CANCER & NEEM

Overview

The National Research Institutes' 1992 report on neem anticipated that continuing research into the components of a tree known in India as a ―village pharmacy‖ would demonstrate useful cures for various ailments, but it's unlikely that even the most optimistic researcher could have predicted the potentially life-saving treatments being identified now for preventing or treating multiple types cancer. Although clinical trials with human beings are still in the future, this early test tube and animal research ―combined with neem's few side effects, easy availability, and low cost in most parts of the world―is cause for tremendous excitement.

Neem contains multiple active compounds that work simultaneously via different mechanisms. This characteristic explains its effectiveness as a pesticide, and appears to be responsible for its potent impact on cancers as well. One of these documented mechanisms is apoptosis (programmed cell death), which directly kills cancer cells and, in so doing, frees material from these cells that enables immune system cells to take over identifying and destroying them as well, a process called ―cross-priming.‖ Neem has also been shown to produce substantially higher levels of antioxidants, including the carcinogen-detoxifying enzyme glutathione. Perhaps the most important – and least surprising – is neem's strengthening impact on the immune system (see separate section at for more detailed data. And neem, or isolated compounds, have shown impressive efficacy against a wide variety of human cancer cell lines, and animal models for human cancers that include colon, stomach, Ehrlich's carcinoma, lung, liver, skin, oral, prostate, and breast cancers.

In addition to studies showing that pretreatment with neem is highly protective against cancer in animals (eg, neem leaf given to mice reduced chemically induced tumors by up to 87%), and demonstrating the efficacy of neem as a stand-alone treatment, two recent reports suggest that neem pretreatment also enhances the activity while reducing the side effects of some conventional cancer treatments.

Much research obviously remains to be done before neem can be recommended for human use, but the consistently spectacular results from these *in vitro* and preclinical studies have already inspired tremendous enthusiasm and hope. The abstracts and links that follow provide an overview of these studies.

Recent Research

Int Immunopharmacol. 2009 Jun;9(6):753-60. Epub 2009 Mar 12.

Induction of type 1 cytokines during neem leaf glycoprotein assisted carcinoembryonic antigen vaccination is associated with nitric oxide production

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Involvement of the nitric oxide (NO) release in CEAM phi NLGP (carcinoembryonic antigen pulsed macrophages with neem leaf glycoprotein) vaccination and its relationship with vaccine induced type 1 immune response were aimed to study in the present communication. Vaccination with CEAM phi NLGP resulted in macrophage activation as evidenced by its increased number and expression of CD69 marker. Activated macrophages demonstrated upregulation in synthesis of IL-12 and downregulation in IL-10, along with excess IFN gamma production in splenic cells, as evidenced from mRNA analysis. Induction of such type 1 immunity was further confirmed by expression of type 1 specific transcription factor, T-bet and enhancement of intracellular glutathione content. Such vaccination also induced greater nitric oxide (NO) production from macrophages. Dependence of induced type 1 immune response on the NO release and vice versa was studied by in vitro neutralization of IFN gamma/IL-12 and in vivo inhibition of NO production by methylene blue. Obtained results clearly demonstrated the interdependence of two anti-tumor immune functions, namely, NO production and generation of type 1 immune response. Understanding of the mechanism of this NO related immune modulation would have great impact in proposing CEAM phi NLGP vaccine in clinic for the treatment of CEA+ tumors.

Invest New Drugs. 2009 Jun;27(3):246-52. Epub 2008 Aug 22.

Nimbolide a limonoid from Azadirachta indica inhibits proliferation and induces apoptosis of human choriocarcinoma (BeWo) cells.

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http://www.springerlink.com/content/b230492841u74p13/

We investigated the cytotoxic effects of nimbolide, a limonoid present in leaves and flowers of the neem tree (Azadirachta indica) on human choriocarcinoma (BeWo) cells. Treatment with nimbolide resulted in dose- and time-dependent inhibition of growth of BeWo cells with IC(50) values of 2.01 and 1.19 microM for 7 and 24 h respectively, accompanied by downregulation of proliferating cell nuclear antigen. Examination of nuclear morphology revealed fragmentation and condensation indicating apoptosis. Increase in the generation of reactive oxygen species (ROS) that was reversed by addition of reduced glutathione suggested ROS involvement in the cytotoxicity of nimbolide. A decrease in Bcl-2/Bax ratio with increased expression of Apaf-1 and caspase-3, and cleavage of poly(ADP-ribose) polymerase provide compelling evidence that nimbolide-induced apoptosis is mediated by the mitochondrial pathway. The results of the present study suggest that nimbolide has immense potential in cancer prevention and therapy based on its antiproliferative and apoptosis inducing effects.

Free Radic Res. 2009 May;43(5):492-504.

The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by modulating xenobiotic-metabolizing enzymes, DNA damage, antioxidants, invasion and angiogenesis.

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http://www.ncbi.nlm.nih.gov/sites/entrez

The neem tree has attracted considerable research attention as a rich source of limonoids that have potent antioxidant and anti-cancer properties. The present study was designed to evaluate the chemopreventive potential of the neem limonoids azadirachtin and nimbolide based on in vitro antioxidant assays and in vivo inhibitory effects on 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. Both azadirachtin and nimbolide exhibited concentration-dependent anti-radical scavenging activity and reductive potential in the order: nimbolide > azadirachtin > ascorbate. Administration of both azadirachtin and nimbolide inhibited the development of DMBA-induced HBP carcinomas by influencing multiple mechanisms including prevention of procarcinogen activation and

oxidative DNA damage, upregulation of antioxidant and carcinogen detoxification enzymes and inhibition of tumour invasion and angiogenesis. On a comparative basis, nimbolide was found to be a more potent antioxidant and chemopreventive agent and offers promise as a candidate agent in multitargeted prevention and treatment of cancer.

PMID: 19391054 [PubMed - in process]

Chemotherapy. 2009;55(3):137-44. Epub 2009 Apr 6.

Neem *(Azadirachta Indica)* Leaf Preparation Prevents Leukocyte Apoptosis Mediated by Cisplatin plus 5-Fluorouracil Treatment in Swiss Mice

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BACKGROUND: Neem (Azadirachta indica) is widely regarded as a wonder tree because of its diverse medicinal applications. We investigated the ability of neem leaf preparation (NLP) to protect against apoptosis of circulating blood cells induced by cisplatin and 5-fluorouracil (cis + 5-FU) in carcinoma-bearing mice. METHODS: Apoptosis was studied by annexin V- propidium iodide method. Total white blood cell count was performed using 3% glacial acetic acid on hemocytometer. Cytotoxicity was determined by LDH release assay and T/NK cell status was determined by flow cytometry. RESULTS: In comparison to untreated control, during cis + 5-FU therapy, significant down-regulation of leukocyte apoptosis was noted in mice pretreated with NLP or granulocyte colony stimulating factor (GCSF) during cis + 5-FU therapy. This enhanced cytotoxicity may be associated with NLP-induced increase of the cytotoxic T and NK cell pool. CONCLUSIONS: Efficacy of NLP is comparable to GCSF in its ability to protect against leukocyte apoptosis induced by cis + 5-FU. NLP would be a better choice of treatment because GCSF is tumor promoting, angiogenic and expensive.

Hum Immunol. 2009 Jan;70(1):6-15. Epub 2008 Nov 5.

Neem leaf glycoprotein directs T-bet–associated type 1 immune commitment

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http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T3B-4TVHFJD-1&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=842f2956545a47bdff39d4abf76f69be

Neem leaf glycoprotein (NLGP)-mediated immune activation and associated immune polarization was studied. NLGP-induced activation is reflected in upregulation of early activation marker CD69 on lymphocytes, monocytes, and dendritic cells. Activation is also denoted by CD45RO enhancement, with a decrease in CD45RA phenotype and CD62L (L- selectin). NLGP-activated T cells secrete greater amount of signature T-helper (Th)1 cytokines interferon-gamma and a lower amount of the Th2 cytokine interleukin (IL)-4. Similar type 1 directiveness is also observed in antigen-presenting monocytes and dendritic cells by upregulation of IL-12, tumor necrosis factor -alpha and downregulation of IL-10. Creation of the type 1 microenvironment is also assisted by NLGP-induced downregulation of FoxP3(+) T-Reg cells. A type 1-specific transcription factor, T-bet, is upregulated in circulating immune cells after their stimulation with NLGP. In the creation of type 1 immune network, increased phosphorylation of STAT1 and STAT4 with decreased phosphorylation of STAT3 might have significance. We conclude that NLGP may be effective in maintaining normal immune homeostasis by upregulating type 1 response in immunosuppressed hosts, which may have significant role in the induction of host protective antitumor functions.

J Immunother. 2009 Jan;32(1):42-53.

Neem leaf glycoprotein induces perforin-mediated tumor cell killing by T and NK cells through differential regulation of IFNgamma signaling.

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http://www.ncbi.nlm.nih.gov/sites/entrez

We have demonstrated augmentation of the CD3-CD56+ natural killer (NK) and CD8+CD56_ T-cell-mediated tumor cell cytotoxicity by neem leaf glycoprotein (NLGP). These NK and T cells were isolated from the peripheral blood of head and neck squamous cell carcinoma patients with a state of immunosuppression. NLGP induces TCRalphabeta-associated cytotoxic T lymphocyte (CTL) reaction to kill oral cancer (KB) cells. This CTL reaction is assisted by NLGP-mediated up-regulation of CD28 on T cells and HLA-ABC, CD80/86 on monocytes. CTL-mediated killing of KB cells and NK-cell-mediated killing of K562 (erythroleukemic) cells are associated with activation of these cells by NLGP. This activation is evidenced by increased expression of early activation marker CD69 with altered expression

of CD45RO/CD45RA. NLGP is a strong inducer of IFNgamma from both T and NK cells; however, IFNgamma regulates the T-cell-mediated cytotoxicity only without affecting NK- cell-mediated one. Reason of this differential regulation may lie within up-regulated expression of IFNgamma-receptor on T-cell surface, not on NK cells. This NLGP-induced cytotoxicity is dependent on up-regulated perforin/granzyme B expression in killer cells, which is again IFNgamma dependent in T cells and independent in NK cells. Although, FasL expression is increased by NLGP, it may not be truly linked with the cytotoxic functions, as brefeldin A could not block such NLGP-mediated cytotoxicity, like, concanamycin A, a perforin inhibitor. On the basis of these results, we conclude that NLGP might be effective to recover the suppressed cytotoxic functions of NK and T cells from head and neck squamous cell carcinoma patients.

PMID: 19307993 [PubMed - indexed for MEDLINE]

J Med Chem. 2008 Oct 23;51(20):6495-502. Epub 2008 Sep 25.

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http://pubs.acs.org/doi/abs/10.1021/jm8007486

Gedunin (1), a tetranortriterpenoid isolated from the Indian neem tree (Azadirachta indica), was recently shown to manifest anticancer activity via inhibition of the 90 kDa heat shock protein (Hsp90) folding machinery and to induce the degradation of Hsp90-dependent client proteins similar to other Hsp90 inhibitors. The mechanism of action by which gedunin induces client protein degradation remains undetermined, however, prior studies have demonstrated that it does not bind competitively versus ATP. In an effort to further probe the mechanism of action, 19 semisynthetic derivatives of gedunin were prepared and their antiproliferative activity against MCF-7 and SkBr3 breast cancer cells determined. Although no compound was found to exhibit antiproliferative activity more effective than the natural product, functionalities critical for antiproliferative activity have been identified.

