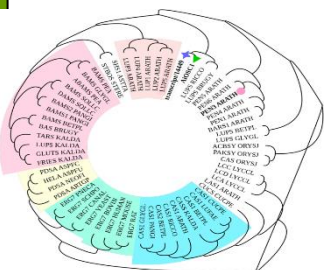
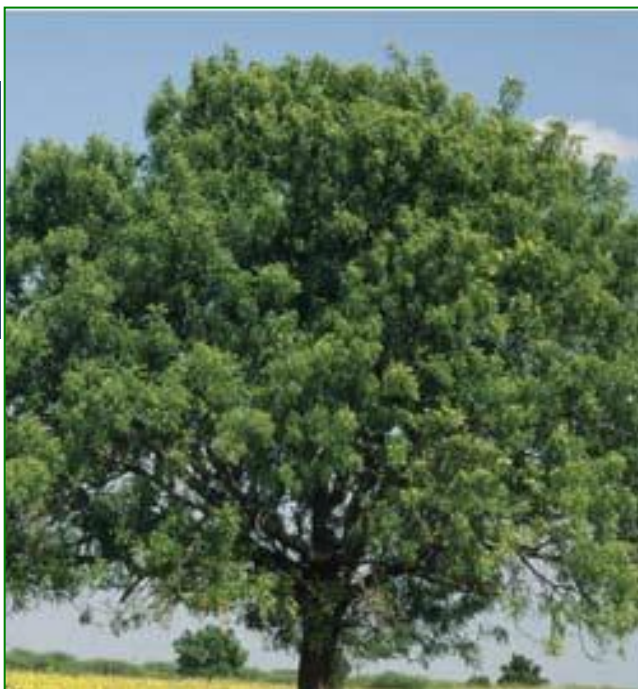
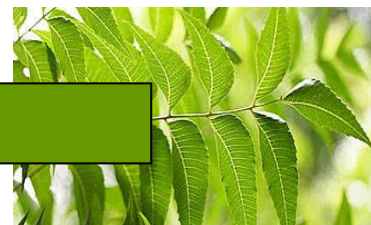




Neem Research Newsletter Volume 3, Issue 7, 2023



WORLD NEEM ORGANISATION (WNO)



From
The Editor's Desk.....

At the outset, I would like to express my deepest condolences to Dr. Nirmala Kothari, General Secretary and Core Founding Member of WNO on the demise of her husband, a committed neem enthusiast and valued guiding principle of WNO. My his soul rest in peace!

This month several research articles on the potential of neem in diverse fields have been published. Notably, neem tree has been identified to tolerate environmental pollution and therefore useful for particulate matter suppression and heavy metal stabilization in and around thermal power plants. In silico and in vivo analysis revealed that neem leaf extracts leaves can be used as an effective drug to manage inflammation. Nimbin and its semi-natural analogue N3 were found to be promising as potential candidates used alone or combined with existing drugs for further investigation and development as anti-cancer agents. Deacetylepoxiazadiradione was shown to protect zebrafish larvae from toxicity due to bisphenol (A) that leaches from plastic products by ameliorating inflammatory responses. Methanolic extract of neem leaf was demonstrated to be effective against bovine ticks.

S. Nagini

Core Founding Member, WNO
Chief Scientific Coordinator &
Regional Director, South India

