Details of Potential Therapeutic Activities of Nerolidol

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According to the ScienceDirect website, a plethora of research into the therapeutic aspects of Nerolidol related to pharmacology, toxicology, and pharmaceutical science has been published in the past three years. 43 articles were published in 2019, 47 in 2020 and 18 during the first two months of 2021. Most of these studies extend previous work of other researchers from in vitro to in vivo in animals- primarily rats and mice. Due to the increased interest and the development of essential oils containing nerolidol (NER) as the primary constituent, a preliminary understanding of the mechanisms of NER may be helpful to those seeking to use the essential oils in the next phase of testing via human trials. This paper seeks to elaborate on the existing NER research, the significance to pharmacology, and how systems in the human body are bolstered by NER in the effort to fight off infections, viruses, and toxic substances.

Acute Kidney Injury (AKI)

In laboratory testing of LPS-treated rats and cells, nerolidol played a critical role in anti-inflammatory effects by protecting kidney cells and decreasing blood urea nitrogen and creatine levels. It also *inhibited increase of TNFα and IL-1β* and mRNA expression of TNFα and IL-1β, and *suppressed increase of TLR4 expression, phosphorylation, and nuclear translocation of p65 NF-κB* (Zhang,2017)

Explanation of Inflammatory Processes

Cytokine is a broad and loose category of small peptide proteins which are important in cell signaling and act through cell surface receptors to regulate immune responses. Cytokines also enhance or inhibit other cytokines. They are important to infection, inflammation, trauma, sepsis, cancer, and reproduction, but cannot pass the bilayer lipid layer and enter the cytoplasm of cells (Wikipedia,2021). Various proteins, receptors, cytokines, and enzymes interact to either increase or decrease inflammation in the human body.

NF-kB Signaling Pathway

The NF-kB Signaling pathway is significant because it is critical for cell growth and enables the body to respond to stress, free radicals, UV radiation, bacterial and viral antigens. It plays an important role in the immune response to infections. Incorrect regulation of the NF-kB signaling pathway are related to various cancer and immune diseases. NF-kappa-B is a DNA binding protein which is present in almost all cell types and is the endpoint of a series of events that are initiated of stimuli related to many biological processes such *as inflammation, immunity, differentiation, cell growth, the production of tumors, and apoptosis.* It is essential in communicating locations of inflammation to T-cells (Bettelli,2005) (UniProtKB Q04206,2020) The activation of NF-κB involves two major signaling pathways: the canonical pathways

and the noncanonical (or alternative) pathway. Both pathways are important for *regulating immune and inflammatory responses* despite their differences in signaling mechanism (Jewison SMP0063813,2014).

TLR4 (Toll-like receptor 4) effects:

TLR4 is a receptor that resides in the cytoplasm and cooperates with proteins LY96 and CD14 to *mediate the innate immune response to bacterial lipopolysaccharide* (LPS) (Tatematsu,2016). It is responsible for NF-kappa-B activation, cytokine secretion and the inflammatory response (Medzhitov,1997) (Arbour,2000) (Tatematsu,2016). TLR4 is also involved in LPS-independent *inflammatory responses triggered by free fatty acids*, such as palmitate, and Ni^{2+}. Both *Mycoplasma tuberculosis* HSP70 (dnaK) and HSP65 (groEL-2) act via this protein to stimulate NF-kappa-B expression (Bulut,2005). In complex with TLR6, it *promotes sterile inflammation* in monocytes/ macrophages in response to oxidized low-density lipoprotein (oxLDL) or amyloid-beta 42. This event induces the formation of a heterodimer of TLR4 and TLR6, which is rapidly internalized and triggers inflammatory response, leading to the NF-kappa-B-dependent production of CXCL1, CXCL2 and CCL9 cytokines, via MYD88 signaling pathway, and CCL5 cytokine, via TICAM1 signaling pathway, as well as IL1B secretion. *Binds electronegative LDL (LDL^-) and mediates the cytokine release induced by LDL^-* (Estruch,2013). *Stimulation of monocytes* in vitro with *M. tuberculosis* PstS1 induces p38 MAPK and ERK1/2 activation primarily via TLR2, but also partially via this receptor (Jung,2006) (UniProtKB O00206,2020). Normal expression of TLR4 is expected in the body, whereas overexpression is often a function of toxins or pathogens.

