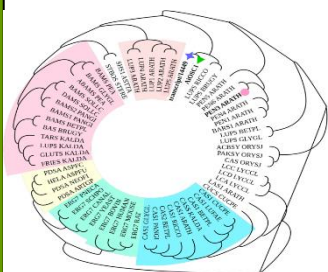


# Neem Research Newsletter

## Volume 2, Issue 2, 2022



**WORLD NEEM ORGANISATION (WNO)**



From  
The Editor's Desk.....

Neem Greetings! The Neem Newsletter, an initiative of the World Neem Organisation (WNO) was started in 2021 with a view to showcase research conducted on neem in diverse domains. Considering the voluminous data generated on neem, it was decided to bring out quarterly issues from 2022. The first issue of the Newsletter brought out in the first quarter of 2022 documented the abstracts of over 50 research papers published on neem. Since then, there has been a steady increase in publications on neem as reflected by 25 papers within a month, emphasising the ever-expanding repertoire of neem's potential that has captured the growing attention of researchers worldwide. While the effect of neem on traditional areas such as agriculture continues to be pursued, several findings also indicate the efficacy of neem in combating COVID-19 and snake bite. There is also increasing research interest in unravelling components in neem responsible for its multifarious activities.

S. Nagini

Core Founding Member, WNO  
Chief Scientific Coordinator &

Regional Director, South India



## Neem in Agriculture

**Insect repellent pellets - An application of botanicals against red flour beetle- its antifungal activity during storage and as a potential fumigant.**

**Mangang IB, Manickam L.**

*J Sci Food Agric.* 2022 May 27. doi: 10.1002/jsfa.12037.

**Background:** The protection of grains from insect infestation is the critical point during storage. Insect repellent pellets (IRP) are a potential technique to repel insects by hindering insect movement towards the grains. The basic principle of IRP is the use of active components found in the oils of lemongrass, eucalyptus and neem leaves for controlled release of fumes thereby avoiding reapplication after a few days. Here, we examined the antifungal activity; lethal dose as well as the repelling effect of IRP against red flour beetle, *Tribolium castaneum* for 30days efficacy. **Results:** We observed that IRP possessed antifungal properties and was able to repel the adults of *T. castaneum*. This insect ultimately died from the fumes if they manage to stay longer near IRP ( $LD_{50} = 2$  and  $LD_{99} = 7$ days). The active components (Phenol, 2,4-di-tert-butyl-, Citral, Neral, Geraniol, n-Hexadecanoic acid) present in IRP during the initial stage were also found after the storage period of 35days. **Conclusion:** The active components present in IRP are accountable for antifungal, repellent, and fumigant properties. IRP can thus be termed as a potent botanical insecticide and an alternative to synthetic insecticides.

**Neem-Based Insecticides Deter Oviposition Activity in Spotted Wing Drosophila (Diptera: Drosophilidae) in Sweet Cherries.**

**Gyawaly S, Rijal J.**

*J Econ Entomol.* 2022 May 23:toac074. doi: 10.1093/jee/toac074. Online ahead of print.

PMID: 35604376

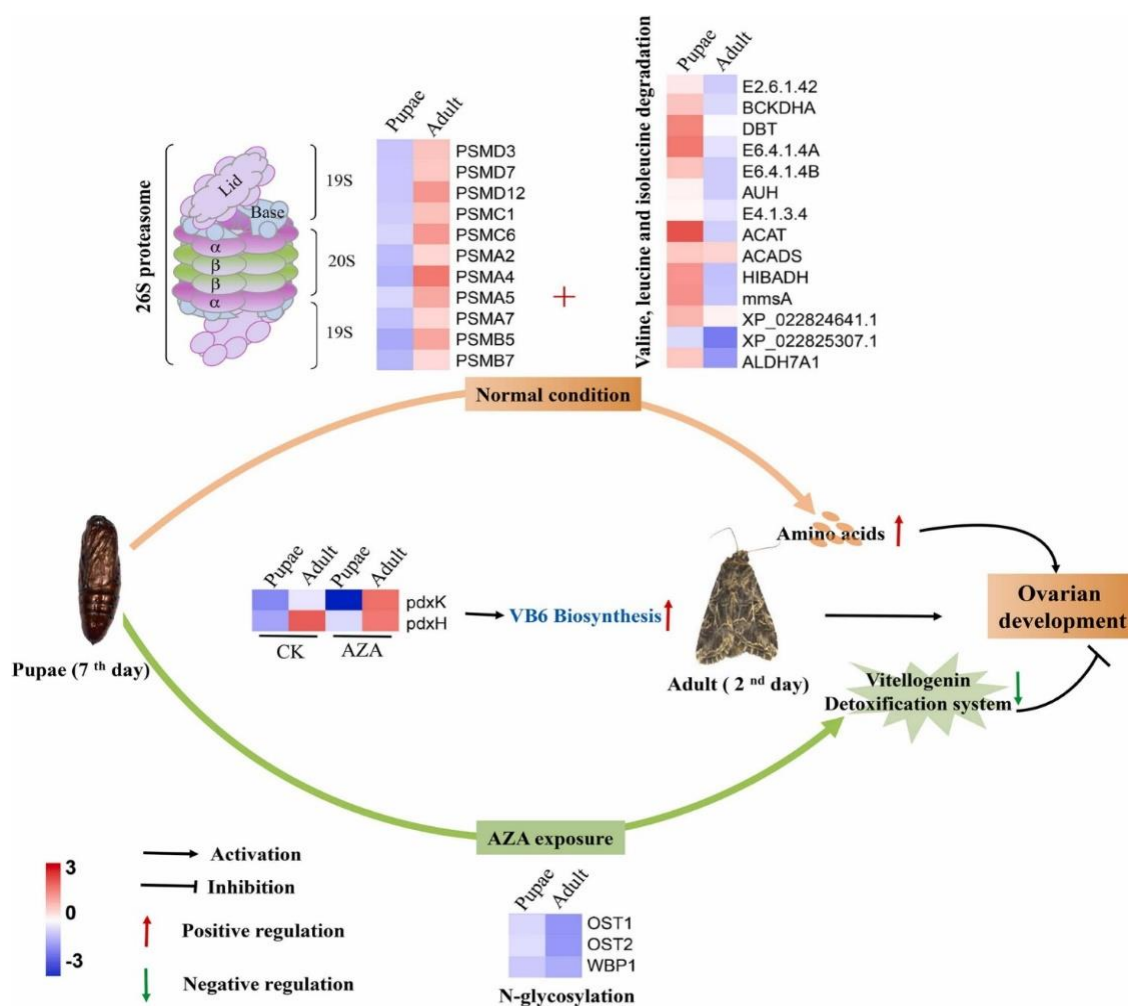
Potted wing drosophila, *Drosophila suzukii* (Matsumura), is an important invasive insect pest in the United States. Spotted wing drosophila females have serrated ovipositor that enables them to deposit eggs inside healthy, ripening softskinned fruits. In many berry and fruit crops, spotted wing drosophila is primarily controlled using synthetic insecticides in the organophosphate, pyrethroid, and spinosyn groups. However, alternative, reduced-risk insecticides with short residuals are necessary to minimize the possibilities of insecticide resistance and address other negative effects of synthetic insecticides. Neem-based products as alternative plant-based insecticides have antifeedant, repellent, antiovipository, and molting interference effects on insects and have been shown to affect the oviposition activities of some tephritid flies. We conducted laboratory studies to evaluate the oviposition deterrent activities of selected neem-based insecticides that contain either azadirachtin, or CHENO, or a combination of both, on spotted wing drosophila using sweet cherry in California. The results of the study showed that some of the neem oil products could reduce the degree of spotted wing drosophila oviposition by half compared with the control.

