000; 8:76-81.

Abstract

Objective: To determine whether the potentized homoeopathic drug Arsenicum Album-30 can induce enzymatic and some other biochemical changes to repair tissue damage caused by the injection of arsenic trioxide in mice.

Design: Controlled laboratory study.

Methods: Mice injected with arsenic trioxide and then orally administered the homoeopathic drug were compared with control animals who either received saline only, or injections of arsenic trioxide, or injections of arsenic trioxide followed by orally administered dilute alcohol. Activities of the enzymes acid and alkaline phosphatases, lipid peroxidation and reduced glutathione, which are used as 'marker' enzymes for cytotoxicity levels, were assessed by standard methods. Histopathological slide preparations of liver were made by routine microtechnique method of tissue sectioning and staining with haematoxylin- eosin for histological examination.

Results: The mice fed homoeopathic drug showed positive results of tissue recovery both in terms of enzymatic and histological changes, compared to controls.

Conclusions: The homoeopathic drug is capable of preventing or repairing liver damage induced by arsenic trioxide and the positive changes were also confirmed by the activities of the enzymatic markers.

Article Link: http://www.ncbi.nlm.nih.gov/pubmed/10859599

Efficacy of a potentized homoeopathic drug (Arsenicum Album-30) in reducing cytotoxic effects produced by of arsenic trioxide in mice. IV. On certain pathological conditions, gel electrophoretic protein profiles, DNA and RNA

Kundu SN, Mitra K, **Khuda-Bukhsh AR**. Comp Ther Med. 2000;8:157-65.

Abstract

Objective: To examine if the potentized homeopathic drug Arsenicum Album-30 can help restore the damage produced in protein profiles, DNA and RNA contents in liver and testis as a result of arsenic treatment in mice.

Design: Sets of mice were injected with arsenic trioxide, one set was fed with Ars. Alb-30, another with Alcohol-30 and the final set was fed neither. The gel electrophoretic protein profiles and DNA and RNA contents in these three sets were studied.

Methods: Protein profiles were studied by SDS-PAGE method; the DNA and RNA contents were assayed by the standard methods through diphenylamine and orcinol reactions respectively.

Results: Arsenic trioxide injection produced some pathological conditions, drastic changes (mainly reduction of protein bands) in protein sub-fractions, reduced DNA and RNA contents in both liver and testis; Ars. Alb-30-fed arsenic-intoxicated mice showed revival and restoration in both liver and testis as revealed by gel patterns and quantitative assay of DNA and RNA.

Conclusion: Efficacy of the homeopathic drug Ars. Alb-30 in reducing arsenic-induced damage to protein and nucleic acids is substantiated and the mechanism of action of the homeopathic drug through expression of regulatory genes inferred.

Efficacy of a potentized homeopathic drug (Arsenicum Album - 30) in reducing genotoxic effects produced by arsenic trioxide in mice: comparative studies of pre-, post-, pre- and post-oral administration and comparative efficacy of two microdoses

Datta S, Mallick P, **Khuda-Bukhsh** AR. Com Ther Med. 1999; 7:62-75.

Abstract

Objectives: To pilot procedures to be used in a randomized controlled trial of acupuncture for low back pain.

Design: Uncontrolled clinical trial.

Setting: Primary care and acupuncture clinics in York, England.

Subjects: 20 patients with low back pain lasting I month or more.

Interventions: 10 sessions of individualized acupuncture from a traditional acupuncturist.

Main outcome measures: Change in Oswestry low back pain disability questionnaire; present pain intensity scale; effect on daily living scale, and SF-36 general health questionnaire at post-treatment and 6 months after the end of treatment.

Results: 14 patients completed follow-up. Patients had similar severity scores at baseline to those referred to an NHS outpatient clinic. Post-treatment, there were statistically significant improvements in Oswestry, present pain intensity, effect on daily living and the physical functioning, social functioning, bodily pain, vitality and mental health sub-scales of the SF36. Similar results were found at the six month follow-up. Oswestry scores showed reduced levels of pain at 6 months compared to than at post-treatment, falling approximately 40% from baseline.

Conclusions: Though the improvements in pain and quality in life may be due to the natural course of back pain, the promising responses justify further research. The procedures used in the study are appropriate for a randomized controlled trial. Drop-out could be reduced by more careful patient monitoring.