Vaccine. 2008 Aug 12;26(34):4352-62. Epub 2008 Jun 30.

Gedunin, a Novel Hsp90 Inhibitor: Semisynthesis of Derivatives and Preliminary Structure−Activity Relationships

Neem leaf glycoprotein helps to generate carcinoembryonic antigen specific anti-tumor immune responses utilizing macrophage-mediated antigen presentation.

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In an objective to generate effective carcinoembryonic antigen (CEA) specific anti-tumor immune response in Swiss mice, CEA was presented using macrophages with adjuvant help from neem leaf glycoprotein (NLGP). Such vaccination generates significantly higher antibody (IgG2a) and T cell response than immunization protocol without NLGP. NLGP controls the function of both B cells and macrophages by altering the expressions of various regulatory molecules, like, CD19, CD11b, etc. NLGP also directs CEA vaccination towards Th1 bias, by modulating cytokine secretion. This NLGP-generated anti-CEA immune response would be effective as a vaccine to lyse CEA(+) tumors in vitro and in vivo.

Food Chem Toxicol. 2008 Jul;46(7):2332-43. Epub 2008 Mar 18.

Evaluation of Azadirachta indica leaf fractions for in vitro antioxidant potential and in vivo modulation of biomarkers of chemoprevention in the hamster buccal pouch carcinogenesis model.

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We evaluated the chemopreventive potential of Azadirachta indica (neem) leaf fractions based on in vitro antioxidant assays, and in vivo inhibitory effects on 7,12- dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. In addition we also identified the major constituents in neem leaf fractions by HPLC. Analysis of the free radical scavenging activities and reducing potential of crude ethanolic extract (CEE), ethyl acetate fraction (EAF) and methanolic fraction (MF) of neem leaf revealed a

concentration-dependent increase in antioxidant potential that was in the order EAF>MF>CEE. Administration of neem leaf fractions reduced the incidence of DMBA- induced HBP carcinomas at a lower concentration compared to the crude extract. Chemoprevention by neem leaf fractions was associated with modulation of phase I and phase II xenobiotic-metabolising enzymes, lipid and protein oxidation, upregulation of antioxidant defences, inhibition of cell proliferation and angiogenesis, and induction of apoptosis. However, EAF was more effective than MF in terms of antiproliferative and antiangiogenic effects, and expression of CYP isoforms. The greater efficacy of EAF may be due to higher content of constituent phytochemicals as revealed by HPLC analysis. The results of the present study suggest that the antioxidant properties of neem leaf fractions may be responsible for modulating key hallmark capabilities of cancer cells such as cell proliferation, angiogenesis and apoptosis in the HBP carcinogenesis model.

Int Immunopharmacol. 2008 Feb;8(2):330-40. Epub 2007 Nov 20.

Neem leaf glycoprotein restores the impaired chemotactic activity of peripheral blood mononuclear cells from head and neck squamous cell carcinoma patients by maintaining CXCR3/CXCL10 balance.

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http://www.ncbi.nlm.nih.gov/pubmed/18182249?ordinalpos=1&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

Interaction between CXCL10 and CXCR3 is dysregulated in head and neck squamous cell carcinoma (HNSCC) and hampers chemotaxis of cytotoxic cells at tumor site. In continuation of the demonstration of significant immunomodulatory effects of neem leaf preparation (NLP), the active ingredient of NLP is characterized as a glycoprotein (NLGP). NLGP is responsible for in vivo immunomodulation to restrict the growth of mice tumors. Effect of NLGP in rectification of the dysregulated IFN gamma dependent chemokine and its receptor CXCR3 splice variants was investigated. Upregulated expression of CXCR3B in HNSCC-PBMC were downregulated following in vitro NLGP treatment. Unchanged expression of CXCR3A+B by NLGP with downregulation of the CXCR3B indirectly suggests the upregulation of the CXCR3A, responsible for cellular migration. However, stimulation of healthy-PBMC with NLGP maintains physiological homeostasis of CXCL10 and increases IFN gamma secretion. The suppressed chemotaxis of HNSCC-PBMC could be restored either by in vitro treatment with NLGP or during use of NLGP stimulated PBMC supernatant as a chemoattractant.

Neutralization studies confirmed that the chemoattraction process is guided by both receptor (CXCR3A) and its ligand (CXCL10). Neutralization of the IFN gamma in PBMC culture in presence of NLGP unexpectedly increases the intracellular release of CXCL10, suggesting the NLGP mediated IFN gamma independent release of CXCL10. Interestingly, downregulation of the CXCL10 release was detected after IFN gamma neutralization in absence of NLGP and IFN gamma receptor neutralization in presence of NLGP. Efficacy of NLGP in restoration of the dysregulation of the chemokine signaling may be utilized to design new immunotherapeutic protocol.

PMID: 18182249 [PubMed - indexed for MEDLINE]

Phytother Res. 2007 Oct;21(10):914-20.

Neem leaf preparation induces apoptosis of tumor cells by releasing cytotoxic cytokines from human peripheral blood mononuclear cells.

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A neem leaf preparation (NLP) was investigated for its role in the induction of tumor cell apoptosis to elucidate the mechanism of NLP mediated immunoprophylaxis in tumor growth restriction. As NLP did not induce direct apoptosis of human tumor cell lines KB, MCF7 and K562, it was used instead to stimulate human peripheral blood mononuclear cells (PBMC) for 72 h. The PBMC derived culture supernatant (NLP-CS) was observed to induce the restriction of tumor cell proliferation as well as apoptosis. An enzyme linked immunosorbant assay revealed the presence of cytotoxic cytokines, IFN-gamma and TNF-alpha, in the NLP-CS. The inhibition of secretion of IFN-gamma and TNF-alpha in NLP-CS caused a significant decrease in tumor cell apoptosis. Furthermore, stimulation of these tumor cells with NLP-CS resulted in upregulation of the caspase 3 and downregulation of the Bcl 2 and cyclin D1. These observations suggested that NLP could induce tumor cellular apoptosis by releasing cytotoxic cytokines from human PBMC.

PMID: 17562567 [PubMed - indexed for MEDLINE]

Hum Immunol. 2007 Oct;68(10):823-31. Epub 2007 Aug 31.

Natural killer cell mediated cytotoxicity of tumor cells initiated by neem leaf preparation is associated with CD40-CD40L-mediated endogenous production of interleukin-12.

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Neem leaf preparation (NLP) was found to activate natural killer (NK) cells (CD56(+)CD3(-)) to enhance their cytotoxic ability to tumor cells and stimulate the release of interleukin-12 (IL- 12) from macrophages from healthy individuals and head-and-neck squamous cell carcinoma patients. NLP upregulated cytotoxic (CD16(+) and CD56(dim)) NK cells, and the cytotoxicity of NK-sensitive K562 cells by NLP-stimulated peripheral blood mononuclear cells decreased significantly after IL-12 neutralization. This NK-mediated cytotoxicity was manifest by upregulation of IL-12-dependent intracellular expression of the perforin-granzyme B system. Moreover, NK cytotoxic function was abolished after use of concanamycin A, a perforin inhibitor, but not by brefeldin A, a Fas inhibitor, confirming the participation of the perforin- granzyme B system. In addition NLP upregulated the expression of CD40 in CD14(+) monocytes and CD40L in CD56(+) lymphocytes. Neutralization of CD40 and CD40L in NLP- stimulated peripheral blood mononuclear cells culture resulted in significant downregulation of IL-12 release and cytotoxicity of NK cells, demonstrating the role of a CD40-CD40L interaction in the observed functions. Signals involved in the NLP-induced release of IL-12, and thereby induction of NK cell cytotoxicity, are mediated by activating p38MAPK pathway, but not through the ERK1/2 signaling pathway. Overall the results suggest that NLP effects NK cellular cytotoxicity by CD40-CD40L-mediated endogenous production of IL-12, which critically controls perforin-dependent tumor cell cytotoxicity.
PMID: 17961770 [PubMed - indexed for MEDLINE]

Cancer Immun. 2007 Mar 30;7:8.

Neem leaf preparation enhances Th1 type immune response and anti-tumor immunity against breast tumor associated antigen.

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An 85-kDa breast tumor associated antigen (BTAA) has been identified and partially characterized from human breast tumors. As BTAA is poorly immunogenic, enhancement of the anti-tumor immunity induced by BTAA is required to obtain an objective clinical response. The potent immune activation by an aqueous preparation of neem (Azadirachta indica) leaf (NLP) suggests its possible utility for enhancing immune responses to tumor vaccines. Mice (Swiss and Balb/c) and rats (Sprague Dawley) immunized with BTAA and NLP have a higher IgG antibody response and a lower IgM response than mice immunized with BTAA alone.

Antibody generated by immunization with BTAA and NLP can induce antibody-dependent cellular cytotoxicity (ADCC) and cytotoxic T cell (CTL) response towards BTAA-expressing MCF-7 cells. Antibody produced by vaccination with BTAA alone generated little cytotoxic response. The occurrence of ADCC and CTL response induced by BTAA plus NLP vaccination was possibly assisted by the induction of a Th1 response, as evidenced by the enhanced secretion of IFN-gamma and decreased release of IL-10 from spleen cells and the greater production of IgG2a antibody in immunized mice. As NLP is nontoxic, abundantly available in the Indian subcontinent and can be extracted by a cost-effective method, this preparation may be considered a promising immune enhancer for BTAA vaccine.

PMID: 17394284 [PubMed - indexed for MEDLINE]

Phytother Res. 2007 Mar;21(3):245-50.

Antiproliferative effect on human cancer cell lines after treatment with nimbolide extracted from an edible part of the neem tree (Azadirachta indica). **Roy MK**, **Kobori M**, **Takenaka M**, **Nakahara K**, **Shinmoto H**, **Isobe S**, **Tsushida T**. National Food Research Institute, 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan.
http://www.ncbi.nlm.nih.gov/pubmed/17163581?ordinalpos=4&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

Nimbolide, a triterpenoid extracted from the flowers of the neem tree (Azadirachta indica), was found to have antiproliferative activity against some cancer cell lines. Treatment of cells with 0.5-5.0 microm concentrations of nimbolide resulted in moderate to very strong growth inhibition in U937, HL-60, THP1 and B16 cell lines. Flow cytometric analysis of U937 cells showed that nimbolide treatment (1-2.5 microm) resulted in cell cycle disruption by decreasing the number of cells in G0/G1 phase, with initial increases in S and G2/M phases. Cells exposed to a higher dose of nimbolide for a longer period displayed a severely damaged DNA profile, resulting in a remarkable increase in the number of cells in the sub-G1 fraction, with a reciprocal decrease of cells in all phases. Quantification of the expression of phosphatidylserine in the outer cell membrane showed that doses of nimbolide higher than 0.4 microm exerted remarkable lethality, with over 60% of cells exhibiting apoptotic features after exposure to 1.2 microm nimbolide. The antiproliferative effect of nimbolide and its apoptosis- inducing property raise hope for its use in anticancer therapy by enhancing the effectiveness of cell cycle disruption.