# Proteomic profiling for ovarian development and azadirachtin exposure in *Spodoptera litura* during metamorphosis from pupae to adults.

Sun R, Xu Y, Liu J, Yang L, Cui G, Zhong G, Yi X.

*Ecotoxicol Environ Saf.* 2022 Jun 1;237:113548. doi: 10.1016/j.ecoenv.2022.113548. Epub 2022 Apr 26. PMID: 35487172

Azadirachtin is one of the most successful botanical pesticides in agricultural pest control. To build a repertoire of proteins and pathways in response to azadirachtin exposure during ovarian development, iTRAQ-based comparative proteomic was conducted. 1423 and 1686 proteins were identified as differentially accumulated proteins (DAPs) by comparing the protein abundance in adult ovary with that in pupal ovary under normal and azadirachtin exposure condition, respectively. Bioinformatics analysis indicated that pupae-to-adult transition requires proteins related to proteasome and branched chain amino acids (BCAAs) degradation for ovary development. Azadirachtin exposure strongly affected glycosylation-related pathway. And proteins related to vitamin B6 synthesis were necessary for ovary development under normal and AZA-exposure condition. RNAi assays confirmed the essential roles of DAPs related to glycosylation and vitamin B6 synthesis in moth growth and ovary development. The results enhance our understanding of the molecular regulatory network for ovary development and provide valuable resources for using AZA-responsive proteins to develop novel bio-rational insecticides.





## **Evaluation of Bacterial Perpetuation Assays and Plant Biomolecules Antimicrobial Activity against Cotton Blight Bacterium *Xanthomonas citri* subsp. *malvacearum*; An Alternative Source for Food Production and Protection.**

**Naqvi SAH, Iqbal S, Hafeez-Ur-Rehman, Farooq U, Hassan MZ, Shahid MN, Noor Shah A, Abbas A, Mubeen I, Farooq A, Ghareeb RY, Kalaji HM, Alrefaei AF, Ahmed MAA.**

*Plants (Basel)*. 2022 May 10;11(10):1278. doi: 10.3390/plants11101278.PMID: 35631704

Cotton (*Gossypium hirsutum*) is a global cash crop which has gained importance in earning foreign exchange for each country. Bacterial blight caused by *Xanthomonas citri* subsp. *malvacearum* (*Xcm*) has been a serious disease in Pakistan's cotton belt on multiple occasions. Bacterium was isolated and identified through various biochemical and diagnostic tests where hypersensitivity reaction, Gram staining, KOH (potassium hydroxide), catalase, starch hydrolysis, lecithinase and Tween 80 hydrolysis tests confirmed bacterium as Gram-negative and plant pathogenic. *Xcm* perpetuation assays were evaluated on various cotton varieties under glasshouse conditions in completely randomized design by three different methods, wherein the scratch method proved to be the best upon CIM-496 and showed 83.33% disease incidence as compared with the other two methods, where Bt-3701 responded with 53.33% incidence via the spray gun method, and 50% with the water splash method on CIM-616, as compared with the control. Similarly, for disease severity percentage, Bt-3701 was pragmatic with 47.21% through scratch method, whereas, in the spray gun method, 45.51% disease severity was noted upon Bt-802, and 31.27% was calculated on Cyto-179 through the water splash method. Owing to the unique antibacterial properties of aqueous plant extracts, the poison food technique showed *Aloe vera*, *Mentha piperita*, *Syzygiumcumini* and *Azadirachta indica* with 17.77, 29.33, 18.33 and 20.22 bacterial colonies counted on nutrient agar medium petri plate, respectively, as compared with the control. Measurement of the inhibition zone by disk diffusion technique showed *Mentha piperita*, *Syzygiumcumini*, *Citrus limon*, *Moringa oleifera* and *Syzygium aromaticum* to present the most promising results by calculating the maximum diameter of the inhibition zone, viz., 8.58, 8.55, 8.52, 8.49 and 8.41 (mm), respectively, at the highest tested concentration (75 ppm, parts per million) compared with the control. It is probable that the decoction's interaction with the pathogen population on the host plant will need to be considered in future experiments. However, at this moment, more research into the effective management of cotton bacterial blight by plant extracts in terms of concentration determination and development of biopesticides will provide future avenues to avoid environmental pollution.

## **Effect of Different Physical Factors on efficacy of *Thevetia Peruviana* leaf extract and bio-formulations.**

**Raj Meena B, Chittora D, Meena S, Jain T, Sharma K.**

*Biochem Biophys Rep*. 2022 May 9;30:101271. doi: 10.1016/j.bbrep.2022.101271.

*eCollection 2022 Jul*. PMID: 35586247

Plant extract possess various secondary metabolites which are antifungal in nature and can be used as a safer alternative to the synthetic fungicides. As we all know that the chemical fungicides are harmful not only for humans but also for animals, other vegetation and for complete ecosystem. To overcome this problem, we have to focused on another alternative which are biologically libel and nonhazardous also. In the present study, herbal formulation was prepared in various combination ratios with *Thevetia peruviana* leaf extracts, cow dung

and neem oil cake. The major aim of this short study is to check the stability of the said plant extracts and prepared herbal formulation on various physical factors like heat, temperature, pH, sunlight and storage etc. The extracts and herbal formulations were exposed to varying conditions of the parameters selected for a precise time period, and then observing the effect as a function of change in the crude extract activity, herbal formulation activity and change minimum inhibitory concentration of plant extract against the *Alternaria solani*. Control set of MIC, and extract free medium were maintained for comparison in each set of experiment against *Alternaria solani*. Results suggested that efficacy of leaf extracts and different formulations was not affected by wet heat up to 100 °C while slight reduction in antifungal activity of the plant extract and herbal formulations were observed with dry heat at 100 °C. In addition, slight reduction in activity of extract and herbal formulations was observed with change in pH. However antifungal activity of plant extract as well as herbal formulations, remain unaffected at alkaline pH (pH 9) and neutral pH (pH7). Storage for 6 and 12 months had no negative effect on extract and herbal formulation efficacy and the antifungal activity was observed similar to freshly prepared extract activity. The present study concluded that the plant disease or plant pathogens can be controlled by plant extract and plant based bioformulations by increasing the shelf life with some little changes in the physical parameters such as light, temperature, pH and storage.