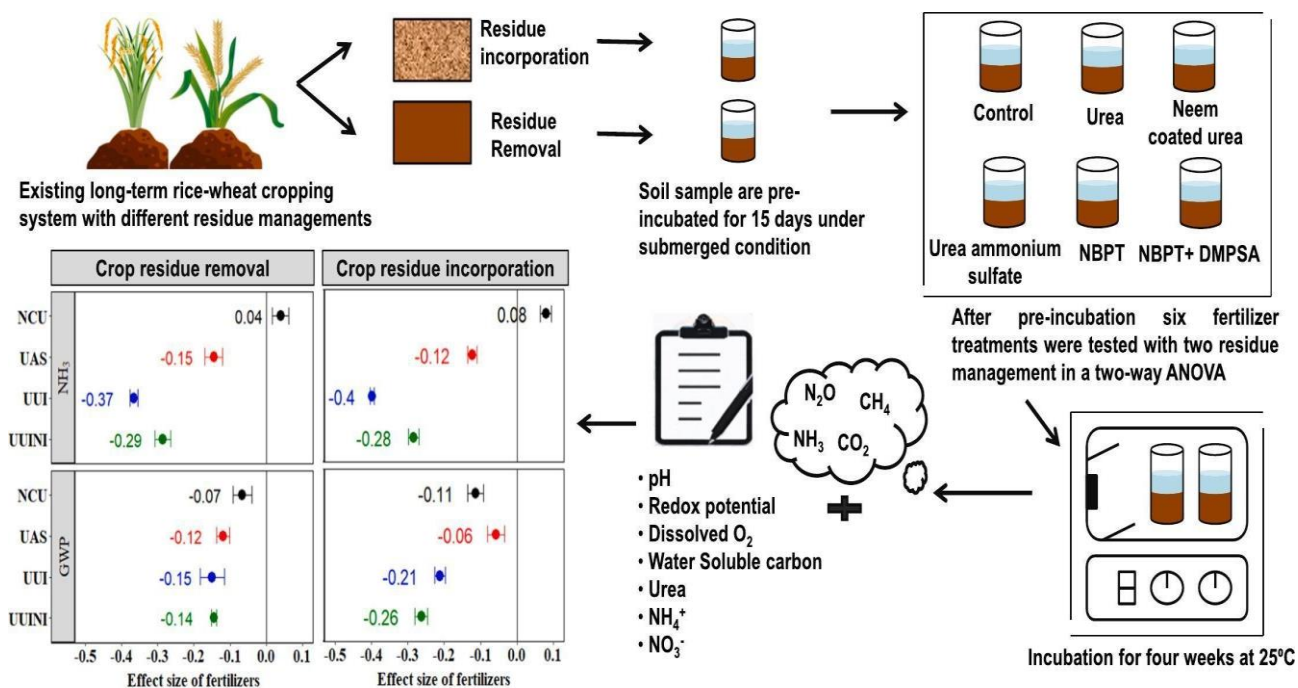


Neem in Agriculture & Aquaculture

Nitrification and urease inhibitors mitigate global warming potential and ammonia volatilization from urea in rice-wheat system in India: A field to lab experiment.

Chakraborty R, Purakayastha TJ, Pendall E, Dey S, Jain N, Kumar S. *Sci Total Environ.* 2023 Jul 15:165479. doi: 10.1016/j.scitotenv.2023.165479. PMID: 37459989

The efficacy of alternative nitrogenous fertilizers for mitigating greenhouse gas and ammonia emissions from a rice-wheat cropping system in northern India was addressed in a laboratory incubation experiment using soil from a 10-year residue management field experiment (crop residue removal, CRR, vs. incorporation, CRI). Neem coated urea (NCU), standard urea (U), urea ammonium sulfate (UAS), and two alternative fertilizers, urea + urease inhibitor NBPT (UUI) and urea + urease inhibitor NBPT + nitrification inhibitor DMPA (UUIINI) were compared to non-fertilized controls for four weeks in incubation under anaerobic condition. Effects of fertilizers on global warming potential (GWP) and ammonia volatilization were dependent on residue treatment. Relative to standard urea, NCU reduced GWP by 11 % in CRI but not significantly in CRR; conversely, UAS reduced GWP by 12 % in CRR but not significantly in CRI. UUI and UUIINI reduced GWP in both residue treatments and were more effective in CRI (21 % and 26 %) than CRR (15 % and 14 %). Relative to standard urea, NCU increased ammonia volatilization by 8 % in CRI but not significantly in CRR. Ammonia volatilization was reduced most strongly by UUI (40 % in CRI and 37 % in CRR); it was reduced 28-29 % by UUIINI and 12-15 % by UAS. Overall, the urease inhibitor, alone and in combination with the nitrification inhibitor, was more effective in mitigating greenhouse gas and ammonia emissions than NCU. However, these products need to be tested in field settings to validate findings from the controlled laboratory experiment.



ISSR markers endorsed genetically stable plants regeneration of neem (*Azadirachta indica* A. Juss.) through indirect organogenesis using different explants.

Kader A, Sinha SN, Ghosh P.