PMID: 17163581 [PubMed - indexed for MEDLINE]

Int Immunopharmacol. 2007 Mar;7(3):306-12. Epub 2006 Dec 12.

Antibody response against neem leaf preparation recognizes carcinoembryonic antigen.

Sarkar K, **Bose A**, **Laskar S**, **Choudhuri SK**, **Dey S**, **Roychowdhury PK**, **Baral R**. Department of Immunoregulation and Immunodiagnostics, Chittaranjan National Cancer Institute, Kolkata-700026, India. http://www.ncbi.nlm.nih.gov/pubmed/17276888?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

An immune serum generated in Swiss mice against an aqueous preparation from neem leaf was reactive with carcinoembryonic antigen (CEA) and a peptide sequence derived from it. Using ELISA, we have demonstrated that CEA reactive antibody titer (chiefly IgG2a) was significantly decreased after absorption of the immune sera with CEA. Neem leaf preparation (NLP) generated immune sera was also reactive with CEA in immunoblotting and CEA reactive component in the NLP sera can be immunoprecipitated. Identical recognition of CEA expressed on human colorectal cancer specimens, by anti-CEA monoclonal antibody and NLP sera was documented by immunohistochemistry. NLP generated sera could also react with NLP in ELISA and this reactivity was decreased after absorption of the NLP with anti-CEA antibody. Estimation of protein in NLP revealed the presence of it, at least 10% of the total dry weight. In addition, existence of flavone and quercetin was also evidenced from LC-MS analysis. Further studies are needed to identify the antigenic component may have some homology with CEA molecule. This unique property of neem may be utilized for the immunotherapy of CEA positive tumors.

PMID: 17276888 [PubMed - indexed for MEDLINE]

International Journal of Oncology

2006 Nov;29(5):1269-78.

Chemopreventative strategies targeting the MGMT repair protein: augmented expression in human lymphocytes and tumor cells by ethanolic and aqueous extracts of several Indian medicinal plants.

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O6-alkylguanines are potent mutagenic, pro-carcinogenic and cytotoxic lesions induced by exogenous and endogenous alkylating agents. A facilitated elimination of these lesions by increasing the activity of O6-methylguanine-DNA methyltransferase (MGMT) is likely to be a

beneficial chemoprevention strategy, which, however, has not been examined. Because, a marginal enhancement of this protein may be adequate for genomic protection, we studied alterations in MGMT activity and expression in human peripheral blood lymphocytes and cancer cell lines induced by water-soluble and alcohol-soluble constituents of several plants with established antioxidant and medicinal properties. Both the ethanolic and aqueous extracts from neem (Azadirachta indica), holy basil (Ocimum sanctum), winter cherry (Withania somnifera), and oregano (Origanum majorana) increased the levels of MGMT protein and its demethylation activity in a time-dependent manner with a maximum of 3-fold increase after 72-h treatment. The extracts from gooseberry (Emblica officinalis), common basil (Ocimum basilicum), and spearmint (Mentha viridis) were relatively less efficient in raising MGMT levels. Increased levels of MGMT mRNA accounted at least, in part, for the increased activity of the DNA repair protein. The herbal treatments also increased glutathione S-transferase-pi (GSTP1) expression, albeit to a lesser extent than MGMT. These data provide the first evidence for the upregulation of human MGMT by plant constituents and raise the possibility of rational dietary approaches for attenuating alkylation-induced carcinogenesis. Further, they reveal the putative antioxidant responsiveness of the MGMT gene in human cells.

PMID: 17016661 [PubMed - in process]

Clin Biochem. 2006 Nov;39(11):1080-7. Epub 2006 Aug 5.

Expression of PCNA, cytokeratin, Bcl-2 and p53 during chemoprevention of hamster buccal pouch carcinogenesis by ethanolic neem (Azadirachta indica) leaf extract.

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Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. http://www.ncbi.nlm.nih.gov/pubmed/16989797?ordinalpos=8&itool=EntrezSystem2.PEntrez. Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

OBJECTIVES: To evaluate the effect of ethanolic neem leaf extract (ENLE) on cell proliferation, differentiation and apoptosis associated proteins during 7,12- dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. DESIGN AND METHODS: Hamsters were divided into four groups. The right buccal pouches of animals in group 1 were painted with 0.5% DMBA three times a week. Animals in group 2 painted with DMBA as in group 1, received in addition, intragastric administration of ENLE (200 mg/kg bw) on days alternate to DMBA application. Group 3 animals were given ENLE (200 mg/kg bw) alone. Animals in group 4 served as control. All the animals were sacrificed after an experimental period of 14 weeks. The expression of proliferating cell nuclear antigen (PCNA), cytokeratin, Bcl-2 and p53 in the buccal pouch tissues were

investigated using immunohistochemical staining. In addition, the expression of p53 was confirmed by Western blot analysis. RESULTS: Topical application of DMBA for 14 weeks induced buccal pouch carcinomas associated with increased expression of PCNA, mutant p53 and Bcl-2 and decreased expression of cytokeratin. Administration of ENLE significantly inhibited the development of HBP carcinomas as revealed by decreased expression of PCNA, mutant p53 and Bcl-2 and overexpression of cytokeratin. CONCLUSION: These findings suggest that ENLE exerts its anticancer properties by inhibiting cell proliferation and inducing differentiation and apoptosis.

PMID: 16989797 [PubMed - indexed for MEDLINE]

Asian Pacific Journal of Cancer Prevention

2006 Jul-Sep;7(3):467-71.

Antioxidative and Modifying Effects of a Tropical Plant Azadirachta indica (Neem) on Azoxymethane-induced Preneoplastic Lesions in the Rat Colon.

Chaimuangraj S, **Arakaki J**, **Suzui M**, **Morioka T**, **Kinjo T**, **Kaneshiro T**, **Inamine M**, **Sunagawa N**, **Nishimaki T**, **Yoshimi N**.
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The purpose of the present study was to examine whether Neem leaf (Azadirachta indica) has short-term chemopreventive effects on endpoint preneoplastic lesions involved in rat colon carcinogenesis and might also exert antioxidative activity. Forty- two male F344 rats were randomly divided into 6 experimental groups. Groups 1 to 4 were given a subcutaneous injection of azoxymethane (AOM, 20 mg/kg body weight) once a week for 2 weeks. Starting one week before the first injection of AOM, rats in groups 2 to 4 received an aqueous extract of Neem leaf (20, 100, and 250 mg/kg, respectively) by gavage 3 times per week, for 5 weeks. Rats in group 5 also were given the Neem extract by gavage feeding 3 times per week for 5 weeks, while group 6 served as untreated controls. The experiment was terminated 5 weeks after the start. Dietary feeding of the Neem extract at all dose levels significantly inhibited the induction of aberrant crypt foci (ACF) ($P<0.0002$), when compared to the AOM-treated group (group 1). In groups 2 to 4, treatment of rats with the Neem extract also significantly decreased the proliferating cell nuclear antigen (PCNA) labeling indices ($P<0.0006$) of colon epithelium and ACF. Moreover, the Neem extract also showed antioxidative activity. The finding that dietary Neem has possible chemopreventive effects in the present short-term colon carcinogenesis bioassay suggests that longer-term exposure may cause suppression of tumor development.

PMID: 17059347 [PubMed - in process]

Phytother Res. 2006 Sep;20(9):814-8.

Pretreatment with neem (Azadirachta indica) leaf preparation in Swiss mice diminishes leukopenia and enhances the antitumor activity of cyclophosphamide.

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Cancer chemotherapy is associated with several life threatening complications, including bone marrow suppression and leucopenia. To overcome this problem, colony stimulating factor (CSF), granulocyte colony stimulating factor (GCSF) and granulocyte macrophage colony stimulating factor (GMCSF), can be used, however, these therapeutics are expensive and have several disadvantages, including tumor growth promoting activities. This study attempted to use an immunostimulatory neem (Azadirachta indica) leaf preparation (NLP) to prevent the cyclophosphamide (CYP) induced reduction in the WBC count. Pretreatment of mice with NLP reduced the extent of leucopenia and neutropenia in normal and tumor bearing CYP treated mice. NLP pretreatment enhanced in vitro tumor cell cytotoxicity by peripheral blood mononuclear cells (PBMC) from CYP treated mice in either normal or tumor bearing conditions. Similarly, NLP pretreatment of mice enhanced the CYP mediated in vivo tumor growth inhibition and survivability of the host. Based on these observations, it is concluded that NLP would be an effective tool to reduce CYP-induced hematological complications. Copyright (c) 2006 John Wiley & Sons, Ltd.

PMID: 16807877 [PubMed - indexed for MEDLINE]

Planta Med. 2006 Aug;72(10):917-23. Epub 2006 Jul 20.

Inhibition of colon cancer (HT-29) cell proliferation by a triterpenoid isolated from Azadirachta indica is accompanied by cell cycle arrest and up-regulation of p21.

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http://www.ncbi.nlm.nih.gov/pubmed/16858664?ordinalpos=9&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

Nimbolide, a natural triterpenoid present in the edible parts of the neem tree (Azadirachta indica), was found to be growth-inhibitory in human colon carcinoma HT-29 cells. Nimbolide treatment of cells at 2.5 - 10 microM resulted in moderate to very strong growth inhibition. Flow cytometric analysis of HT-29 cells showed that nimbolide treatment (2.5 microM, 12 h)

caused a 6.5-fold increase in the number of cells (55.6 %) in the G2/M phase compared with the control cells (8.8 %). At 48 h, the cell population in the G2/M phase decreased to 18 %, while that in the G0/G1 phase increased to 52.3 %. Western blot analysis revealed that nimbolide-mediated G2/M arrest was accompanied by the up-regulation of p21, cyclin D2, Chk2; and down-regulation of cyclin A, cyclin E, Cdk2, Rad17. At G0/G1 cell cycle arrest, modulation in the expression of the cell cycle regulatory molecules was also observed. We found that nimbolide-induced growth inhibition and cell cycle arrest were not associated with cellular differentiation. Quantification of cells with respect to the expression of phosphatidylserine in the outer cell membrane showed an increase in apoptotic cells by about 13 % after 48 h of nimbolide treatment.

PMID: 16858664 [PubMed - indexed for MEDLINE]

Bioorg Med Chem Lett. 2006 Aug 15;16(16):4391-4. Epub 2006 Jun 21. **Synthesis and biological activity of amide derivatives of nimbolide.**

Sastry BS, **Suresh Babu K**, **Hari Babu T**, **Chandrasekhar S**, **Srinivas PV**, **Saxena AK**, **Madhusudana Rao J**.