### **Toxic and repellent impacts of botanical oils against *Callosobruchus maculatus* (Bruchidae: Coleoptera) in stored cowpea [*Vigna unguiculata* (L.) Walp.].**

**Nisar MS, Ali S, Hussain T, Ramzan H, Niaz Y, Haq IU, Akhtar F, Alwahibi MS, Elshikh MS, Kalaji HM, Telesiński A, Ahmed MAA, Mackled MI.**

*PLoS One*. 2022 May 23;17(5):e0267987. doi: 10.1371/journal.pone.0267987. eCollection 2022. PMID: 35605009

Cowpea (*Vigna unguiculata*) is an important legume which is consumed globally for protein intake, particularly in Asian states. It is a well-known source of dietary fiber, protein, minerals, and vitamins. The cowpea grains are stored after harvest and used till the next harvest. However, the grains are infested by storage pests, primarily *Callosobruchus maculatus*. Hence, effective management strategies are needed to protect the stored grains from the pests. This study assessed the efficacy of some edible oils in suppressing *C. maculatus* infestation in stored cowpea grains. Four different botanical oils (i.e., mustard, neem, poppy, and pumpkin) at four different concentrations (i.e., 0.5, 1.0, 1.5 and 2.0 ml per 100 g grain) were included in the study. A control treatment without any botanical oil was also included for comparison. The relevant concentrations of botanical oils were poured into plastic containers containing 100 g cowpea grains and ten *C. maculatus* adults were released. The jars were sealed and placed at room temperature. Data relating to mortality, oviposition, F1 adult emergence, and seed weight loss were recorded. The tested botanical oils and their concentrations significantly affected mortality after one day. Mortality after 2nd and 3rd days remained unaffected by botanical oils and their different concentrations. The highest mortality was recorded in neem oil-treated grains followed by poppy, pumpkin, and mustard oils. Increased oviposition rate was observed in the grains treated with mustard and pumpkin oils, while those treated with neem and poppy oil recorded decreased oviposition. The control treatment had increased oviposition rate compared to tested botanical oils. All botanical oils significantly inhibited egg laying percentage. The highest germination was

recorded for the grains treated with mustard oil followed by pumpkin, poppy, and neem oils, respectively. The lowest germination was recorded for control treatment. Significant differences were noted for *C. maculatus* repellency among botanical oils. No emergence of adults (F1 progeny) was recorded in all tested botanical oils; thus, F1 progeny was inhibited by 100%. Weight loss, damage percentage, and holes in the grains were not recorded since F1 progeny did not emerge. It is concluded that tested botanical oils are promising and could be utilized to control *C. maculatus* in cowpea grains during storage.

## Neem in Aquaculture

**Effect of dietary supplementation of Neem, *Azadirachta indica* leaf extracts on enhancing the growth performance, chemical composition and survival of rainbow trout, *Oncorhynchus mykiss*.**

**Abidin ZU, Hassan HU, Masood Z, Rafique N, Paray BA, Gabol K, Shah MIA, Gulnaz A, Ullah A, Zulfiqar T, Siddique MAM.**

*Saudi J Biol Sci.* 2022 Apr;29(4):3075-3081. doi: 10.1016/j.sjbs.2022.01.046. Epub 2022 Jan 25. PMID: 35531238

Rainbow trout *Oncorhynchus mykiss* has a great nutritional value and delicious taste. A 90-days experimental trial was conducted to investigate the effect of dietary leaf extract of neem tree *Azadirachta indica* as a feeding supplement on the growth performance and proximate composition of *O. mykiss*. Four experimental diets were designed as T<sub>1</sub> (with 5% *A. indica* leaf extract), T<sub>2</sub> (with 7% of *A. indica* leaf extract), T<sub>3</sub> (with 10% *A. indica* leaf extract), and T<sub>4</sub> (control group feed with a regular diet with 0% *A. indica* leaf extract). The average initial weight of fry  $0.4 \pm 0.14$  g was stocked at 25 fish/tank with two replicates per treatment ( $4 \times 2 = 8$ ). After 90 days of the experimental trial, One-way ANOVA showed significant differences in final body weight, weight gain, specific growth rate, feed conversion ratio, and survival rate among the treatment groups ( $p < 0.05$ ). The highest final body weight (48.10 g) and weight gain (47.70 g) was observed in T<sub>2</sub> with 7% *A. indica* leaf extract, which was significantly different from the other treatments ( $p < 0.05$ ). The lowest FCR was recorded in T<sub>2</sub> (1.90), which was significantly different compared to other treatment groups ( $p < 0.05$ ). Inclusion of *A. indica* leaf extract in formulated feed for rainbow trout had significant effects in the hepatosomatic index, viscerosomatic index and Fulton's condition factor ( $p < 0.05$ ), but there was no significant difference in the survival rate of rainbow trout fry treated with different experimental diets ( $p > 0.05$ ). The phenomenal regression indicates that 7.5% *A. indica* inclusion is optimum for best growth performance for rainbow trout under a controlled environment. Thus, the present study suggests that the dietary leaf extract has performed an excellent nutritional supplement by enhancing growth performance and health conditions of rainbow trout in the hatchery conditions.

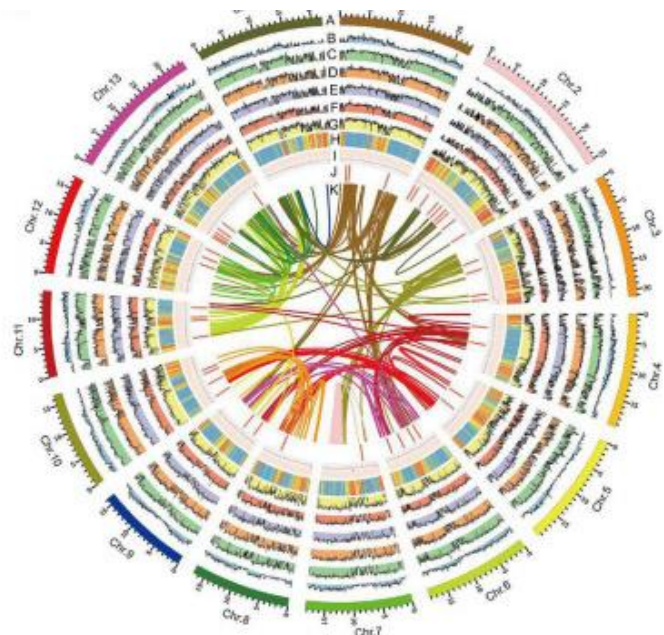
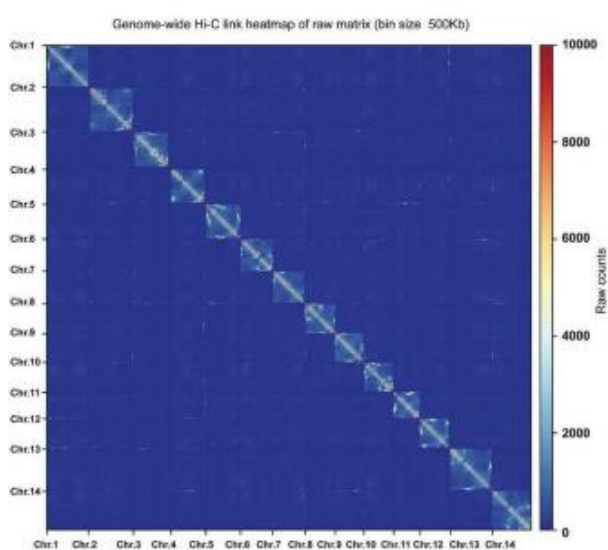
# Neem- Genomic Analysis

## Genomic Analysis Based on Chromosome-Level Genome Assembly Reveals an Expansion of Terpene Biosynthesis of *Azadirachta indica*.