Mol Biol Rep. 2023 Jul 11. doi: 10.1007/s11033-023-08655-3. PMID: 37432543

Background: Various parts of neem (*Azadirachta indica*) have high demand in several industries. However, the inadequate supply of sources hampers the commercialization of different neem products. In this scenario, the current research was undertaken to produce genetically stable plants through indirect organogenesis. **Methods and results:** Several explants like shoot tips, internodal segments, and leaves, were cultivated on MS media with different growth regulators. Maximum callus formation was achieved using 1.5 mg/L NAA, 0.5 mg/L 2,4-D and 0.2 mg/L both for Kn and BAP in combination with shoot tip (93.67%). These calli showed an organogenic potentiality on MS medium having coconut water (15%) without growth regulators. This medium along with 0.5 mg/L Kn and 0.1 mg/L both for BAP and NAA yielded the maximum adventitious shoot production with shoot tip-derived callus (95.24%). These calli further produced the most buds per shoot (6.38) and highest average shoot length (5.46 cm) with 0.5 mg/L both for BAP and Kn and 0.1 mg/L NAA in combination after the fifth subculture. The 1/3 strength of MS media was found to be best along with 0.5 mg/L IBA and 0.1 mg/L Kn in combination to generate maximum root response (92.86%), roots per shoot (5.86) and longest average root length (3.84 cm). The mean plant survival after initial hardening was 83.33% which increased to 89.47% after secondary hardening. The lack of variation in ISSR markers among the regenerated trees is evidence of clonal fidelity between hardened plants. **Conclusions:** This protocol will accelerate the propagation of neem for utilization of its sources.

Acaricide exposure impairs predatory behavior of the phytoseiid mite *Neoseiulus idaeus* (Acari: Phytoseiidae).

de Sousa Neto EP, Mendes JA, Filgueiras RMC, de Lima DB, Guedes RNC, Melo JWS.

J Econ Entomol. 2023 Jul 4:toad127. doi: 10.1093/jee/toad127. PMID: 37402615

Predation is an important interaction that can change the structure of arthropod communities across both temporal and spatial scales. In agricultural systems predation can reduce the population levels of several arthropod pest species of a community. This predator-prey interaction involves the predator searching and handling behaviors. Several factors can affect this interaction, such as pesticide exposure, which is a frequent feature in agroecosystems. Thus, the hypothesis of our study is that the predatory behavior of the phytoseiid mite *Neoseiulus idaeus* Denmark & Muma, an important natural enemy of spider mites, is affected by acaricide exposure. To test that hypothesis, the predatory mite was exposed to the acaricides abamectin, fenpyroximate, and azadirachtin in 4 exposure scenarios. The predatory behavior of *N. idaeus* was negatively affected by acaricide exposure when the leaf surface containing both prey and predator was sprayed leading to a reduction in the frequency of transitions between predator walking and meeting preys. Prey handling and consumption were also compromised by acaricide exposure through contaminated leaf surface and prey, and contaminated leaf surface, prey, and predator. Abamectin compromised predation regardless of the exposure scenario. Acaricide-exposure reduced the number of prey found, number of attacks, and number prey killed by

N. idaeus. Moreover, partial prey consumption was observed with acaricide-exposed mites. Thus, caution is necessary while attempting to integrate acaricide applications and mass release of *N. idaeus* for spider mite management.

Using *Azadirachta indica* protein hydrolysate as a plant protein in Nile tilapia (*Oreochromis niloticus*) diet: Effects on the growth, economic efficiency, antioxidant-immune response and resistance to *Streptococcus agalactiae*.

Abdel Rahman AN, Amer SA, Behairy A, Younis EM, Abdelwarith AA, Osman A, Moustafa AA, Davies SJ, Ibrahim RE.