Natural Products Laboratory, Division of Organic Chemistry-I, Indian Institute of Chemical Technology, Hyderabad 500 007, India. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPl us&list_uids=16793266&query_hl=3&itool=pubmed_docsumNimbolide (1), a limonoid isolated from Azadirachta indica, is the chief cytotoxic principle in Neem leaves extract. Using nimbolide as a lead compound for anti-cancer analogue design, a series of nimbolide derivatives have been synthesized and evaluated for in vitro cytotoxic activity against a panel of human cancer cell lines. Out of 10 compounds screened 2g, 2h and 2i showed potent activity.

PMID: 16793266 [PubMed - in process]

Phytother Res. 2006 Aug 14

Preventive effects of Azadirachta indica on benzo(a)pyrene-DNA adduct formation in murine forestomach and hepatic tissues.

Gangar SC, **Sandhir R**, **Rai DV**, **Koul A**.

Department of Biophysics, Basic Medical Sciences Block, Panjab University, Chandigarh 160014, India. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPl us&list_uids=16909440&query_hl=2&itool=pubmed_docsum

In the present investigation, the effects of aqueous Azadirachta indica leaf extract (AAILE) on (3)H-benzo(a)pyrene-DNA [(3)H-B(a)P-DNA] adduct formation, the status of biotransformation enzymes and reduced glutathione (GSH) content were evaluated in the forestomach and liver of Balb/c mice. Two weeks of AAILE treatment reduced the (3)H- B(a)P-DNA adduct levels by 31.6% in forestomach tissue. Similarly, (3)H-B(a)P-DNA adduct levels were decreased by 34.7% in the liver of AAILE treated mice compared with their control counterparts. After AAILE treatment, the cytochrome P450 content decreased, whereas the GSH content increased significantly in the hepatic tissue. In the forestomach as well as in the liver, the cytochrome b5 content declined, whereas an increase in glutathione-S-transferase (GST) activity was observed in both tissues. These observations suggested that AAILE may have reduced the metabolic activation of (3)H-B(a)P with enhanced detoxification of its active metabolites, hence the observed decrease in the levels of (3)H-B(a)P-DNA adducts. These molecular and biochemical modulations observed at the initiation phase of carcinogenesis seems to be significant and could be correlated with the chemopreventive effects of A. indica against B(a)P induced forestomach tumorigenesis. Copyright 2006 John Wiley & Sons, Ltd. PMID: 16909440 [PubMed - indexed for MEDLINE]

Planta Med. 2006 Aug;72(10):917-23. Epub 2006 Jul 20.

Inhibition of Colon Cancer (HT-29) Cell Proliferation by a Triterpenoid Isolated from Azadirachta indica is Accompanied by Cell Cycle Arrest and Up- Regulation of p21.

Roy MK, **Kobori M**, **Takenaka M**, **Nakahara K**, **Shinmoto H**, **Tsushida T**.
National Food Research Institute, Kannondai, Tsukuba, Japan.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16858664&query_hl=3&itool=pubmed_docsum

Nimbolide, a natural triterpenoid present in the edible parts of the neem tree(AZADIRACHTA INDICA), was found to be growth-inhibitory in human colon carcinoma HT-29 cells. Nimbolide treatment of cells at 2.5 - 10 muM resulted in moderate to very strong growth inhibition. Flow cytometric analysis of HT-29 cells showed that nimbolide treatment (2.5 muM, 12 h) caused a 6.5-fold increase in the number of cells (55.6 %) in the G2/M phase compared with the control cells (8.8 %). At 48 h, the cell population in the G2/M phase decreased to 18 %, while that in the G0/G1 phase increased to 52.3 %. Western blot analysis revealed that nimbolide-mediated G2/M arrest was accompanied by the up-regulation of p21, cyclin D2, Chk2; and down-regulation of cyclin A, cyclin E, Cdk2, Rad17. At G0/G1 cell cycle arrest, modulation in the expression of the cell cycle regulatory molecules was also observed. We found that nimbolide-induced growth inhibition and cell cycle arrest were not associated with cellular differentiation. Quantification of cells with respect to the expression of

phosphatidylserine in the outer cell membrane showed an increase in apoptotic cells by about 13 % after 48 h of nimbolide treatment.
PMID: 16858664 [PubMed - as supplied by publisher]

Immunobiology. 2006;211(9):721-31. Epub 2006 Jun 8.

Neem (Azadirachta indica) leaf preparation induces prophylactic growth inhibition of murine Ehrlich carcinoma in Swiss and C57BL/6 mice by activation of NK cells and NK-T cells.

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http://www.ncbi.nlm.nih.gov/pubmed/17015147?ordinalpos=7&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

We have reported earlier that pretreatment of mice with neem leaf preparation (NLP) causes prophylactic growth inhibition of murine Ehrlich's carcinoma (EC) and B16 melanoma. Using adoptive cell transfer technology, here we have established that NLP-mediated activation of immune cells may be involved in tumor growth restriction. Mononuclear cells from blood and spleen of NLP-activated Swiss and C57BL/6 mice causes enhanced cytotoxicity to murine EC cells in vitro. Fractionation of spleen cells exhibited greater percentage of tumor cell lysis in macrophage and B-cell-depleted NK and T-cell-rich fractions. Flow cytometric analysis revealed in both blood and spleen, NK cells (DX5+ or NK1.1+) and NK-T cells (CD3+/DX5+ or CD3+/NK1.1+) were increased in number in Swiss, C57BL/6 and athymic nude mice after pretreatment with NLP. NLP-stimulated spleen cells showed greater secretion of TNFalpha and IFNgamma. Thus, NLP-activated NK and NK-T cells in mice may regulate tumor cell cytotoxicity by enhancing the secretion of different cytotoxic cytokines.

PMID: 17015147 [PubMed - indexed for MEDLINE]

Phytother Res. 2006 Jun 28; [Epub ahead of print]

Pretreatment with neem (Azadirachta indica) leaf preparation in swiss mice diminishes leukopenia and enhances the antitumor activity of cyclophosphamide.

Ghosh D, **Bose A**, **Haque E**, **Baral R**.
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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16807877&query_hl=3&itool=pubmed_docsum

Cancer chemotherapy is associated with several life threatening complications, including bone marrow suppression and leucopenia. To overcome this problem, colony stimulating factor (CSF), granulocyte colony stimulating factor (GCSF) and granulocyte macrophage colony stimulating factor (GMCSF), can be used, however, these therapeutics are expensive and have several disadvantages, including tumor growth promoting activities. This study attempted to use an immunostimulatory neem (Azadirachta indica) leaf preparation (NLP) to prevent the cyclophosphamide (CYP) induced reduction in the WBC count. Pretreatment of mice with NLP reduced the extent of leucopenia and neutropenia in normal and tumor bearing CYP treated mice. NLP pretreatment enhanced in vitro tumor cell cytotoxicity by peripheral blood mononuclear cells (PBMC) from CYP treated mice in either normal or tumor bearing conditions. Similarly, NLP pretreatment of mice enhanced the CYP mediated in vivo tumor growth inhibition and survivability of the host. Based on these observations, it is concluded that NLP would be an effective tool to reduce CYP-induced hematological complications. Copyright (c) 2006 John Wiley & Sons, Ltd.

PMID: 16807877 [PubMed - as supplied by publisher]

World J Gastroenterol. 2006 May 7;12(17):2749-55.

Modulatory effects of Azadirachta indica on benzo(a)pyrene-induced forestomach tumorigenesis in mice.

Gangar SC, **Sandhir R**, **Rai DV**, **Koul A**.
Department of Biophysics, Basic Medical Sciences Block, Panjab University, Chandigarh, PIN-160014, India. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16718763&query_hl=3&itool=pubmed_docsum

AIM: To evaluate the chemopreventive effects of aqueous Azadirachta indica (A indica) leaf extract (AAILE) against benzo(a)pyrene [B(a)P]-induced forestomach tumorigenesis in Balb/c mice. METHODS: Female Balb/c mice were divided into four groups of 10-12 animals each. For induction of forestomach tumors, starting from d 14 of the experi-ment, mice of B(a)P and B(a)P+A indica groups were given intra-gastric instillations of B(a)P (40 mg/kg), twice a week for four weeks. Mice of A indica and B(a)P+A indica groups were orally administered with AAILE (100 mg/kg), two weeks prior to B(a)P instillations till the end of the experiment. After 22 wk of the first B(a)P instillation, mice were sacrificed and the forestomachs were analyzed for development of tumors, scanning electron microscopy (SEM) and histopathology. RESULTS: Tumor incidence was observed to be 100% in mice that received only B(a)P.

However, treatment with AAILE reduced the tumor incidence by 58.4% as observed in mice of B(a)P+A indica group when compared to that of B(a)P group. Similarly, the tumor burden and multiplicity were seen to decrease by 87.3% and 69.6% respectively in mice of B(a)P+A indica group when compared to those of B(a)P group. Scanning electron microscopy analysis showed that AAILE treatment itself did not cause any abnormalities on the surface architecture of forestomach epithelium. In tumorous forestomach, surface disruption was observed. Over the forestomach tumors of B(a)P group of mice certain rounded structures were seen in addition to closely placed tongue-shaped squamous cells. Interestingly, these rounded structures were not observed in B(a)P + A indica group of mice. Histopathalogically, the tumors were identical and diagnosed to be papillomas. Mice from control and A indica groups of mice did not develop any forestomach tumors and showed normal histo-architecture. CONCLUSION: The present data suggest that A indica exerts chemopreventive effects against B(a)P-induced forestomach tumors in murine model. Because of lack of toxicity and ubiquitous bioavailability, A indica may play a promising role in future drug discovery and development as far as chemoprevention of cancer is concerned.

PMID: 16718763 [PubMed - indexed for MEDLINE]

In Vivo. 2006 Mar-Apr;20(2):247-51.

Enhancement of immune responses to neem leaf extract (Azadirachta indica) correlates with antineoplastic activity in BALB/c-mice.

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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16634526&query_hl=3&itool=pubmed_docsum

An aqueous plant extract from Azadirachta indica and its chromatographic fraction F1 (neem extract) exerted immunomodulating and antimetastatic activities in BALB/ c-mice. Regular subcutaneous administration of neem extract yielded significantly increased spleen weight and significant enhancement of peritoneal macrophage activity in the chemiluminescence assay, and activation marker CD-44 expression. The thymus weight and thymocyte counts did not show significant differences in the control and neem extract-treated groups, however, determination of peripheral blood cells revealed significant up-regulations of leukocyte subsets, the lymphocytes and monocytes. Flow cytometric analysis of lymphocyte supopulations documented increased counts of CD-4 and CD-8 cells and an inreased activation marker expression on lymphocytes (CD-25) and monocytes (MAC-3) in neem-treated mice compared to the control animals. To evaluate the antimetastatic activity of neem extract, sarcoma L-1 cells and lymphosarcoma RAW cells were intravenously inoculated into

BALB/c-mice. In these model systems the number of experimental lung and liver metastases decreased relevantly, however, biometrically non-significantly in neem extract-treated animals, as compared to the control mice which received injections of saline solutions. Neem extract can be regarded as an immunomodulating and antimetastatic substance which holds promise for further experimental and clinical investigation.

PMID: 16634526 [PubMed - indexed for MEDLINE]

J Ethnopharmacol. 2006 Apr 21;105(1-2):246-50. Epub 2005 Dec 27.