Du Y, Song W, Yin Z, Wu S, Liu J, Wang N, Jin H, Qiao J, Huo YX.

Front Plant Sci. 2022 Apr 18;13:853861. doi: 10.3389/fpls.2022.853861. eCollection 2022.PMID: 35528946

*Azadirachta indica* (neem), an evergreen tree of the Meliaceae family, is a source of the potent biopesticide azadirachtin. The lack of a chromosome-level assembly impedes an in-depth understanding of its genome architecture and the comparative genomic analysis of *A. indica*. Here, a high-quality genome assembly of *A. indica* was constructed using a combination of data from Illumina, PacBio, and Hi-C technology, which is the first chromosome-scale genome assembly of *A. indica*. Based on the length of our assembly, the genome size of *A. indica* is estimated to be 281 Mb anchored to 14 chromosomes (contig N50 = 6 Mb and scaffold N50 = 19 Mb). The genome assembly contained 115 Mb repetitive elements and 25,767 protein-coding genes. Evolutional analysis revealed that *A. indica* didn't experience any whole-genome duplication (WGD) event after the core eudicot  $\gamma$  event, but some genes and genome segment might likely experienced recent duplications. The secondary metabolite clusters, TPS genes, and CYP genes were also identified. Comparative genomic analysis revealed that most of the *A. indica*-specific TPS genes and CYP genes were located on the terpene-related clusters on chromosome 13. It is suggested that chromosome 13 may play an important role in the specific terpene biosynthesis of *A. indica*. The gene duplication events may be responsible for the terpene biosynthesis expansion in *A. indica*. The genomic dataset and genomic analysis created for *A. indica* will shed light on terpene biosynthesis in *A. indica* and facilitate comparative genomic research of the family Meliaceae.





## Neem For Sustainable Environment

**Phyto-cleaning of particulate matter from polluted air by woody plant species in the near-desert city of Jodhpur (India) and the role of heme oxygenase in their response to PM stress conditions.**

**Popek R, Mahawar L, Shekhawat GS, Przybysz A.**

*Environ Sci Pollut Res Int.* 2022 May 18. doi: 10.1007/s11356-022-20769-y. Online ahead of print. PMID: 35585451

Particulate matter (PM) is one of the most dangerous pollutants in the air. Urban vegetation, especially trees and shrubs, accumulates PM and reduces its concentration in ambient air. The aim of this study was to examine 10 tree and shrub species common for the Indian city of Jodhpur (Rajasthan) located on the edge of the Thar Desert and determine (1) the accumulation of surface and in-wax PM (both in three different size fractions), (2) the amount of epicuticular waxes on foliage, (3) the concentrations of heavy metals (Cd and Cu) on/in the leaves of the examined species, and (4) the level of heme oxygenase enzyme in leaves that accumulate PM and heavy metals. Among the investigated species, *Ficus religiosa* L. and *Cordia myxa* L. accumulated the greatest amount of total PM. *F. religiosa* is a tall tree with a lush, large crown and leaves with wavy edge, convex veins, and long petioles, while *C. myxa* have hairy leaves with convex veins. The lowest PM accumulation was recorded for drought-resistant *Salvadora persica* L. and *Azadirachta indica* A. Juss., which is probably due to their adaptation to growing conditions. Heavy metals (Cu and Cd) were found in the leaves of almost every examined species. The accumulation of heavy metals (especially Cu) was positively correlated with the amount of PM deposited on the foliage. A new finding of this study indicated a potentially important role of HO in the plants' response to PM-induced stress. The correlation between HO and PM was stronger than that between HO and HMs. The results obtained in this study emphasise the role of plants in cleaning polluted air in conditions where there are very high concentrations of PM.

**Neem extract-blended nanocellulose derived from jackfruit peel for antibacterial packagings.**

**Rajasekharan R, Bahuleyan AK, Madhavan A, Philip E, Sindhu R, Binod P, Kumar Awasthi M, Pandey A.**

*Environ Sci Pollut Res Int.* 2022 May 4. doi: 10.1007/s11356-022-20382-z. Online ahead of print. PMID: 35507222

The use of jackfruit peel as a source for natural and fully biodegradable "nanocellulose" (NC) for the production of bioplastics with *Azadirachta indica* (*A. indica*) extracts and polyethylene glycol (PEG) for the antibacterial properties is investigated. The characterization of the biocomposite using FT-IR and WXR D was reported. The physicochemical properties including thickness, moisture content, water holding capacity, swelling, porosity, and biodegradability in soil were investigated. The incorporation of *A. indica* extract revealed an increased shelf life due to the strong antibacterial activity, and these biocomposites were

degraded in soil within 60 days after the end use without any harm to the environment. Jackfruit-derived nanocellulose film blended with *A. indica* extract exhibited strong antibacterial activity against gram-positive and gram-negative food spoilage bacteria. Disc diffusion assay, live/dead assay, and CFU analysis confirmed the antibacterial property of the synthesized film. Moreover, the films clearly prevented the biofilm formation in bacteria. Thus, the developed bioplastics can be utilized as appropriate substitutes to food packaging materials and also for biomedical applications such as wound dressings.



## Neem For Human Health

### **Native Iranian Medicinal Plants with Anti-Vaginal Infection Properties: A Systematic Review.**

**Rashidipour M, Shakib P, Goudarzi G, Pournia Y, Karimi M, Sarlak M.**

*Infect Disord Drug Targets.* 2022 May 1. doi: 10.2174/1871526522666220501171551. Online ahead of print. PMID: 35490427

**Background:** The use of herbs has recently been considered an effective method in the treatment of infections. The purpose of this study was to introduce Iranian medicinal plants with an effect on vaginal infections. **Methods:** In our systematic review study, keywords including 'medicinal plants', 'Iranian medicinal plants', 'vaginal infection', 'essential oils', and 'extracts' were searched in PubMed, Web of Science Direct, Scopus, Scientific Information Database, and Google Scholar. This study focused on the articles published from 2000 to 2019. **Results and discussion:** According to our investigation, *Stachys lavandulifolia*, *Thymus vulgaris* L., *Origanum vulgare* L., *Allium jesdianum*, *Cichorium intybus*, *Trigonella foenum-graecum*, *Azadirachta Indica*, *Stachys lavandulifolia*, *Coriandrum sativum* L., *Rosmarinus officinalis*, *Ferula gummosa* L., *Origanum syriacum*, *Eucalyptus globulus*, *Myrtus communis*, *Zingiber officinale*, *Punica granatum*, *Vitex agnus-castus*, *Mentha suaveolens*, *Quercus Brantii* Lindl, *Zataria multiflora*, *Berberis vulgaris*, *Thymus vulgaris* L., *Echinophora platyloba*, *Stachys lavandulifolia*, *Viola odorata*, *Menthe piperita*, *Eucalyptus camaldulensis*, *Ziziphus mauritiana*, *Quercus infectoria*, *Hypericum perforatum*, *Glycyrrhiza glabra* L., *Calendula officinalis*, *Origanum vulgare*, *Ziziphus nummularia*, *Satureja Bachtiarica*, *Foeniculum vulgare*, *Nigella sativa*, *Anethum graveolens*, *Salvia officinalis*, and *Calendula officinalis* are used to treat vaginal infections. **Conclusion:** Therefore, native plants of Iran have been reported to have antimicrobial properties for the treatment of vaginal infections and can be used as a suitable and effective alternative to antibiotics in future research.