J Anim Physiol Anim Nutr (Berl). 2023 Jul 11. doi: 10.1111/jpn.13857. PMID: 37431590

A feeding trial for 90 days was conducted on Nile tilapia (*Oreochromis niloticus*) (average weight: 25.50 ± 0.05 g) to evaluate the effect of dietary inclusion of *Azadirachta indica* seed protein hydrolysate (AIPH). The evaluation included the impact on the growth metrics, economic efficiency, antioxidant potential, hemato-biochemical indices, immune response, and histological architectures. A total of 250 fish were randomly distributed in five treatments (n = 50) and received diets included with five levels of AIPH (%): 0 (control diet, AIPH0), 2 (AIPH2), 4 (AIPH4), 6 (AIPH6) or 8 (AIPH8), where AIPH partially replace fish meal by 0, 8.7%, 17.4%, 26.1%, and 34.8%, respectively. After the feeding trial, a pathogenic bacterium (*Streptococcus agalactiae*, 1.5 × 10⁸ CFU/mL) was intraperitoneally injected into the fish and the survival rate was recorded. The results elucidated that AIPH-included diets significantly (p < 0.05) enhanced the growth indices (final body weight, total feed intake, total body weight gain, and specific growth rate) and intestinal morpho-metrics (villous width, length, muscular coat thickness, and goblet cells count) in comparison to the control diet, with the AIPH8 diet recording the highest values. Dietary AIPH inclusion significantly improved (p < 0.05) the economic efficacy indicated by reduced feed cost/kg gain and increased performance index. The fish fed on the AIPH diets had noticeably significantly higher (p < 0.05) protein profile variables (total proteins and globulin) and antioxidant capabilities (superoxide dismutase and total antioxidant capacity) than the AIPH0 group. The dietary inclusion of AIPH significantly (p < 0.05) boosted the haematological parameters (haemoglobin, packed cell volume %, and counts of red blood cells and white blood cells) and immune indices (serum bactericidal activity %, antiprotease activity, and immunoglobulin M level) in a concentration-dependent manner. The blood glucose and malondialdehyde levels were significantly (p < 0.05) lowered by dietary AIPH (2%-8%). The albumin level and hepatorenal functioning parameters (aspartate aminotransferase, alanine aminotransferase, and creatinine) were not significantly (p > 0.05) altered by AIPH diets. Additionally, AIPH diets did not adversely alter the histology of the hepatic, renal or splenic tissues with moderately activated melano-macrophage centres. The mortality rate among *S. agalactiae*-infected fish declined as dietary AIPH levels rose, where the highest survival rate (86.67%) was found in the AIPH8 group (p < 0.05). Based on the broken line regression model, our study suggests using dietary AIPH at the optimal level of 6%. Overall, dietary AIPH inclusion enhanced the growth rate, economic efficiency, health status, and resistance of Nile tilapia to the *S. agalactiae* challenge. These beneficial impacts can help the aquaculture sector to be more sustainable.

Neem for Sustainable Environment

Evaluation of plant species for air pollution tolerance and phytoremediation potential in proximity to a coal thermal power station: implications for smart green cities.

Sawarkar R, Shakeel A, Kumar T, Ansari SA, Agashe A, Singh L.

Environ Geochem Health. 2023 Jun 27. doi: 10.1007/s10653-023-01667-9. PMID: 37368173

In metropolitan areas, air pollution poses a significant threat, and it is crucial to carefully select plant species that can tolerate such conditions. This requires a scientific approach based on systematic evaluation before recommending them to executive bodies. This study aimed to determine the air pollution tolerance index (APTI), dust retention capacity, and phytoremediation ability of 10 plant species growing in and around a lignite-based coal thermal power station. The results showed that *Ficus benghalensis* L. had the highest APTI, followed by *Mimusops elengi* L., *Ficus religiosa* L., *Azadirachta indica* A. Juss., and *Annona reticulata* L. *F. benghalensis* also showed the highest pH of leaf extract, relative water content, total chlorophyll, and ascorbic acid content, as well as the highest dust capturing capacity. Among the ten plant species, *F. benghalensis*, *M. elengi*, *F. religiosa*, *A. indica* and *F. racemosa* were identified as a tolerant group that can be used for particulate matter suppression and heavy metal stabilization in and around thermal power plants. These findings can inform the selection of plants for effective green infrastructure in smart green cities, promoting the health and well-being of urban populations. This research is relevant to urban planners, policymakers, and environmentalists interested in sustainable urban development and air pollution mitigation.

Air mapping during COVID-19 and association between air pollutants and physiochemical parameters of the plants using structural equal modeling: a case study.

Das CP, Goswami S, Swain BK, Panda BP, Das M.