Abnormal amounts of two proteins TNFα and IL-1β contribute significantly to inflammation.

TNFα (tumor necrosis factor) effects:

TNFα is a cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR and is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. *It is a potent pyrogen causing fever by direct action* or by stimulation of interleukin-1 secretion and is *implicated in the induction of cachexia*. Under certain conditions it can stimulate cell proliferation and induce cell differentiation. *Impairs regulatory T-cells functionally defective* in individuals with rheumatoid arthritis (Nie,2013). *Induces insulin resistance* in fat cells via inhibition of insulin-induced IRS1 tyrosine phosphorylation and insulin-induced glucose uptake. *Prevents TNF-induced insulin resistance* (Ando,2015). Plays a role in the formation of new blood vessels (Nakahara,2003) (UniProtKB P01375,2020). *TNFα triggers the expression of the pro-inflammatory cytokine interleukin-12* by activated human dendritic cells (Friedmann, 2006)

IL-1β (Interleukin-1 beta) effects:

IL-1β *Potent proinflammatory cytokine*. Initially discovered as the major *endogenous pyrogen, induces prostaglandin synthesis, neutrophil influx and activation, T-cell activation and cytokine production, B-cell activation and antibody production, and fibroblast proliferation and collagen production*. Promotes Th17 differentiation of T-cells. Synergizes with IL12/interleukin-12 to induce IFNG synthesis from T-helper 1 (Th1) cells (Tominaga,2000)" (UniProtKB P01584,2020). Plays a role in angiogenesis by inducing VEGF production synergistically with TNF and IL6 (Nakahara,2003)"

NER has been shown to reduce interleukin-1β (IL-1β) production in lipopolysaccharide-induced peritoneal macrophages (Fonsêca,2016).

According to Fonseca et al (2016), in laboratory cultures NER does not reduce the levels of TNFα and IL-1β and prevent normal cellular functions, but rather prevents overproduction caused by cancers and other harmful toxins. The body's normal response to pathogens, toxins, and injury is inflammation. Reducing inflammation which interferes with therapeutic treatments is the desired outcome of anti-inflammatory constituents. However, reducing naturally occurring inflammation without addressing the cause of the inflammation can be ultimately harmful.

ALI (acute lung injury)

NER also prevents LPS from inhibiting the actions of several important enzymes, acting upon the AMPK/Nrf-2/H 0-1 pathway in the treatment of ALI (acute lung injury) to prevent decrease of the enzymatic activities of superoxide dismutase, catalase, and glutathione peroxidase (Ni,2019), (Choi,1996). *NER protects against ALI, alveolar-capillary barrier disruption and leukocyte infiltration, lung edema, lipid peroxidation in the lungs*. In addition, it counteracts AOE (SOD, CAT, GPx)(Leung,2017) inhibition, prevented the LPS-induced repression of Nrf-2 and HO-1 expression, and prevented repression of AMPK phosphorylation.

Explanation of the AMPK/N rf-2/H 0-1 pathway

AMPK effects:

Adenosine 5'-monophosphate-activated protein kinase (AMPK) is referred to as a *metabolic master switch*, phosphorylating key target proteins that *control flux through metabolic pathways of hepatic ketogenesis, cholesterol synthesis, lipogenesis, and triglyceride synthesis, adipocyte lipolysis, and skeletal muscle fatty acid oxidation*. Recent evidence also implicates AMPK as being *responsible for mediating the stimulation of glucose uptake induced by muscle contraction*. In addition, the *secretion of insulin by insulin-secreting (INS-1) cells* in culture is modulated by AMPK activation. The net effect and significance of AMPK activation is *stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipolysis and lipogenesis, stimulation of skeletal muscle fatty acid oxidation and muscle glucose uptake, and modulation of insulin secretion by pancreatic beta-cells*. In skeletal muscle, AMPK is activated by contraction. Type 2 diabetes mellitus is likely to be a disease of numerous etiologies. However, defects or disuse (due to a sedentary lifestyle) of the AMPK signaling system would be predicted to result in many of the metabolic perturbations observed in *Type 2 diabetes mellitus*. Increased recruitment of the AMPK signaling system, either by exercise or pharmaceutical activators, *may be effective in correcting insulin resistance in patients with forms of impaired glucose tolerance and Type 2 diabetes resulting from defects in the insulin signaling cascade*. (Winder,1999)