Anticancer effects of ethanolic neem leaf extract on prostate cancer cell line (PC-3).

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Prostate cancer (PC) is the most prevalent cancer and the leading cause of male cancer death. Azadirachta indica (neem tree) has been used successfully centuries to reduce tumors by herbalists throughout Southeast Asia. Here the present study indicated that an ethanolic extract of neem has been shown to cause cell death of prostate cancer cells (PC-3) by inducing apoptosis as evidenced by a dose-dependent increase in DNA fragmentation and a decrease in cell viability. Western blot studies indicated that treatment with neem extract showed decreased level of Bcl-2, which is anti-apoptotic protein and increased the level of Bax protein. So the neem extract could be potentially effective against prostate cancer treatment.

PMID: 16378700 [PubMed - in process]

Phytother Res. 2006 Mar;20(3):169-77.

Chemomodulatory effects of Azadirachta indica on the hepatic status of skin tumor bearing mice.

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Department of Biophysics, Basic Medical Sciences Block, Panjab University, Chandigarh- 160014, India. ashwanik@pu.ac.in http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16521106&query_hl=3&itool=pubmed_docsum

The liver plays an important role in the modulation of the process of carcinogenesis, as it is the primary site for the biotransformation of xenobiotics including carcinogens as well as anticancer drugs. The present study was designed to evaluate the biochemical alterations occurring in the liver of 7,12-dimethylbenz(a)anthracene (DMBA) induced skin tumor bearing male Balb/c mice and their modulation by aqueous Azadirachta indica leaf extract (AAILE). It was observed that skin tumor induction caused hepatic damage characterized by a decreased hepatosomatic index and significantly increased ($p < 0.001$) activities of the hepatic tissue injury marker enzymes, namely alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. However, upon treatment with AAILE, the above-mentioned alterations, including the increased activities of hepatic tissue injury marker enzymes, were significantly reversed, which signified the hepato-protective efficacy of Azadirachta indica. Increased oxidative stress was also observed in the hepatic tissue of skin tumor bearing mice as revealed by a significant increase ($p < 0.001$) in lipid peroxidation levels and a decrease in reduced glutathione contents and activities of various antioxidant enzymes studied, namely glutathione- S-transferase, glutathione peroxidase and glutathione reductase. The AAILE treatment reduced oxidative stress by decreasing lipid peroxidation levels and enhancing the reduced glutathione contents and activities of various antioxidant enzymes. The activities of the xenobiotic biotransformation enzymes, namely cytochrome P450, cytochrome b5 and glutathione-S- transferase, were found to be decreased in the hepatic tissue of tumor bearing mice. Treatment with AAILE further caused a decrease in the activity of cytochrome P450 and cytochrome b5, whereas it up-regulated the activity of glutathione-S-transferase. The significance of these observations with respect to the progress of the process of carcinogenesis is explained in the present research article.

PMID: 16521106 [PubMed - indexed for MEDLINE]

Mol Cell Biochem. 2006 Feb;283(1-2):47-55.

Inhibitory effects of Azadirachta indica on DMBA-induced skin carcinogenesis in Balb/c mice.

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ashwanik@pu.ac.in http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16444585&query_hl=3&itool=pubmed_docsum

Male Balb/c mice were divided into four groups on the basis of their respective treatments wherein mice of Group I served as controls. For induction of skin tumors, mice of Group II and IV were injected sub-cutaneously with 7,12-dimethylbenz(a)anthracene (DMBA). Mice of

Group III and IV were administered aqueous Azadirachta indica leaf extract (AAILE) thrice a week throughout the experiment. After 14 weeks of the first DMBA injection, Group II and IV mice developed tumors. In the tumor-bearing mice that received AAILE (Group IV), a significant reduction in mean tumor burden and tumor volume was observed. The tumors were confirmed to be papillomas and interestingly, the extent of hyper-chromatia was observed to be much more in skin tumors of Group II mice vis a vis the mice receiving AAILE. An increase in the extent of lipid peroxidation was observed in tumorous tissue of Group IV when compared to that of Group II mice. Glutathione (GSH) content and the activities of GSH-based antioxidant enzymes viz. glutathione peroxidase (GPx) and glutathione reductase (GR) increased significantly in the skin tissues of all the groups of mice when compared to control counterparts. Catalase activity was found to decrease significantly in the skin of mice, which received AAILE treatment only (Group III). Activity of super-oxide dismutase (SOD) decreased significantly in all the tumorous tissues (Group II and IV mice). In light of the above observations, the role of AAILE in inhibition of DMBA-induced skin carcinogenesis is discussed in the present study.

PMID: 16444585 [PubMed - indexed for MEDLINE]

Immunopharmacol Immunotoxicol. 2006;28(1):33-50.

Prophylactic dose of neem (Azadirachta indica) leaf preparation restricting murine tumor growth is nontoxic, hematostimulatory and immunostimulatory. **Haque E**, **Mandal I**, **Pal S**, **Baral R**.
Department of Immunoregulation and Immunodiagnostics, Chittaranjan National Cancer Institute, Kolkata, India. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16684666&query_hl=3&itool=pubmed_docsum

Significant restriction of growth of Ehrlich's carcinoma was observed following prophylactic treatment on Swiss albino mice with neem leaf preparation (NLP-1 unit) once weekly for four weeks. Toxic effects of this particular dose (1 unit), along with 0.5 unit and 2 units of NLP doses, were evaluated on different murine physiological systems. One hundred percent of mice could tolerate 4 injections of 0.5 and 1 unit NLP doses. Body weight, different organ-body weight ratios and physical behavior of treated mice remained completely unchanged during treatment with different NLP doses. All of these NLP doses were observed to stimulate hematological systems as evidenced by the increase in total count of RBC, WBC and platelets and hemoglobin percentage. As histological changes as well as elevation in serum alkaline phosphatase, SGOT, SGPT were not observed in mice treated with three different doses of NLP, the nonhepatotoxic nature of NLP was proved. The level of serum urea remained

unaltered and normal architecture of the cortical and medullary parts of the kidney were also preserved after NLP treatment. Increased antibody production against B16 melanoma antigen was detected in mice immunized with 0.5 unit and 1 unit of NLP. Number of splenic T lymphocytes (CD4+ and CD8+) and NK cells were also observed to be increased in mice injected with 0.5 unit and 1 unit of NLP. However, NLP dose of 2 units could not exhibit such immunostimulatory changes; NLP mediated immunostimulation was correlated well with the growth restriction of murine carcinoma. In other words, tumor growth restriction was observed only when mice were injected with immunostimulatory doses of NLP (0.5 unit and 1 unit). PMID: 16684666 [PubMed - indexed for MEDLINE]

Asian Pac J Cancer Prev. 2005 Oct-Dec;6(4):515-20.

Ethanolic neem (Azadirachta indica) leaf extract induces apoptosis in the hamster buccal pouch carcinogenesis model by modulation of Bcl-2, Bim, caspase 8 and caspase 3.

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Induction of apoptosis is one of the most active strategies in cancer chemoprevention and the ability of medicinal plants in this regard has attracted major research interest. The present study was designed to investigate the apoptosis inducing capacity of an ethanolic neem leaf extract (ENLE) during 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis using the apoptosis-associated proteins Bcl-2, Bim, caspase 8 and caspase 3 as markers. Topical application of DMBA to the hamster cheek pouch for 14 weeks resulted in well developed squamous cell carcinomas associated with increased expression of Bcl-2 and decreased expression of Bim, caspase 8 and caspase 3. Administration of ENLE inhibited DMBA-induced hamster buccal pouch (HBP) carcinogenesis, as revealed by the absence of neoplasms, with induction of Bim and caspases 8 and 3 and inhibition of Bcl-2 expression. Our results suggest that the chemopreventive effects of ENLE may be mediated by induction of apoptosis.

PMID: 16436003 [PubMed - indexed for MEDLINE]

Asian Pac J Cancer Prev. 2005 Jul-Sep;6(3):263-9.

Quinone reductase inducers in Azadirachta indica A. Juss flowers, and their mechanisms of action.

Sritanaudomchai H, **Kusamran T**, **Kuakulkiat W**, **Bunyapraphatsara N**, **Hiransalee A**, **Tepsuwan A**, **Kusamran WR**.
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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16235984&query_hl=3&itool=pubmed_docsum

We have previously shown that the flowers of neem tree (Azadirachta indica A. Juss, family Meliaceae), Thai variety, strongly induced the activity of glutathione S-transferase (GST) while resulting in a significant reduction in the activities of some cytochrome P(450)- dependent monooxygenases in rat liver, and possess cancer chemopreventive potential against chemically-induced mammary gland and liver carcinogenesis in rats. In the present study, 2 chemicals possessing strong QR inducing activity were fractionated from neem flowers using a bioassay based on the induction of QR activity in mouse hepatoma Hepa 1c1c7 cultured cells. Spectroscopic characteristics revealed that these compounds were nimbolide and chlorophylls, having CD (concentration required to double QR specific activity) values of 0.16 and 3.8 mug/ml, respectively. Nimbolide is a known constituent of neem leaves, but was found for the first time here in the flowers. Both nimbolide and chlorophylls strongly enhanced the level of QR mRNA in Hepa 1c1c7 cells, as monitored by northern blot hybridization, indicating that the mechanism by which these constituents of neem flowers induced QR activity is the induction of QR gene expression. These findings may have implication on cancer chemopreventive potential of neem flowers in experimental rats previously reported.

PMID: 16235984 [PubMed - indexed for MEDLINE]

Cell Biochem Funct. 2005 Jul-Aug;23(4):229-38.

Ethanolic leaf extract of neem (Azadirachta indica) inhibits buccal pouch carcinogenesis in hamsters.

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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15473007&query_hl=3&itool=pubmed_docsum

We evaluated the chemopreventive effects of ethanolic neem leaf extract in the initiation and post-initiation phases of 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. The frequency of bone marrow micronuclei as well as the concentrations of lipid peroxides, ratio of reduced to oxidized glutathione (GSH/GSSG), and

the activities of the GSH-dependent enzymes glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in the buccal pouch, liver and erythrocytes were used as biomarkers of chemoprevention. All the hamsters painted with DMBA alone for 14 weeks developed buccal pouch carcinomas that showed diminished lipid peroxidation and enhanced antioxidant status associated with increased frequencies of bone marrow micronuclei. In the liver and erythrocytes of tumour-bearing animals, enhanced lipid peroxidation was accompanied by compromised antioxidant defences. Administration of ethanolic neem leaf extract effectively suppressed DMBA-induced HBP carcinogenesis as revealed by the absence of tumours in the initiation phase and reduced tumour incidence in the post-initiation phase. In addition, ethanolic neem leaf extract modulated lipid peroxidation and enhanced antioxidant status in the pouch, liver and erythrocytes and reduced the incidence of bone marrow micronuclei. The results of the present study, demonstrate that ethanolic neem leaf extract inhibits the development of DMBA-induced HBP tumours by protecting against oxidative stress.