Environ Monit Assess. 2023 Jul 26;195(8):997. doi: 10.1007/s10661-023-11614-x. PMID: 37493963

In urban areas around the world, air pollution introduced by vehicular movement is a key concern. However, restricting vehicular traffic during the COVID-19 shutdown improved air quality to some extent. This study was conducted out in the smart city of Bhubaneswar, which is also the state capital of Odisha, India. The study has tried to map Bhubaneswar by collecting the air quality data before, during, and after the COVID lockdown of six air quality monitoring stations present in Bhubaneswar established under "National Ambient Air Monitoring Program" (NAMP). Furthermore, plants, which are the most vulnerable to air pollution, can show a variety of visible changes depending on their level of sensitivity. Moreover, leaves of *Mangifera indica*, *Monoon longifolium*, *Azadirachta indica*, *Millettia pinnata*, *Aegle marmelos* were collected from nearby of six air monitoring stations to assess the "Air Pollution Tolerance Index." *M. indica* was found to be intermediately tolerant, and all of the other species were found to be sensitive. The structural equation modeling results also revealed a significant relationship between total chlorophyll content, relative water content, ascorbic acid content, leaf extract pH, APTI with species, air quality index, and PM₁₀.

Neem for Human Health

Pharmacoinformatics-Based Approach for Uncovering the Quorum-Quenching Activity of Phytochemicals against the Oral Pathogen, *Streptococcus mutans*.

Marimuthu SCV, Murugesan J, Babkiewicz E, Maszczyk P, Sankaranarayanan M, Thangamariappan E, Rosy JC, Ram Kumar Pandian S, Kunjiappan S, Balakrishnan V, Sundar K.

Molecules. 2023 Jul 19;28(14):5514. doi: 10.3390/molecules28145514.PMID: 37513386

Streptococcus mutans, a gram-positive oral pathogen, is the primary causative agent of dental caries. Biofilm formation, a critical characteristic of *S. mutans*, is regulated by quorum sensing (QS). This study aimed to utilize pharmacoinformatics techniques to screen and identify effective phytochemicals that can target specific proteins involved in the quorum sensing pathway of *S. mutans*. A computational approach involving homology modeling, model validation, molecular docking, and molecular dynamics (MD) simulation was employed. The 3D structures of the quorum sensing target proteins, namely SecA, SMU1784c, OppC, YidC2, CiaR, SpaR, and LepC, were modeled using SWISS-MODEL and validated using a Ramachandran plot. Metabolites from *Azadirachta indica* (Neem), *Morinda citrifolia* (Noni), and *Salvadora persica* (Miswak) were docked against these proteins using AutoDockTools. MD simulations were conducted to assess stable interactions between the highest-scoring ligands and the target proteins. Additionally, the ADMET properties of the ligands were evaluated using SwissADME and pkCSM tools. The results demonstrated that campesterol, melianol, stigmasterol, isofucosterol, and ursolic acid exhibited the strongest binding affinity for CiaR, LepC, OppC, SpaR, and YidC2, respectively. Furthermore, citrostadienol showed the highest binding affinity for both SMU1784c and SecA. Notably, specific amino acid residues, including ASP86, ARG182, ILE179, GLU143, ASP237, PRO101, and VAL84 from CiaR, LepC, OppC, SecA, SMU1784c, SpaR, and YidC2, respectively, exhibited significant interactions with their respective ligands. While the docking study indicated favorable binding energies, the MD simulations and ADMET studies underscored the substantial binding affinity and stability of the ligands with the target proteins. However, further in vitro studies are necessary to validate the efficacy of these top hits against *S. mutans*.

Antimalarial Activities of a Therapeutic Combination of *Azadirachta indica*, *Mangifera indica* and *Morinda lucida* Leaves: A Molecular View of its Activity on *Plasmodium falciparum* Proteins.

Abdulai SI, Ishola AA, Bewaji CO.