### **Scrutinizing the role of Genetically Modified Herbs (GMHs) in the Treatment of Various Diseases.**

**Sindhu RK, Verma R, Salgotra T, Madaan P, Chandel P, Batiha GE, Cavalu S.**

*Recent Pat Biotechnol.* 2022 May 12. doi: 10.2174/1872208316666220512113921. Online ahead of print. PMID: 35549856

**Background:** Medicinal plants are being manipulated by the variety of methods with genes. Genetic engineering is the alteration of genetic material of an organism via biotechnology techniques. Wide range of genetically modified medicinal plants are diligently examined possessing therapeutic efficacy for the treatment of various diseases, like diabetes, cardiovascular diseases, rheumatoid arthritis, and cancer. **Objective:** This review discloses the numerous genetically modified herbs employed in the treatment of diseases, such as diabetes, cardiovascular diseases, rheumatoid arthritis, and cancer. In addition, the review highlights the therapeutically active constituents present in these herbs, which are liable for their specific pharmacological action. **Methods:** Several review, research papers, and patents highlighting the enormous aptitude of genetically modified herbs in the treatment of

various diseases were collected from medical databases, and are exhaustively studied for writing this review paper. **Results:** Due to rapid advancement in the phytochemical investigation of therapeutically active plants, and because of their potential to treat these disorders, they are being rapidly promoted and favoured nowadays. Moreover, a range of active constituents can be isolated from natural plants that can be employed in the treatment of various disorders, and due to their less toxicity they are preferred over synthetic products. **Conclusion:** Utilizing genetically modified herbs, like *Azadirachta indica*, *Allium cepa*, *Acacia arabica*, *Allium sativum*, and herbal formulations like flaxseed oil, green tea, and many more appear to be a pioneer strategy in the therapy of these diseases, but still further investigation is required to overcome the negative outcomes of these herbs.

## Antibacterial Effects

### In-vitro and in-silico antibacterial activity of *Azadirachta indica* (Neem), methanolic extract, and identification of Beta.d-Mannofuranoside as a promising antibacterial agent

Altayb HN, Yassin NF, Hosawi S, Kazmi I.

*BMC Plant Biol.* 2022 May 25;22(1):262. doi: 10.1186/s12870-022-03650-5. PMID: 35610569

**Background:** Antimicrobial resistance became the leading cause of death globally, resulting in an urgent need for the discovery of new, safe, and efficient antibacterial agents. Compounds derived from plants can provide an essential source of new types of antibiotics. *A. indica* (neem) plant is rich in antimicrobial phytoconstituents. Here, we used the sensitive and reliable gas chromatography-mass spectrometry (GC-MS) approach, for the quantitative and quantitative determination of bioactive constituents in methanolic extract of neem leaves grown in Sudan. Subsequently, antibacterial activity, pharmacokinetic and toxicological properties were utilized using in silico tools. **Results:** The methanolic extract of neem leaves was found to have antibacterial activity against all pathogenic and reference strains. The lowest concentration reported with bacterial activity was 3.125%, which showed zones of inhibition of more than 10 mm on *P. aeruginosa*, *K. pneumoniae*, *Citrobacter* spp., and *E. coli*, and 8 mm on *Proteus* spp., *E. faecalis*, *S. epidermidis*, and the pathogenic *S. aureus*. GC-MS analysis revealed the presence of 30 chemical compounds, including fatty acids (11), hydrocarbons (9), pyridine derivatives (2), aldehydes (2), phenol group (1), aromatic substances (1), coumarins (1), and monoterpenes (1). In silico and in vitro tools revealed that beta.d-Mannofuranoside, O-geranyl was the most active compound on different bacterial proteins. It showed the best docking energy (-8 kcal/mol) and best stability with different bacterial essential proteins during molecular dynamic (MD) simulation. It also had a good minimum inhibitory concentration (MIC) (32 µg/ml and 64 µg/ml) against *S. aureus* (ATCC 25,923) and *E. coli* (ATCC 25,922) respectively. **Conclusion:** The methanolic extract of *A. indica* leaves possessed strong antibacterial activity against different types of bacteria. Beta.d-Mannofuranoside, O-geranyl was the most active compound and it passed 5 rules of drug-likeness properties. It could therefore be further processed for animal testing and clinical trials for its possible use as an antibacterial agent with commercial values.



### Evaluation of the Nimbamrithadhi Panchathiktha Kashayam against SARS CoV-2 based on Network Pharmacology and Molecular Docking analysis.

Murali M, Nair B, V R V, T P A, Nath LR.

*Comb Chem High Throughput Screen.* 2022 May 19. doi:

10.2174/1386207325666220519112846. Online ahead of print. PMID: 35598237

**Background:** Nimbamrithadhi Panchathiktha Kashayam (NPK) is an Ayurvedic formulation of potent plant ingredients with immune-modulating effects and anti-viral activities.

**Objectives:** The present study is intended to identify the key target involved in immune and inflammatory response against SARS-CoV-2 via network pharmacology and also investigates the potent phytoconstituent within NPK in combating or modulating target response via molecular docking. **Methods:** Active phytoconstituents of NPK were filtered based on overall bioavailability and drug-likeness by Lipinski's and ADMETOX prediction.

**Results:** Results indicate that IRF 7 can be selected as an efficient target in regulating immunomodulatory and anti-viral activity via network pharmacology. Molecular docking studies show that apigenin (22.22 Kcal /mol), thiamine (24.89 Kcal /mol) and esculetin (25.21 Kcal /mol) within Nimbamrithadhi Panchathiktha Kashayam(NPK) possess better binding affinity in comparison with standard drug gemcitabine (14.56 Kcal /mol). Even though docking score is more for Esculetin and Thiamine, Apigenin within Solanum Virgianum (Yellow nightshade) and Azadirachta Indica (Neem) is considered as the active phytoconstituent in modulating immune responses and anti-viral activities based on the number and nature of amino acid interaction. **Conclusion:** To the best of our knowledge, no scientific validation has been done on NPK against COVID-19. The study indicates that NPK can be a better alternative prophylaxis strategy against SARS-COV-2 infection if further validated via suitable preclinical studies.

### Impact of the COVID-19 pandemic on immune boosting food consumption and overall dietary pattern among selected Indian adults: An observational study.

Gonella K, Nayak SP, Garg M, Kotebagilu NP.

*Clin Epidemiol Glob Health.* 2022 May-Jun;15:101056. doi: 10.1016/j.cegh.2022.101056.