Article Link: http://www.complementarytherapiesinmedicine.com/article/S0965-2299(99)80084-3/abstract

Efficacy of a potentized homoeopathic drug (Arsenicum Album-30) in reducing cytotoxic effects produced by of arsenic trioxide in mice: II. On alterations in body weight, tissue weight and total protein

Mitra K, Kundu SN, **Khuda-Bukhsh AR.** CompTher Med. 1999;7:24-34

Summary

Objective: To study the alterations in body weight, tissue weight and total protein in mice, caused by a single sublethal injection of arsenic trioxide and to investigate whether treatment by microdoses of arsenic has any antidotal effect.

Methods: For each fixation interval, altogether 36 individuals of Swiss albino mice, Mus musculus, were used, 27 were injected with As2O3 in a single sub-lethal dose (@1.0 mg/kg body weight) and were divided into three batches. One batch was fed with diluted potentized alcohol (Alcohol control), one batch was fed with potentized homoeopathic drug Ars.Alb-30 (Active treatment), while the remaining one neither fed with potenized alcohol nor with the potentized homoeopathic drug (As-intoxicated control). The remaining batch of nine mice were injected with normal saline which served as negative control (Saline control). The mean body weights before and after injections and weights of different tissues like liver, kidney, spleen and testis were recorded at seven fixation intervals, 12 hours, 24 hours, 48 hours, 7 days, 21 days, 30 days, and 90 days.

Results: In arsenic treated mice orally administered with the homoeopathic drug statistically significant increases were noted in the weights of individual tissue weight, protein content as well as the mean body weight as compared to their respective controls.

Conclusions: Arsenicum album can be considered as an antidote to arsenic poisoning.

Article Link: http://www.complementarytherapiesinmedicine.com/article/S0965-2299(99)80055-7/abstract

Efficacy of a potentized homoeopathic drug (Arsenicum Album-30) in reducing cytotoxic effects produced by of arsenic trioxide in mice. I. On rate of accumulation of arsenic in certain vital organs

Mitra K, Kundu SN, **Khuda-Bukhsh AR**. Comp Ther Med. 1998;6(4):178-184.

Abstract

Objective: The widespread occurrence of arsenic poisoning in West Bengal, India led us to examine the extent of deposition of arsenic in different vital organs of mice after a single sublethal injection of arsenic trioxide and if microdoses of arsenic could reduce the deposition effectively in them.

Design: For each fixation interval, 15 mice were injected intramuscularly with As2O3 in a single dose @ 1.0 mg/kg body weight and were divided into three batches and another batch of five mice injected with normal saline served as negative control (saline control). Among arsenic treated mice, one batch was fed with diluted potentized alcohol-30 (alcohol-treated-positive control), one batch with a potentized homoeopathic drug Arsenicum Album-30 in ultra low doses (active treatment) while the remaining one was neither fed with potentized alcohol nor with the potentized homoeopathic drug (as-intoxicated control).

Methods: The accumulation of arsenic was determined by spectrophotometric analysis in four tissues, namely, liver, kidney, spleen and testis at seven different fixation intervals, viz. 12 hours, 24 hours, 48 hour, 7 days, 21 days, 30 days and 90 days.

Results: In arsenic treated mice orally administered with the homoeopathic drug, statistically significant decreases in accumulation were observed in all tissues at most fixation intervals as compared to controls.

Conclusions: This homoeopathic drug can be considered to effectively antagonize and antidote arsenic poisoning.

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Potentized homeopathic drugs act through regulation of gene expression: A hypothesis to explain their mechanism and pathways of action in vitro

Khuda-Bukhsh AR. Comp Ther Med. 1997; 5:43-6.

Abstract

A working hypothesis to explain the mechanism of action of potentized homoeopathic drugs in vivo has been proposed. The model is partly substantiated from our own research data on repair of chromosomal damages in X-irradiated or toxic chemical-treated mice by the oral administration of some potentized homoeopathic drugs, and partly from some of the unpublished and published works of other researchers in the field of homoeopathy. In this model, strong scientific arguments have been made to form the hypothesis that the potentized homoeopathic drugs act through regulation of gene-expression, presumably through hormone—hormone—protein complexes — the sensorgene-integrator gene-receptor gene-producer gene pathway of Britten and Davidson's model, or else through the regulator/mutator gene-operator gene-structural gene pathway of Jacob and Monod's model among some other independent mechanisms. Scientific details of some possible pathways, admittedly speculative for some steps, have also been provided to stimulate research in this direction to verify the correctness of the hypothesis.

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