Acta Parasitol. 2023 Jul 21. doi: 10.1007/s11686-023-00698-7. PMID: 37474844

Background: The search for new antimalarial drugs remains elusive prompting research into antimalarial combinations from medicinal plants due to their cheapness, efficacy and availability. *Azadirachta indica* (AI), *Morinda lucida* (ML) and *Mangifera indica* (MI) have all been reported as potent antimalarial plants. **Purpose:** This study evaluated the efficacy of an antimalarial combination therapeutics prepared from leaves of AI, ML and MI using in vitro, in vivo and molecular methods. **Methods:** Refined extracts of the plants combination

was made by partitioning the aqueous extract of plants combinations (AI + MI, AI + ML, MI + ML, AI + MI + ML) using methanol and ethyl acetate consecutively. The resulting ethyl acetate partitioned fraction was evaluated for its antimalarial activity. Molecular docking and molecular dynamics simulation were employed to determine the possible mechanism of action of the constituent of the most active combination against four important *P. falciparum* proteins. **Results:** The result revealed that the refined extract from combinations AI + ML and MI + ML at 16 mg/kg bodyweight have the highest chemo-suppressive effect of 90.7% and 91.0% respectively compared to chloroquine's 100% at 10 mg/kg. Also, refined extract from MI + ML combination improved PCV levels significantly ($p < 0.05$) compared to controls. Molecular docking revealed oleanolic acid and ursolic acid as multiple inhibitors of plasmepsin II, hiso-aspartic protease, falcipain-2 and *P. falciparum* Eonyl acyl-carrier protein reductase with relative stability during 100 ns of simulation. **Conclusion:** The study unveiled the potentials of ML and MI as good candidates for antimalarial combination therapy and further established their use together as revealed in folklore medicine.

Revolutionizing the effect of *Azadirachta indica* extracts on edema induced changes in C-reactive protein and interleukin-6 in albino rats: in silico and in vivo approach.

Ammara A, Sobia A, Nureen Z, Sohail A, Abid S, Aziz T, Nahaa MA, Rewaa SJ, Ahellah MJ, Nouf SAA, Nehad AS, Manal YS, Amnah AA, Majid A, Abdulhakeem SA, Anas SD, Saad A.

Eur Rev Med Pharmacol Sci. 2023 Jul;27(13):5951-5963. doi: 10.26355/eurrev_202307_32947.PMID: 37458623

Objective: The aim of the present study is to determine the in vivo and in silico anti-inflammatory effect of *Azadirachta indica* (*A. indica*) in carrageenan-induced rats and its blood biomarkers. *A. indica* (Neem) is a widely used medicinal plant across the world, especially in Pakistan. Neem leaves have been traditionally used for the synthesis of drugs and treatment of a wide variety of diseases. **Materials and methods:** In this study, sixty albino rats (160-200 g) were divided into 4 groups: control (group I), standard (group II), ethanolic and aqueous (group III and IV) at doses of 50, 100, 200 and 400 mg/kg. **Results:** Ethanolic and aqueous extracts showed maximum inhibition in paw size at the 5th hour (400 mg/kg). Similarly, biomarkers measured, including Interleukin-6 and C-reactive protein, exhibited significant anti-inflammatory activity at the highest dose of 400 mg/kg in both experimental groups but were more distinct in the group treated with ethanolic extracts. Correlation between C-reactive protein (CRP) and inter-leukin-6 (IL-6) showed positive correlation in group III, while negative in group IV. Similarly, positive and negative correlations were observed between CRP biomarkers and paw size in group III and IV, and the same results were also shown in the case of IL-6 and paw size. In molecular docking, the binding energy value of protein CRP and IL-1 β with the identified ligands quercetin and nimbosterol showed (-8.2 kcal/mol and -7.7 kcal/mol) the best binding affinity as compared to standard drug diclofenac with -7.0 kcal/mol binding energy respectively. **Conclusions:** In conclusion, in silico and in vivo analysis revealed that the extracts of *A. indica* leaves can be used as an effective drug to manage inflammation.

Nimbin (N1) and analog N3 from the neem seeds suppress the migration of osteosarcoma MG-63 cells and arrest the cells in a quiescent state mediated via activation of the caspase-modulated apoptotic pathway.

Sudhakaran G, Velayutham M, Aljarba NH, A

I-Hazani TM, Arokiyaraj S, Guru A, Arockiaraj J.