*Epub* 2022 May 5. PMID: 35527741

**Background:** /Objectives: The rise of the pandemic of the coronavirus SARS-CoV-2 (COVID-19) has upset the diet and lifestyle of individuals. This study was designed to highlight the choices of individuals across different ages to quantify food consumption using the Food Consumption Score, to assess changes, if any, that were caused by the COVID-19 pandemic, and to identify the factors that affected these changes. It also assessed the consumption of immune -boosting foods, including spices and gooseberries, taken as a preventive measure against the virus. **Methods:** An online survey in a questionnaire format was used in this study to collect information from adults aged 18 to 55 (categorized into young and middle-aged adults) across India. The Food Consumption Score, a tool validated by the World Food Programme, was used to assess frequencies of food consumption per food group. **Results:** The study revealed that young adults had higher Food Consumption

Scores than their middle-aged counterparts during the two time points, pre COVID-19 (55.25 & 32,  $p = 0.001$ ) and during COVID-19 (57.25 & 32,  $p < 0.001$ ). The pandemic also led to a tangible rise in the consumption of various foods known for their immune-boosting abilities such as spices, gooseberries, and Neem. **Conclusion:** The findings of the study indicate an increase in awareness with respect to dietary habits, specifically in terms of consumption of immune boosting foods observed during the COVID-19 pandemic across the age groups.

## Anti-Parasitic & Anti-Snake Venom Properties

**Formulation of Neem oil-loaded solid lipid nanoparticles and evaluation of its anti-Toxoplasma activity.**

**Nemati S, Mohammad Rahimi H, Hesari Z, Sharifdini M, Jalilzadeh Aghdam N, Mirjalali H, Zali MR.**

*BMC Complement Med Ther.* 2022 May 4;22(1):122. doi: 10.1186/s12906-022-03607-z.PMID: 35509076

**Background:** Toxoplasmosis is caused by an intracellular zoonotic protozoan, *Toxoplasma gondii*, which could be lethal in immunocompromised patients. This study aimed to synthesize Neem oil-loaded solid lipid nanoparticles (NeO-SLNs) and to evaluate the anti-Toxoplasma activity of this component. **Methods:** The NeO-SLNs were constructed using double emulsification method, and their shape and size distribution were evaluated using transmission electron microscope (TEM) and dynamic light scattering (DLS), respectively. An MTT assay was employed to evaluate the cell toxicity of the component. The anti-Toxoplasma activity of NeO-SLNs was investigated using vital (trypan-blue) staining. Anti-intracellular *Toxoplasma* activity of NeO-SLNs was evaluated in *T. gondii*-infected Vero cells. **Results:** The TEM analysis represented round shape NeO-SLNs with clear and stable margins. DLS analysis showed a mean particle size 337.6 nm for SLNs, and most of nanoparticles were in range 30 to 120 nm. The cell toxicity of NeO-SLNs was directly correlated with the concentration of the component ( $P$ -value = 0.0013). The concentration of NeO-SLNs, which was toxic for at least 50% of alive *T. gondii* (cytotoxic concentration ( $CC_{50}$ )), was > 10 mg/mL. The ability of NeO-SLNs to kill *Toxoplasma* was concentration-dependent ( $P$ -value < 0.0001), and all concentrations killed at least 70% of alive tachyzoites. Furthermore, the viability of *T. gondii*-infected Vero cells was inversely correlated with NeO-SLNs concentrations ( $P$ -value = 0.0317), and in the concentration 100  $\mu$ g/mL at least 75% of *T. gondii*-infected Vero cells remained alive. **Conclusions:** Overall, our findings demonstrated that the NeO-SLNs was able to kill *T. gondii* tachyzoites in concentration 100  $\mu$ g/mL with a cell toxicity lower than 20%. Such results suggest that employing SLNs as carrier for NeO can effectively kill *T. gondii* tachyzoites with acceptable cell toxicity. Our findings also showed that SLNs capsulation of the NeO can lead to prolonged release of the extract, suggesting that NeO-SLNs could be also employed to clear cyst stages, which should be further investigated in animal models.

## **Molecular docking analysis of modified gedunin from neem with snake venom enzymes.**

**Dagar P, Mishra A.**

*Bioinformation. 2021 Sep 30;17(9):776-783. doi: 10.6026/97320630017776. eCollection 2021.PMID: 35539885*

Snakebites are a problem due to the increasing number of deaths and permanent disabilities. There is currently a shortage of antidotes for snakebite. The existing antibody antidote, produced from horse/sheep plasma/sera is expensive, species-dependent, and causes fatal side effects. Therefore, it is of interest use of natural flavonoid named gedunin from the *Azadirachta indica* (Neem) plant species to combat snakebites. Thus, we show the molecular docking analysis of gedunin (C<sub>26</sub>H<sub>31</sub>N<sub>2</sub>O<sub>6</sub>F) with enzymes (common in snake species) such as 5-nucleotidase, acetyl cholinesterase, L-aaO, metalloproteinase, serine, thrombin and phospholipase A<sub>2</sub>. The modified gedunin in the enzyme pocket showed improved pharmacological properties for further consideration in combating snakebites.

## **Dermatological Applications**

### **Vegetable Butters and Oils as Therapeutically and Cosmetically Active Ingredients for Dermal Use: A Review of Clinical Studies.**

**Poljšak N, Kočevar Glavač N.**

*Front Pharmacol. 2022 Apr 25;13:868461. doi: 10.3389/fphar.2022.868461. eCollection 2022.PMID: 35548366*

While the chemical composition of vegetable butters and oils has been studied in detail, there is limited knowledge about their mechanisms of action after application on the skin. To understand their dermal effects better, 27 clinical studies evaluating 17 vegetable oils (almond, argan, avocado, borage, coconut, evening primrose, kukui, marula, mustard, neem, olive, rapeseed, sacha inchi, safflower, shea butter, soybean and sunflower oils) were reviewed in this research. The reviewed studies focused on non-affected skin, infant skin, psoriasis, xerosis, UVB-induced erythema, atopic dermatitis, *molluscum contagiosum*, tungiasis, scars, striae and striae gravidarum. We conclude that in inflammation-affected skin, vegetable oils with a high content of oleic acid, together with the lack of or a low linoleic acid content, may cause additional structural damage of the stratum corneum, while oils high in linoleic acid and saturated fatty acids may express positive effects. Non-affected skin, in contrast, may not react negatively to oils high in oleic acid. However, the frequency and duration of an oil's use must be considered an important factor that may accelerate or enhance the negative effects on the skin's structural integrity.

## **Drug-Aggravated Bullous Pemphigoid in a 47-year-old Asian Woman - A Case Report.**

**N D, H H, N S, Chander J SJU.**

*Curr Drug Saf.* 2022 May 18. doi: 10.2174/1574886317666220518091554. Online ahead of print. PMID: 35593331

**Introduction:** Bullous pemphigoid is the most common chronic recurrent autoimmune subepidermal blistering disorder most prevalent in geriatric population. It varies widely in clinical presentation ranging from tense bullae to intense generalized pruritus. It is immunologic in origin with the presence of IgG antibodies. **Case report:** A 47-year-old female presented to the hospital with complaints of blisters in the lower limbs, which she was self managed with neem oil after which the blisters occurred over the body. The patient also experienced blisters over both upper and lower limbs, gluteal region, painful generalized uclers, necrotic patches, multiple erythematous blisters, ulcer erosions over bilateral legs and upper limbs with few lesions, which were foul-smelling and oozing. On second day of admission, the patient's CRP and ESR levels were tested and the levels were 33.5 and 35mm/hr respectively. The patient was treated with injection meropenem 1 g three times daily, injection dexamethasone 4 mg once daily and injection ofloxacin 400 mg once daily. On the third day of admission, the patient complained of leg swelling and subsequently D-Dimer levels were checked which showed a value of 5,740 and was treated with injection enoxaparin 40 mg for same throughout the course of hospital stay A skin biopsy confirmed the diagnosis of bullous pemphigoid. The culture test showed the growth of Klebsiella pneumonia and Acinetobacter baumani, which were resistant to most of the antibiotics. The patient was managed appropriately with modalities including antibiotics, anti-inflammatory agents, immunosuppressants, multivitamins, fluids, and albumin. The patient responded well to the treatment without new lesions or fever spikes. There was presence of necrotic patches of old lesions alone at the time of discharge. **Conclusion:** This case report was done with the purpose of presenting an exemplary case of bullous pemphigoid aggravated by the application of neem oil and emphasizing the inappropriate use of folk medicine in an autoimmune disease like bullous pemphigoid.