PMID: 15473007 [PubMed - indexed for MEDLINE]

Int Immunopharmacol. 2005 Jul;5(7-8):1343-52. Epub 2005 Apr 12. **Immunostimulatory neem leaf preparation acts as an adjuvant to enhance the efficacy of poorly immunogenic B16 melanoma surface antigen vaccine.**

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http://www.ncbi.nlm.nih.gov/pubmed/15914339?ordinalpos=18&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

Immunogenecity of the poorly immunogenic B16 melanoma cell surface antigen (B16MelSAg) was enhanced by combining B16MelSAg with NLP in C57BL/6 mice, as evidenced by ELISA and flow cytometry. NLP was as effective as Freund's complete and incomplete adjuvant to generate antibodies recognizing the B16MelSAg. The NLP generated antibody was a gamma globulin with a subtype of IgG1. Splenic lymphocytes from B16MelSAg+NLP treated mice proliferated more rapidly in vitro when stimulated by specific (B16MelSAg) and nonspecific (ConA) stimulators, in comparison to the proliferation detected in B16MelSAg and NLP treated groups. Vaccination of mice with B16MelSAg+NLP more efficiently prevented the growth of B16 melanoma tumor than mice immunized with B16MelSAg or NLP alone. In another experiment, the immune sera (B16MelSAg+NLP) was mixed with B16Mel tumors and injected subcutaneously into syngenic C57BL/6 mice. Tumor burden was less in mice receiving a tumor along with B16MelSAg+NLP generated immune

sera than other groups. The B16MelSAg+NLP generated immune sera induced antibody dependent cellular cytotoxicity specifically towards B16Mel tumor cells in vitro. We concluded that NLP might be a potential immune adjuvant for inducing active immunity towards tumor antigens.

PMID: 15914339 [PubMed - indexed for MEDLINE]

J Exp Clin Cancer Res. 2005 Jun;24(2):223-30.

Modulation of xenobiotic-metabolizing enzymes by ethanolic neem leaf extract during hamster buccal pouch carcinogenesis.

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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16110755&query_hl=3&itool=pubmed_docsum

Chemoprevention by medicinal plants is a promising approach for controlling cancer. There is substantial evidence to indicate that chemopreventive agents exert their anticarcinogenic effects by modulation of phase I and phase II xenobiotic-metabolizing enzymes. Therefore, we examined the chemopreventive potential of ethanolic neem leaf extract (ENLE) on 7,12- dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. Hamsters were divided into four groups of six animals each. The right buccal pouches of animals in Group I were painted with 0.5 per cent DMBA in liquid paraffin three times per week. Animals in Group 2 painted with DMBA as in group 1, received in addition, intragastric administration of ENLE at a concentration of 200 mg/kg bw three times per week on days alternate to DMBA application. Group 3 was given ENLE alone. Animals in Group 4 served as controls. All animals were killed after an experimental period of 14 weeks. Five out of six hamsters painted with DMBA alone developed squamous cell carcinomas in the buccal pouch. The HBP tumours showed an increase in phase I carcinogen activation (cytochrome P450 and b5) and phase II detoxification enzyme (glutathione-S-transferase, DT-diaphorase and NADPH-diaphorase) activities. In the liver of tumour-bearing animals, enhanced cytochrome P450 and b5 levels were accompanied by a decrease in phase II detoxification enzyme activities. Administration of ENLE effectively suppressed DMBA-induced HBP tumours, decreased cytochrome P450 and b5 levels, and enhanced phase II enzyme activities in the pouch and liver. Our results suggest that the modulation of DMBA metabolism is a possible mechanism for the chemopreventive effects of ethanolic neem leaf extract.

PMID: 16110755 [PubMed - indexed for MEDLINE]

J Ethnopharmacol. 2005 May 13;99(1):109-12.

Antioxidant activity of Siamese neem tree (VP1209).

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Leaves, fruits, flowers and stem bark extracts from the Siamese neem tree (Azadirachta indica A. Juss var. siamensis Valeton, Meliaceae) were assessed for antioxidant activity in vitro using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay, total antioxidant activity and inhibition of lipid peroxidation in Chago K1 cancer cell culture by the thiobarbituric acid reactive substances (TBARS) method. The results showed that leaf aqueous extract, flower and stem bark ethanol extracts exhibited higher free radical scavenging effect on the DPPH assay with 50% scavenging activity at 26.5, 27.9 and 30.6 microg/ml, respectively. The total antioxidant activity of these extracts was found to be 0.959, 0.988 and 1.064 mM of standard trolox, respectively. At 100 microg/ml, the flower ethanol and leaf aqueous extracts significantly decreased malondialdehyde (MDA) levels (46.0 and 50.6%, respectively) by the TBARS method. The results suggest that extracts from leaf, flower and stem bark of the Siamese neem tree have strong antioxidant potential. This report supports the ethnomedical use of young leaves and flowers of this plant as a vegetable bitter tonic to promote good health.

PMID: 15848028 [PubMed - indexed for MEDLINE]

Med Food. 2004 Fall;7(3):334-9.

Effects of aqueous extracts of garlic (Allium sativum) and neem (Azadirachta indica) leaf on hepatic and blood oxidant-antioxidant status during experimental gastric carcinogenesis.

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The modifying effects of aqueous extracts of garlic and neem leaf during the pre-initiation and post-initiation phases of gastric carcinogenesis induced by N-methyl-N'-nitro-N- nitrosoguanidine were investigated in male Wistar rats. The extent of lipid peroxidation and

the status of phase II biotransformation enzymes such as glutathione peroxidase and glutathione-S-transferase that use reduced glutathione (GSH) as substrate were used to biomonitor the chemopreventive potential of these extracts. Enhanced lipid peroxidation in the liver and blood of tumor-bearing animals was accompanied by significant decreases in the activities of GSH-dependent antioxidants in the pre-initiation as well as in the post-initiation phases. Our results suggest that the modulatory effects of garlic and neem leaf on hepatic and blood oxidant-antioxidant status may play a key role in preventing cancer development at extrahepatic sites.

PMID: 15383228 [PubMed - indexed for MEDLINE]

Trans R Soc Trop Med Hyg. 2004 Jul;98(7):435-7.

An antimalarial extract from neem leaves is antiretroviral.

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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=15138081&query_hl=3&itool=pubmed_docsum

An acetone-water neem leaf extract with antimalarial activity was evaluated in vitro at 5 microg/ml for inhibition of adhesion of malaria parasite-infected erythrocytes and cancer cells to endothelial cells, and at 10 microg/ml for protection of lymphocytes against invasion by HIV. The extract was also evaluated in 10 patients with HIV/AIDS at 1000 mg daily for 30 d. The mean binding of infected erythrocytes and cancer cells per endothelial cell was 15 and 11 respectively in the absence of the extract, and 0 and 2 respectively in with the extract. In the absence and presence of the extract, 0% and 75%, respectively, of lymphocytes were protected. In the treated patients, haemoglobin concentration, mean CD4+ cell count and erythrocyte sedimentation rate, which were initially 9.8 g/dl, 126 cells/microl and 90 mm/h respectively, improved to 12.1 g/dl, 241 cells/microl and 49 mm/h. Mean bodyweight and platelet count, initially 57 kg and 328 x 10(3)/mm3 respectively, increased to 60 kg and 359 x 10(3)/mm3. No adverse effects were observed during the study. The extract showed antiretroviral activity with a mechanism of action that may involve inhibition of cytoadhesion. The results may help in the development of novel antiretroviral and antimalarial drugs. PMID: 15138081 [PubMed - indexed for MEDLINE]

Toxicology. 2004 May 20;198(1-3):83-90.

Pesticide exposure--Indian scene.

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Use of pesticides in India began in 1948 when DDT was imported for malaria control and BHC for locust control. India started pesticide production with manufacturing plant for DDT and benzene hexachloride (BHC) (HCH) in the year 1952. In 1958, India was producing over 5000 metric tonnes of pesticides. Currently, there are approximately 145 pesticides registered for use, and production has increased to approximately 85,000 metric tonnes. Rampant use of these chemicals has given rise to several short-term and long-term adverse effects of these chemicals. The first report of poisoning due to pesticides in India came from Kerala in 1958 where, over 100 people died after consuming wheat flour contaminated with parathion. Subsequently several cases of pesticide-poisoning including the Bhopal disaster have been reported. Despite the fact that the consumption of pesticides in India is still very low, about 0.5 kg/ha of pesticides against 6.60 and 12.0 kg/ha in Korea and Japan, respectively, there has been a widespread contamination of food commodities with pesticide residues, basically due to non-judicious use of pesticides. In India, 51% of food commodities are contaminated with pesticide residues and out of these, 20% have pesticides residues above the maximum residue level values on a worldwide basis. It has been observed that their long-term, low-dose exposure are increasingly linked to human health effects such as immune-suppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer. In this light, problems of pesticide safety, regulation of pesticide use, use of biotechnology, and biopesticides, and use of pesticides obtained from natural plant sources such as neem extracts are some of the future strategies for minimizing human exposure to pesticides.
PMID: 15138033 [PubMed - indexed for MEDLINE]

J Ethnopharmacol. 2004 May;92(1):23-36.

Chemopreventive potential of Azadirachta indica (Neem) leaf extract in murine carcinogenesis model systems.

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Numerous laboratory studies reveal that various naturally occurring dietary substances can modify the patho-physiological process of various metabolic disorders and can be an effective preventive strategy for various diseases, including cancer. Indian Neem tree, Azadirachta indica A. Juss. (family: Meliaceae), contains at least 35 biologically active principles and is widely grown all over the tropics. The effect of two different doses (250 and 500 mg per kilogram body weight) of 80% ethanolic extract of the leaves of Azadirachta indica were examined on drug metabolizing Phase-I and Phase-II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase, and lipid peroxidation in the liver of 7-week-old Swiss albino mice. Also anticarcinogenic potential of Azadirachta indica leaf extract was studied adopting protocol of benzo(a)pyrene-induced fore-stomach and 7,12-dimethyl benz(a)anthracene (DMBA)-induced skin papillomagenesis. Our primary findings reveal its potential to induce only the Phase-II enzyme activity associated mainly with carcinogen detoxification in liver of mice. The hepatic glutathione S-transferase ($P < 0.005$) and DT- diaphorase specific activities ($P < 0.01$) were elevated above basal level. With reference to antioxidant enzymes the investigated doses were effective in increasing the hepatic glutathione reductase (GR), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) activities significantly (from $P < 0.005$ to $P < 0.001$). Reduced glutathione measured as non-protein sulphydryl was found to be significantly elevated in liver ($P < 0.005$) and in extrahepatic organs (from $P < 0.005$ to $P < 0.001$) examined in our study. Glutathione S- transferase (GST) and DT-diaphorase (DTD) showed a dose-dependent increase in extrahepatic organs. Chemopreventive response was measured by the average number of papillomas per mouse, as well as percentage of tumor-bearing animals. There was a significant inhibition of tumor burden, in both the tumor model system studied (from $P < 0.005$ to $P < 0.001$). Tumor incidence was also reduced by both the doses of Azadirachta indica extract.

PMID: 15099843 [PubMed - indexed for MEDLINE]

Med J Malaysia. 2004 May;59 Suppl B:208-9.