Mol Biol Rep. 2023 Jul 14. doi: 10.1007/s11033-023-08627-7. PMID: 37450077

Background: Natural products are considered effective sources for new therapeutic research and development. The numerous therapeutic properties of natural substances in traditional medicine compel us to investigate the anti-cancer properties of Nimbin (N1) and its semi-natural analog Nimbic acid (N3) from *Azadirachta indica* against MG-63 Osteosarcoma cells. **Materials and methods:** The therapeutic efficacy of N1 and N3 were screened for their toxicity and cytotoxic activity using L6 myotubes, zebrafish larvae and MG-63 osteosarcoma cells. The mitochondrial membrane potential was evaluated using the Rhodamine 123 stain. Further, the nuclear and cellular damage was distinguished using Hoechst and Acridine orange/EtBr stain. The mechanism of cell cycle progression, cellular proliferation and caspase cascade activation was screened using scratch assay, flow cytometry, and mRNA expression analysis. **Results:** The Nimbin and analogue N3 were found to be non-toxic to normal L6 cells (Rat skeletal muscles), exhibited cytotoxicity in MG-63 cells, and were exposed to be an active inhibitor of cell proliferation and migration. Analogs N1 and N3 induced negative mitochondrial membrane potential when stained with Rhodamine 123, leading to nuclear damage and apoptosis stimulation using AO/EtBr and Hoechst. Further, N1 and N3 induced cell cycle arrest in G0/G1 phase in flow cytometry using PI staining and induced apoptosis by activating the caspase cascade and upregulated Caspase 3 and caspase 9. **Conclusion:** The study demonstrated cytotoxic activity against MG-63 osteosarcoma cells while being non-toxic to normal L6 cells. These compounds inhibited cell proliferation and migration, induced mitochondrial dysfunction, nuclear damage, and apoptosis stimulation. Furthermore, N1 and N3 caused cell cycle arrest and activated the caspase cascade, ultimately leading to apoptosis. These findings indicate that N1 and N3 hold promise as potential candidates used alone or combined with existing drugs for further investigation and development as anti-cancer agents.

Triterpenes as Potential Drug Candidates for Rheumatoid Arthritis Treatment.

Faustino C, Pinheiro L, Duarte N.

Life (Basel). 2023 Jul 5;13(7):1514. doi: 10.3390/life13071514. PMID: 37511889

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease characterized by joint inflammation, swelling and pain. Although RA mainly affects the joints, the disease can also have systemic implications. The presence of autoantibodies, such as anti-cyclic citrullinated peptide antibodies and rheumatoid factors, is a hallmark of the disease. RA is a significant cause of disability worldwide associated with advancing age, genetic predisposition, infectious agents, obesity and smoking, among other risk factors. Currently, RA treatment depends on anti-inflammatory and disease-modifying anti-rheumatic drugs intended to reduce joint inflammation and chronic pain, preventing or slowing down joint damage and disease progression. However, these drugs are associated with severe side effects upon long-term use, including immunosuppression and development of opportunistic

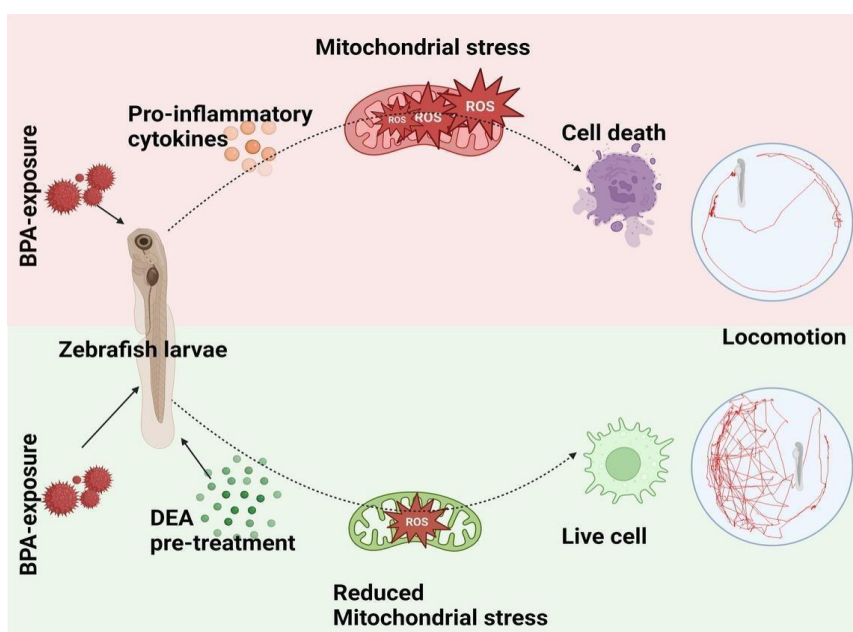
infections. Natural products, namely triterpenes with anti-inflammatory properties, have shown relevant anti-arthritic activity in several animal models of RA without undesirable side effects. Therefore, this review covers the recent studies (2017-2022) on triterpenes as safe and promising drug candidates for the treatment of RA. These bioactive compounds were able to produce a reduction in several RA activity indices and immunological markers. Celastrol, betulinic acid, nimbolide and some ginsenosides stand out as the most relevant drug candidates for RA treatment.