## **Antidiabetic & Anti-Obesity Effects**

### **Molecular modeling and in vitro studies of gedunin a potent alpha-amylase inhibitor and alpha-glucosidase inhibitor.**

**Dagar P, Mishra A.**

*Biofactors.* 2022 May 24. doi: 10.1002/biof.1846. Online ahead of print. PMID: 35608401

The present study explores the potential of the *Azadirachta indica* (Neem) plant parts (stem and bark) component gedunin for inhibition of alpha-amylase and alpha glucosidase. In contrast, Methanol at 50 mg/ml and 65 mg/ml had the lowest IC50 in alpha glucosidase and alpha amylase with noncompetitive and mixed inhibition, respectively. *Azadirachta indica* seeds collected from ICAR showed anti-diabetes activity in vitro and in vivo seeds collected were subjected to soxhlet and nonsoxhlet techniques followed by chromatography. HR-LCMS, HPLC, and FTIR to comprehend phytoconstituents present in the extract were used



to comprehend phytoconstituents present in the extract and showed the presence gedunin. Among many hits observed, gedunin was used for docking studies using ICM software and for molecular dynamic simulation using gromacs. The results show significant alpha-amylase inhibitory activity and alpha glucosidase inhibitory activity and interaction of ligand targeting these enzymes, which can be used for cross-validation, in vitro using ligplot maps and visualization.

### **Protective effect of nimbolide against streptozotocin induced gestational diabetes mellitus in rats via alteration of inflammatory reaction, oxidative stress, and gut microbiota.**

**Ma Y, Xu S, Meng J, Li L.**

*Environ Toxicol.* 2022 Jun;*37*(6):1382-1393. doi: 10.1002/tox.23491. Epub 2022 Feb 25. PMID: 35212444

**Background:** Gestational diabetes mellitus (GDM) is a significant pregnancy-related condition, which showed effect on the development of fetal. Anti-inflammatory and antioxidant therapy commonly used for the treatment of GDM. Nimbolide already confirmed their anti-inflammatory and anti-oxidant effect against various animal disease model. Our objective in this research is to investigate the protective effect of nimbolide against STZ induced GDM and elucidate the mechanism. **Methods:** In this experimental study, pregnant female Wistar rats were used and STZ (40 mg/kg) was used to induce the GDM. Blood glucose level (BGL), body weight and plasma insulin were assessed at regular time (gestational day 0, 9, and 18). Water intake, food intake, fecal and urine output were also estimated. In the female rats, hemoglobin (Hb), glycalated hemoglobin (HbA1c), hepatic glycogen, fructosamine, adiponectin, leptin, lipid, antioxidant and inflammatory cytokines parameters were estimated. In the fetuses, the fetues weight, implementation loss, and fetal weight were estimated. At the completion of the protocol, biochemical parameters were calculated. Gut microbiota was estimated in end of the study. **Results:** Nimbolide treatment significantly ( $p < .001$ ) improved the fetuses level and suppressed the fetal weight and implantation loss. Nimbolide treatment significantly ( $p < .001$ ) suppressed the BGL and enhanced the body weight, insulin level. Nimbolide treatment suppressed the water intake, food intake, urinary and fecal output. Nimbolide significantly ( $p < .001$ ) suppressed the fructosamine, leptin and enhanced the adiponectin level. Nimbolide treatment significantly ( $p < .001$ ) decreased the malonaldehyde (MDA) level and boosted the total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase (CAT); suppressed the level of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and boosted the level of IL-10. Furthermore, nimbolide treatment reversed the gut microbiota alteration induced via STZ in female rats. At the phylum level, the Firmicutes and Bacteroidetes relative abundance was altered via nimbolide treatment. The ratio of F/B boosted in GDM group and nimbolide treatment significantly ( $p < .001$ ) suppressed. Nimbolide considerably suppressed the firmicutes and enhanced the Bacteroidetes, CAG-352, Lacospirace. **Conclusion:** Based on the findings, we may conclude that nimbolide protects the pregnant rats from GDM via alteration of inflammation, oxidative stress, and gut microbiota.

## **Glucosidase Inhibitor Alleviates Inflammation and Fibrosis in Type-1 Diabetic Kidney.** **Juin SK, Pushpakumar S, Sen U.**

*FASEB J. 2022 May;36 Suppl 1. doi: 10.1096/fasebj.2022.36.S1.R4357.PMID: 35555029*

Diabetic nephropathy (DN) is characterized by renal fibrosis leading to chronic kidney disease and end-stage renal disease. A plethora of evidence suggests that chronic inflammation plays a crucial role in the development of renal fibrosis, i.e., the accumulation of extracellular matrix (ECM) proteins in the glomerular and tubulointerstitial region. Moreover, epithelial-mesenchymal transition (EMT), increased macrophage infiltration, elevated pro-inflammatory cytokines and pro-fibrotic factors contribute to renal fibrosis. Previous studies have shown that excess ECM accumulation leads to fibrosis in type-1 diabetic kidney. Nimbidol is a diterpenoid derived from the medicinal plant 'neem' (*Azadirachta indica*), and is reported to have anti-diabetic properties by inhibiting glucosidases. Further, 'neem' extracts are shown to regulate oxidative stress, inflammation and ECM proteins in different disease models. The aim of our study was to understand the regulatory role of the glucosidase inhibitor, Nimbidol on renal inflammation and fibrosis and its underlying signaling mechanisms in type-1 DN. Wild type C57BL6/J (WT) and type-1 diabetic C57BL6/*Ins2<sup>Akita</sup>*/J (Akita) mice (12-14 weeks) were treated without or with Nimbidol (0.4 mg/kg/day) for eight weeks. In diabetic kidney, the expression of CD40, the M1 macrophage marker, was elevated along with pro-inflammatory cytokines viz., TNF $\alpha$ , IL-6, IL-1 $\beta$  and pro-fibrotic factors including MCP-1, TGF- $\beta$ 1,  $\alpha$ -SMA and collagen. Additionally, Akita mice exhibited downregulation of CD206, the M2 macrophage marker and anti-inflammatory cytokine, IL-10 and also E-cadherin in the kidney. The changes were associated with excess collagen deposition in the glomerular and tubulointerstitial region of the diabetic kidney compared to that of WT mice. Furthermore, the expression of NF- $\kappa$ B, p-Smad2/3, p-P38, p-JNK and p-ERK were elevated in the diabetic kidney compared to the WT mice. Nimbidol reversed the above changes to alleviate renal inflammation and fibrosis in Akita mice. Together, our results suggest that Nimbidol protects the diabetic kidney from macrophage-mediated inflammation and fibrosis by inhibiting NF- $\kappa$ B, TGF- $\beta$ 1/Smad and MAPK signaling pathways.