The effect of neem (Azadirachta indica) extract and dietary selenium on distribution of selenium in hepatocarcinogenesis induced rat.

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Neem, Azadirachta indica, is a plant from the family Meliaceae, known as "Pokok Semambu" in Malay community. It has been extensively used in India as traditional Ayurvedic and folklore minedicine for the treatment of various diseases. This study aimed to determine the distribution of selenium in the liver of rats during hepatocarcinogenesis when neem aqueous extract and dietary selenium was supplemented.

PMID: 15468891 [PubMed - indexed for MEDLINE]

Int Immunopharmacol. 2004 Mar;4(3):355-66.

Neem (Azadirachta indica) leaf mediated immune activation causes prophylactic growth inhibition of murine Ehrlich carcinoma and B16 melanoma.

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Conditional growth inhibition of murine Ehrlich carcinoma (EC) and B16 melanoma (B16Mel) was observed, following treatment of mice (Swiss and C57BL/6) with aqueous extract of neem (Azadirachta indica) (1 unit/mice/week for 4 weeks) either before or after inoculation of 1 x 10(6) tumor cells. Tumor inoculation after weekly injections for 4 weeks with neem leaf preparation (NLP) induced significant reduction of tumor growth (both EC and B16Mel) and increased survivability of mice. On the other hand, NLP treatment after tumor inoculation demonstrated no tumor growth inhibition in the NLP treated group in comparison to the PBS treated control. No direct cytotoxic effect of NLP towards EC and B16Mel tumor cells was observed in vitro. The spleen cells of NLP treated mice when mixed with inoculum of B16Mel tumor cells and injected into a group of mice, tumor growth was found to be significantly reduced and survivability of the tumor hosts increased remarkably in comparison to mice inoculated with tumor along with normal spleen cells. Concanavalin A (ConA) induced proliferation of lymphocytes from NLP treated mice was significantly higher than the lymphocytes of untreated mice. In in vitro, NLP by itself had no proliferative effects on lymphocytes but it co-stimulated ConA induced mitogenesis. NLP induced lymphocytosis as evidenced by increased lymphocyte count in blood as well as spleen. Flow cytometric evidence suggested that increase in CD4+ and CD8+ T cells accounted for lymphocytosis. The conditional tumor growth retardation, observed in mice treated with NLP before tumor inoculation, may be regulated by NLP mediated immune activation, having prominent role in

the cellular immune function of the tumor host. PMID: 15037213 [PubMed - indexed for MEDLINE]

Asia Pac J Clin Nutr. 2004;13(Suppl):S170.

The effect of Azadirachta indica on distribution of antioxidant elements and glutathione S-transferase activity in the liver of rats during hepatocarcinogenesis.

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The liver is often the first organ to be infected by metastasizing cancer. Hepatocarcinogenesis is one of the most prevalent and deadly cancers worldwide, which ranks seventh among cancers in order of frequency of occurrence. Numbers of natural and synthetic antioxidants are known to treat initiation and promotion of chemical carcinogenesis in experimental animal models. The effect of 5% w/v of Azadirachta indica extract in diethylnitrosamine and acetylaminofluorene induced hepatocellular carcinoma, which is a vital mechanism in cancer treatment, was studied in male Sprague dawly rats. The result of microscopic observation of the lesion score during hepatocarcinogenesis revealed that cells of cancer group without treatment were severely necrotic at week 12. However, cells of cancer group with Azadirachta indica treatment appeared nearly normal. The tracking of the elements during hepatocarcinogenesis was done using energy filtering transmission electron microscope (EFTEM). According to EFTEM results, some of antioxidant elements such Na, Ca, and P is highly distributed in Azadirachta indica treated normal and cancer group. However, the distribution is too low in normal control and cancer control group without Azadirachta indica treatment. The obtained results have shown a significant, decrease (P=0.05) of liver cytosol Glutathione S-transferase in cancer control group rats. Meanwhile, treatment with Azadirachta indica caused overall increase in liver GST activity nearly to control group. Distinct evidence from this study contribute that oral administration of 5% Azadirachta indica extract demonstrated anticancer activity by increasing the distribution of antioxidant elements and GST activity may to protect cells in preneoplastic nodules in cancer treated groups. However, there was no evidence of side effects of Azadirachta indica towards normal cells indicating Azadirachta indica as a potential preventive agent for cancer.

PMID: 15294745 [PubMed - in process]

Bioorg Med Chem Lett. 2003 Nov 17;13(22):4111-5.

Biological investigation and structure-activity relationship studies on azadirone from Azadirachta indica A. Juss.

Nanduri S, **Thunuguntla SS**, **Nyavanandi VK**, **Kasu S**, **Kumar PM**, **Ram PS**, **Rajagopal S**, **Kumar RA**, **Deevi DS**, **Rajagopalan R**, **Venkateswarlu A**.

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Azadirone 1, a limonoidal constituent of Azadirachta indica is found to possess potent cytotoxic activity against a panel of human cancer cell lines in our in vitro studies. In vitro screening of a number of semi-synthetic analogues of 1 revealed that the alpha,beta- unsaturated enone moiety or its equivalent conjugated system in A-ring, C-7 acetyloxy/chloroacetyloxy or keto group in B-ring and the furan moiety are responsible for the activity of 1 and its analogues. Compound 1 and two of the semi-synthetic analogues 10 and 13 were found to possess good in vivo antitumor activity in modified hollow fiber animal models.

PMID: 14592518 [PubMed - indexed for MEDLINE]

Asian Pac J Cancer Prev. 2003 Jul-Sep;4(3):215-23.

Comment in: Asian Pac J Cancer Prev. 2003 Jul-Sep;4(3):167-8.

Ethanolic neem leaf extract protects against N-methyl -N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis in Wistar rats.

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We evaluated the effects of ethanolic neem leaf extract on N-methyl-N'-nitro-N- nitrosoguanidine (MNNG)-induced gastric carcinogenesis in Wistar rats. The extent of lipid peroxidation and the status of the antioxidants superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) in the stomach, liver and erythrocytes were used as biomarkers of chemoprevention. Animals were divided into four groups of six animals each. Rats in group 1 were given MNNG (150 mg/kg bw) by intragastric intubation three times with a gap of 2 weeks in between the treatments. Rats in group 2 administered MNNG as in group 1, in addition received

intragastric intubation of ethanolic neem leaf extract (200 mg/kg bw) three times per week starting on the day following the first exposure to MNNG and continued until the end of the experimental period. Group 3 animals were given ethanolic neem leaf extract alone, while group 4 served as controls. All the animals were killed after an experimental period of 26 weeks. Diminished lipid peroxidation in the stomach tumour tissue was associated with enhanced antioxidant levels. In contrast to tumour tissue, enhanced lipid peroxidation with compromised antioxidant defences was found in the liver and erythrocytes of tumour bearing animals. Administration of ethanolic neem leaf extract significantly reduced the incidence of stomach tumours, modulated lipid peroxidation and enhanced antioxidant status in the stomach, liver and blood. From the results of our study, we suggest that ethanolic neem leaf extract may exert its chemopreventive effects by modulating lipid peroxidation and enhancing the antioxidant status in the stomach, liver and erythrocytes.

PMID: 14507242 [PubMed - indexed for MEDLINE]

J Nat Prod. 2002 Dec;65(12):1886-91.

Antineoplastic agents. 489. Isolation and structures of meliastatins 1-5 and related euphane triterpenes from the tree Melia dubia.

Pettit GR, **Numata A**, **Iwamoto C**, **Morito H**, **Yamada T**, **Goswami A**, **Clewlow PJ**, **Cragg GM**, **Schmidt JM**.

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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=12502333&query_hl=3&itool=pubmed_DocSum

The bark of the giant neem tree Melia dubia was found to contain 11 euphane-type triterpenes. Five new compounds, meliastatins 1-5 (1-5), proved to inhibit growth of the P388 lymphocytic leukemia cell line (ED(50) 1.7-5.6 microg/mL). Four of the others, the previously known methyl kulonate (8), kulinone (9), 16-hydroxybutyrospermol (10), and kulactone (11), were also found to inhibit (ED(50) 2.5-6.2 microg/mL) the P388 cancer cell line. In addition, two new euphane triterpenes were isolated and named dubione A (6) and dubione B (7). Structures for each of the 11 euphane triterpenes were established by spectral techniques that included HRMS and 2D NMR.

PMID: 12502333 [PubMed - indexed for MEDLINE] Life Sci. 2001 Jan 26;68(10):1153-60.

Cytotoxicity of azadirachtin A in human glioblastoma cell lines.

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The neem toxin azadirachtin A exhibits selective toxicity on insects. Despite its well-proven efficacy, the mode of action of this toxin remains obscure. The toxicity on vertebrate cells compared to insect cells is also not well characterized. We have cultivated six human glioblastoma cell lines G-28, G-112, G-60 (TP53 mutant) and G-44, G-62, G-120 (TP53 wild- type) in the presence of 28 microM of azadirachtin. This toxin concentration was chosen because it represents the 25 to 50% lethal dose in the glioma cells. Toxicity was measured in terms of cell proliferation (binucleation index), formation of micronuclei and cell survival. In the TP53 mutant cell lines, azadirachtin reduced the proportion of dividing cells and induced formation of micronuclei. Except for G-44 which showed a decrease in binucleation index, proliferation in the TP53 wild-type cell lines was unaffected by azadirachtin. In the TP53 wild- type cell lines, the decrease in micronuclei frequency is attributed to fewer cells entering mitosis to produce micronuclei. This is also apparent from the low surviving fractions. Cell survival was suppressed by 25-69% in all cell lines. The reduction of cell survival is a clear indication that azadirachtin affects reproductive integrity and cell division. The induction of micronuclei reflects DNA damage. Similar studies on damage induction in insect cell lines could elucidate the processes which precede the antifeedant and antimoulting effects of azadirachtin and other neem toxins in insects.

PMID: 11228099 [PubMed - indexed for MEDLINE]

Phytother Res. 2000 Jun;14(4):291-3.

Garlic and neem leaf extracts enhance hepatic glutathione and glutathione dependent enzymes during N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric carcinogenesis in rats.

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The protective effect of garlic (Allium sativum L.) and neem leaf (Azadirachta indica A. Juss.) was investigated on hepatic lipid peroxidation and antioxidant status during N-methyl-N'-nitro- N-nitrosoguanidine (MNNG)-induced gastric carcinogenesis in male Wistar rats. Enhanced

lipid peroxidation in the liver of tumour-bearing animals was accompanied by significant decreases in the activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST), gamma-glutamyl transpeptidase (GGT) and reduced glutathione (GSH) levels. Administration of garlic and neem leaf extracts significantly lowered lipid peroxidation and enhanced the hepatic levels of glutathione and glutathione dependent enzymes. We speculate that garlic and neem leaf significantly alter cancer development at extrahepatic sites by influencing hepatic biotransformation enzymes and antioxidants. PMID: 10861977 [PubMed - indexed for MEDLINE]

J Ethnopharmacol. 1999 Nov 1;67(2):189-95.

Chemopreventive potential of neem (Azadirachta indica) on 7,12-dimethylbenz[a]anthracene (DMBA) induced hamster buccal pouch carcinogenesis.