Deacetyl epoxyzadiradione ameliorates BPA-induced neurotoxicity by mitigating ROS and inflammatory markers in N9 cells and zebrafish larvae.

Murugan R, Haridevamuthu B, Kumar RS, Almutairi BO, Arokiyaraj S, Arockiaraj J.

Comp Biochem Physiol C Toxicol Pharmacol. 2023 Jun 30:109692. doi: 10.1016/j.cbpc.2023.109692. PMID: 37394128

Bisphenol A (BPA) leaches from plastic products have become a major inevitable concern among the research society. Human exposure to BPA leads to deleterious effects on multiple organs by the induced hyper inflammatory and oxidative stress responses. Due to the compromised antioxidant mechanism, the brain environment was highly susceptible and required special concern to ameliorate the effects of BPA. Hence, this study investigates the potential of neem-derived semi natural deacetyl epoxyzadiradione (DEA) against the oxidative stress and inflammatory response induced by BPA exposure in N9 cells and zebrafish larvae. The results from the in vitro analyses showed a decrease in cell viability in the MTT assay and a decline in mitochondrial damage in BPA-exposed N9 cells. Further in vivo, results revealed that pre-treatment of DEA to zebrafish larvae has significantly reduced the level of superoxide anion and increased the production of antioxidant enzymes such as SOD, CAT, GST, GPx and GR. We also found a significant decrease in the production of nitric oxide ($p < 0.0001$) and iNOS gene expression at 150 μM concentration. Further, DEA pre-treatment improved the behaviour of zebrafish larvae by ameliorating the production of the AChE enzyme. In conclusion, DEA protected zebrafish larvae from BPA toxicity by ameliorating oxidative stress and inflammatory responses.



Acaricidal activity of paste of methanolic extract of leaf of *Azadirachta indica*, *Eucalyptus* hybrid, *Saraca asoca* and *Murraya koenigii* against the bovine ticks in in-vitro condition.

Kumar N, Solanki JB, Patel DC, Thakuria N, Varshney N.

J Parasit Dis. 2023 Sep;47(3):513-519. doi: 10.1007/s12639-023-01593-8. Epub 2023 May 15. PMID: 37520197

The objective of this study was to evaluate the acaricidal activity of a paste made from freeze-dried methanolic extracts of air-dried leaf powders of *Azadirachta indica*, *Eucalyptus* hybrid, *Saraca asoca*, and *Murraya koenigii* against *Rhipicephalus (Boophilus) microplus* on cellulose paper. The extracts were tested in both single form (100% and 50% concentration) and dual combination (prepared by mixing equal proportions of the extracts in 100% concentration). The results showed a direct proportional relationship ($p < 0.05$) between the concentration of the extracts and the mortality percentage at 72 h post-treatment, as well as the inhibition of oviposition (I.O.) percentage. The highest mortality rate of $98.75 \pm 1.25\%$ and I.O. of $44.47 \pm 0.87\%$ was observed with the *A. indica* extract at 100% concentration, followed by *E. hybrid*, *S. asoca*, and *M. koenigii*. The combination of *A. indica* and *E. hybrid* extracts had a mortality rate of $87.5 \pm 5.59\%$ and I.O. of $42.91 \pm 0.44\%$, followed by the combinations of *S. asoca*: *E. hybrid*, *A. indica*: *S. asoca*, *E. hybrid*: *M. koenigii*, and *A. indica*: *M. koenigii*. The extracts of *A. indica* and *E. hybrid* demonstrated the highest mortality and inhibition of oviposition percentages compared to the other extracts in both single and dual combinations. These extracts required 72 h to reach their maximum mortality.