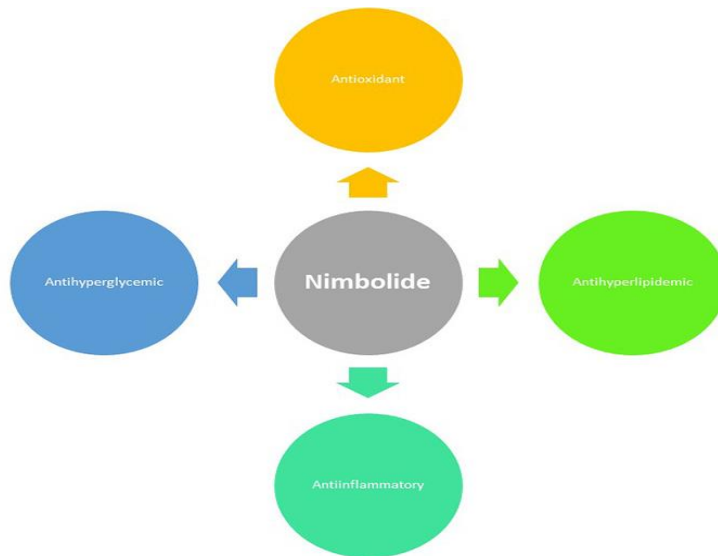
## **Protective Effect of Nimbolide against High Fat Diet-induced Obesity in Rats via Nrf2/HO-1 Pathway.**

**Zhang L, Li Y, Sun D, Bai F.**

*J Oleo Sci. 2022;71(5):709-720. doi: 10.5650/jos.ess21389.PMID: 35491096*

Current time obesity is the major challenges globally and the incidence of the obesity has raised dramatically in current years. The obesity enhanced the various metabolic diseases such as diabetes, cardiac, cancer and steatohepatitis. Natural drug having the long history to ameliorate the obesity and its related metabolic disorder. In this experimental study, we scrutinized the anti-obesity effect of nimbolide against high fat diet (HFD) induced obesity in rats. Wistar rats were divided into 5 groups and each group contains 10 rats. The body weight, tissue weight was estimated at regular time. Carbohydrate, lipid, hepatic, inflammatory cytokines, antioxidant and inflammatory parameters were estimated. The mRNA expression was also estimated. Nimbolide treated groups significantly ( $p < 0.001$ ) suppressed the body weight at dose dependent manner. Nimbolide significantly ( $p < 0.001$ ) reduced the hepatic parameters and altered the antioxidant parameters such as

thiobarbituric acid reactive substances (TBARS), glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), superoxide mutase (SOD), glutathione S transferase (GST); decreased the level of inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ). Nimbolide suppressed the mRNA expression of glucose-6-phosphatase HO-1 and nuclear factor erythroid-2 related factor-2 (Nrf2). Collectively, we can say that nimbolide having the capability to suppress the HFD induced obesity via Nrf2/HO-1 pathway.



## Anticancer Effects

**Nimbolide attenuates gut dysbiosis and prevents bacterial translocation by improving intestinal barrier integrity and ameliorating inflammation in hepatocellular carcinoma.**

**Ram AK, Vairappan B, Srinivas BH.**

*Phytother Res.* 2022 Mar 1. doi: 10.1002/ptr.7434. Online ahead of print. PMID: 35229912

Gut microbiota imbalance plays a key pathological role in hepatocellular carcinoma (HCC) progression; however, the mechanism is poorly understood. We previously showed nimbolide impede tumor development by improving hepatic tight junction (TJ) proteins expression and attenuating inflammation in HCC mice. Here, we aimed to study the role of nimbolide in regulating gut microbiota imbalance and bacterial translocation (BT) through modulating intestinal TJ proteins in an experimental hepatocarcinogenesis. Nimbolide (6 mg/kg) was administered orally for 4 weeks following induction of HCC in mice at the 28th week. Nimbolide treatment attenuated the gut microbiota imbalance by decreasing 16 s rRNA levels of *Escherichia coli*, *Enterococcus*, *Bacteroides* and increasing *Bifidobacterium*, and *Lactobacillus* in the intestinal tissue, which was otherwise altered in HCC mice. Furthermore, nimbolide improved intestinal barrier integrity in HCC mice by upregulating TJ proteins such as occludin and ZO-1 expression and subsequently prevented hepatic BT and decreased BT markers such as LBP, sCD14, and procalcitonin in the plasma of HCC mice. Moreover, nimbolide ameliorated intestinal and hepatic inflammation by downregulating TLR4, MyD88, and NF- $\kappa$ B protein expression in HCC mice. Thus, nimbolide represents a novel therapeutic drug for HCC treatment by targeting the gut-liver axis, which plays an imperative role in HCC pathogenesis.

## Neem in Veterinary Science

### **Influence of a Polyherbal Choline Source in Dogs: Body Weight Changes, Blood Metabolites, and Gene Expression.**

**Mendoza-Martínez GD, Hernández-García PA, Plata-Pérez FX, Martínez-García JA, Lizarazo-Chaparro AC, Martínez-Cortes I, Campillo-Navarro M, Lee-Rangel HA, De la Torre-Hernández ME, Gloria-Trujillo A.**

*Animals (Basel)*. 2022 May 20;12(10):1313. doi: 10.3390/ani12101313.PMID: 35625159

Choline chloride is used to provide choline in dog foods; however, in other domestic species, it has been replaced with a polyherbal containing phosphatidylcholine. A polyherbal containing *Achyranthes aspera*, *Trachyspermum ammi*, *Citrullus colocynthis*, *Andrographis paniculata*, and *Azadirachta indica* was evaluated in adult dogs through body weight changes, subcutaneous fat thickness, blood metabolites, and gene expression. Forty dogs (4.6 ± 1.6 years old) who were individually housed in concrete kennels were randomly assigned to the following treatments: unsupplemented diet (377 mg choline/kg), choline chloride (3850 mg/kg equivalent to 2000 mg choline/kg diet), and polyherbal (200, 400, and 800 mg/kg) for 60 days. Blood samples were collected on day 59 for biochemistry, biometry, and gene expression analysis through microarray assays. Intake, final body weight, and weight changes were similar for the two choline sources. Feed intake variation among dogs ( $p = 0.01$ ) and dorsal fat ( $p = 0.03$ ) showed a quadratic response to herbal choline. Dogs that received the polyherbal diet had reduced blood cholesterol levels (Quadratic,  $p = 0.02$ ). The gene ontology analysis indicated that 15 biological processes were modified ( $p \leq 0.05$ ) with implications for preventing cardiovascular and metabolic diseases, cancer prevention, inflammatory and immune response, and behavior and cognitive process. According to these results that were observed in a 60 day trial, the polyherbal form could replace choline chloride in dog diets at a concentration of 400 mg/kg.