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http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=10619383&query_hl=3&itool=pubmed_DocSum

The inhibitory effect of the aqueous extract of neem (Azadirachta indica A. Juss.) on 7,12-dimethylbenz[a]anthracene (DMBA) induced buccal pouch carcinogenesis was investigated in Syrian male hamsters. All hamsters painted on their buccal pouch with DMBA for 14 weeks developed squamous cell carcinoma. Administration of neem leaf extract effectively suppressed oral carcinogenesis initiated with DMBA as revealed by the reduced incidence of neoplasms. Lipid peroxidation, glutathione (GSH) content and the activities of glutathione peroxidase (GPx), glutathione S-transferase (GST) and gammaglutamyl transpeptidase (GGT) were used to biomonitor the chemopreventive potential of neem. Lipid peroxidation was found to be significantly decreased, whereas GSH, GPx, GST and GGT were elevated in the oral mucosa of tumour bearing animals. Our data suggest that neem may exert its chemopreventive effects in the oral mucosa by modulation of lipid peroxidation, antioxidants and detoxification systems.

PMID: 10619383 [PubMed - indexed for MEDLINE]

Mutat Res. 1998 Jun 18;402(1-2):247-58.

Antimutagenic and anticarcinogenic potentials of some Thai vegetables.

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Fifteen kinds of commonly consumed Thai vegetables were sequentially extracted with hexane, chloroform and methanol, and then tested for antimutagenic activities against direct- acting (AF-2 and NaN3) and indirect-acting (AFB1 and B(a)P) mutagens using Ames' Salmonella mutagenicity test with Salmonella typhimurium TA100 as tester strain. It was found that only the methanol extract of neem leaves contain weak antimutagen inhibiting the mutagenicities of both direct-acting mutagens. Interestingly, all vegetables studied were found to contain chemical compounds, mainly nonpolar ones, capable of inhibiting the mutagenicity of AFB1, while only some vegetables contain chemical compounds capable of inhibiting the mutagenicity of B(a)P, which is also an indirect-acting mutagen. Studies on anticarcinogenic potentials demonstrated that Thai bitter gourd fruits, but not sweet basil leaves, at the concentration of 6.25% and 12.5% in the diet, partially inhibited DMBA-induced mammary gland carcinogenesis in female Sprague-Dawley rats when fed to the animals 2 weeks prior to DMBA. Results in the present study therefore demonstrated that most Thai vegetables contain antimutagens inhibiting the mutagenicity of some indirect-acting mutagen, particularly AFB1. The mechanism of their antimutagenicity may probably be the inhibition of the activity of metabolic-activating enzymes in rat liver homogenates. Very interestingly, our results clearly reveal that Thai bitter gourd fruits, which possess Phase II enzymes inducing property, as well as the ability to reduce Phase I enzyme activities in rat liver, contain some anticarcinogens or chemopreventive agents. However, sweet basil leaves that possess both Phase I and Phase II enzyme-inducing properties may not contain any anticarcinogen, at least against DMBA- induced mammary gland carcinogenesis.

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Cytotoxicity of nimbolide, epoxyazadiradione and other limonoids from neem insecticide.

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Neem seed preparations contain not only azadirachtin as the active insect antifeedant or growth regulator but also a variety of their limonoids, some of which are cytotoxic to N1E-115 neuroblastoma (mouse), 143B.TK- osteosarcoma (human) and Sf9 (insect) cultured cell lines. The most potent of these limonoids is nimbolide with an IC50 ranging from 4 to 10 microM, and averaging 6 microM for the three cell lines. Other limonoids of decreasing potency and their average IC50 values (microM) are epoxyazadiradione 27 microM, salannin 112 microM, and nimbin, deacetylnimbin and azadirachtin each >200 microM (practically nontoxic). Nimbolide at 10 microM acts rapidly in the neuroblastoma cells to induce blebbing associated with disruption of plasma membranes almost instantaneously and 50% loss of cell viability with 30 min. At 5 microM nimbolide, the cells become elongated and assume a neuronal shape accompanied by spikes and lamellipodia within 1-2 hr followed shortly thereafter by extensive cytological changes and and vacuolization associated with irreversible processess leading to cell death. Calcium is apparently not involved the cytotoxic effect since a calcium-free medium, leading to profound morpholigical changes, does not alter the sensitivity to nimbolide. In contrast, epoxyazadiradione requires higher concentrations and a few hr for 50 % viability loss without major morphological changes, indicating a difference in mode of action for nimbolide and epoxyazadiradione. and epoxyazadiradione.

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Progress in male contraceptive research.

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PIP: 80% of the world's contraceptive users are women. This gender-based usage has occurred due to the emphasis of family planning programs and contraception research on female methods. Even if men desired to take responsibility for contraception, only the condom and vasectomy are available and have a reasonable assurance of protection. The Population Council has been researching male contraception through its Center for Biomedical Research. An oral contraceptive derived from gossypol, a cottonseed plant pigment, is being tested after successful clinical trials were performed in China during the 1970s. Also being investigated are male hormonal methods that regulate sperm production while protecting against loss of potency, loss of libido, and changes in secondary sex characteristics. A hormonal implant, effective for one year, has been in Phase I clinical trials since 1993. A small Phase I clinical trial is in process for a vaccine/implant for men that is effective for one year. Testing with injectables for men has suggested that different hormonal mixes could increase cardiovascular risk for men and exacerbate prostate cancer. Research has focused on new materials for

condoms. Kraton-type materials are made from block copolymers and polyurethanes, and these condoms have shown some promise. The advantages of these products are that they are allergen-free, less susceptible to oxidation, and can be of thinner construction, which would increase sensitivity and acceptability. The percutaneous chemical method of no-scalpel vasectomy has been studied as a means of blocking passage of sperm in the vas deferens. In China and India, injections with liquid silicone, polyurethane, neem-oil, and n-butyl- cyanoacrylate mixed with phenol are being studied. Zinc injections that cause the epididymis to atrophy are being tested on animals in the US. Lasers and fiber cautery are other methods under investigation. Increased funding is essential for these and other research efforts. PMID: 12288916 [PubMed - indexed for MEDLINE]

IDRC Rep. 1995 Jan;22(4):10.
India's vaccine inventor: Gursaran Talwar.
Rai U. http://www.ncbi.nlm.nih.gov/pubmed/12288547?ordinalpos=35&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

PIP: Dr Gursaran Talwar, 68, has worked for almost 20 years to find a safe, long-lasting, and reversible contraceptive vaccine. The Director of India's National Institute of Immunology began his research in the mid 1970s with financial support from the Indian government and IDRC. Toxicology studies were conducted for 10 years. The vaccine increases production of antibodies against human chorionic gonadotropin (HCG), a hormone which assists in preparing the uterus for embryo implantation. The vaccine blocks this process and prevents pregnancy. Without the vaccine, 50-75% of embryos fail to be implanted because of antibodies to HCG; with the vaccine, 100% do. The vaccine is administered once a month for 3 months. Although another form of contraception must be used during this time, protection afterwards lasts for a year. Boosters are given annually. In a clinical study of 88 vaccinated women, 1 pregnancy occurred in 821 menstrual cycles. Fertility returns with discontinuation of the vaccinations. Dr Talwar is also working on a contraceptive for the 3-month period using the purified extract of the neem tree, a male contraceptive, a treatment for prostate cancer, and a vaccine against leprosy.

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Exploration of the frontiers of tradomedical practices: basis for development of alternative medical healthcare services in developing countries.

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http://www.ncbi.nlm.nih.gov/pubmed/8410912?ordinalpos=36&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_RVDocSum

The study is a brief exploration of the functions and roles of the traditional healers in the total health care delivery system as a basis for tapping the salient features of this age old art: for the purpose of refining, and establishing it as an alternative medical health-care service. The investigation is considered relevant particularly in the developing countries where, in addition to the dearth of orthodox medical services, institutions and personnel, it is relatively cheaper, socio-culturally accessible and acceptable. Refining and developing some aspects of the traditional healers' services will serve the interest of the health consumers whose main concern is with service and not the source. Furthermore, it is hoped that the study will stimulate purposeful discussions on the need for an unbiased examination of the materials, methods and techniques of the traditional healers including, eventually, compiling a native pharmacopoeia. A more comprehensive account of the traditional healers contributions to the battle against diseases and maintenance of health and well being is envisaged.

PIP: In traditional healing, practitioners use barks, leaves, nuts, fruit juices and roots, and parts of domestic animals. They practice their craft mostly in Africa, Asia, and other Third World countries, and they are variously called juju priests, diviners, herbalists, and witch doctors. Cases of achievements in their contributions to preventive and curative health have been documented. In Nigeria, clients regularly patronize both orthodox and traditional medical practitioners. Their remedies include healing the bite of the very poisonous carpet viper, chronic bronchitis, peptic ulcer, and heart problems, as well as performing uvulectomy and tonsillectomy. Quinine, the cure for malaria, was originally the ritual medicine of the Incas of Peru. It was confirmed that Azadirachta Indica (Meliaceae), the neem tree, used against malaria in Nigeria, India, and Asia, had a potent antiplasmodial activity. The plant Streblus asper, Linn (Shakhotoha Siora) is well known in Indian Ayurvedic medicine to treat fever, filariasis, dysentery, and diarrhea. The alkaloids derived from the Madagascan periwinkle Catharanthus roseus (Apocynaceae), used in a West Indian remedy for diabetes mellitus, have antitumor activity. The drug Maytensine, obtained from Mytenus ovatus Loes (Celastraceae), was found to be a powerful antitumor agent in animals. Tea made from the leaves of Osyris wightiana stimulated the flow of breast milk and also acted as a labor-inducing agent. Saponaria officinalis and Enterobbium cyclocarpum are both used in Egypt and Tanzania as spermicide contraceptives. A 1985 survey in Cross River State, Nigeria, demonstrated that 165 (61%) of respondents went to traditional healers for treatment. Part of their continued popularity is the person-centered approach that is virtually lacking in orthodox hospitals, although this humanistic approach to therapy is gradually gaining inroads into Western medical education. The services of both kinds of medicine could be harmonized by open-

minded appraisal, identification of positive aspects, and acceptance of their complimentary nature.
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Plant products as protective agents against cancer.

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Out of various spices and leafy vegetables screened for their influence on the carcinogen- detoxifying enzyme, glutathione-S-transferase (GST) in Swiss mice, cumin seeds, poppy seeds, asafoetida, turmeric, kandathipili, neem flowers, manathakkali leaves, drumstick leaves, basil leaves and ponnakanni leaves increased GST activity by more than 78% in the stomach, liver and oesophagus, - high enough to be considered as protective agents against carcinogenesis. Glutathione levels were also significantly elevated in the three tissues by these plant products. All of them except neem flowers, significantly suppressed (in vivo) the chromosome aberrations (CA) caused by benzo(a)pyrene in mouse bone marrow cells. Multiple CA and exchanges reflecting the severity of damage within a cell were significantly suppressed by these nine plant products. The results suggest that these nine plant products are likely to suppress carcinogenesis and can act as protective agents against cancer.

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