NRF2 effects:

NRF2 plays a significant role in the response to oxidative stress, detoxification, and cytoprotection. Nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor that binds to antioxidant response (ARE) elements present in the promoter region of many *cytoprotective genes*, such as phase 2 detoxifying enzymes, and promotes their expression, thereby *neutralizing reactive electrophiles* (Huang,2000), (Eggler,2009), (Huppke,2017), (Sanghvi,2019). The NFE2L2/NRF2 pathway is also activated in response to selective autophagy: autophagy promotes interaction between KEAP1 and

SQSTM1/p62 and subsequent inactivation of the BCR(KEAP1) complex, leading to NFE2L2/NRF2 *nuclear accumulation and expression of cytoprotective genes* (Jain,2010), (UniProtKB Q16236,2020) The multifunctional regulator (Nrf2) is *considered not only as a cytoprotective factor regulating the expression of genes coding for anti-oxidant, anti-inflammatory and detoxifying proteins, but it is also a powerful modulator of species longevity*. The major characteristics of Nrf2 are to some extent mimicked by Nrf2-dependent genes and their proteins including heme oxygenase-1 (HO-1), which besides *removing toxic heme, produces biliverdin, iron ions and carbon monoxide*. Sic (Activity of) Nrf2 and HO-1 across different phyla suggesting their conservative role as stress-protective and anti-aging factors." (Loboda,2106) Nrf2, sometimes referred to as the master regulator of antioxidant, detoxification, and cell defense gene expression upregulates a series of phase II detoxification and antioxidant genes (CYP450), as well as cell survival, anti-inflammatory, energy metabolism, and other groups of genes that contain a cis-acting element in their promoter region and are recognized as the antioxidant response element (ARE) or electrophile response element (EpRE) (Goa,2014). Deficiency: Immunodeficiency, developmental delay, hypohomocysteinemia (Huppke,2017)

HO-1 or HMOX1 effects:

Heme oxygenase-1 (HO-1 or HMOX1) is an enzyme found in the endoplasmic reticulum of macrophages. It opens the heme ring during the *recycling of red blood cells* at the end of their useful lifespan (Jewison,2014). Heme oxygenase cleaves the heme ring at the alpha methene bridge to form biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. Under physiological conditions, the activity of heme oxygenase is highest in the spleen, where *senescent erythrocytes are sequestrated and destroyed*. Exhibits *cytoprotective effects since excess of free heme sensitizes cells to undergo apoptosis*. "HO-1 and their products exert beneficial effects through the *protection against oxidative injury, regulation of apoptosis, modulation of inflammation as well as contribution to angiogenesis*. On the other hand, the disturbances in the proper HO-1 level are associated with the pathogenesis of some age-dependent disorders, including neurodegeneration, cancer, or macular degeneration "(Loboda,2106). The upregulation of this enzyme is beneficial by preventing the effects of a deficiency and by aiding the recycling of red blood cells.

A deficiency of HMOX1 is a disease characterized by impaired stress hematopoiesis, resulting in marked erythrocyte fragmentation and intravascular hemolysis, coagulation abnormalities, endothelial damage, and iron deposition in renal and hepatic tissues. Clinical features include persistent hemolytic anemia, asplenia, nephritis, generalized erythematous rash, growth retardation and hepatomegaly (UniProtKB P09601,2020).

CAT (catalase) effects:

Catalase occurs in almost all aerobically respiring organisms and serves to *protect cells from the toxic effects of hydrogen peroxide*. *Promotes growth of cells* including T-cells, B-cells, myeloid leukemia cells, melanoma cells, mastocytoma cells and normal and transformed fibroblast cells (Jewison SMP0000024,2014). Catalase *attacks the hydrogen peroxide produced by SODs and converts it into oxygen and Water* (UniProtKB P04040,2020).

SOD (superoxide dismutase) effects

SOD is a cytoplasmic protein and mitochondrial protein that is one of two isozymes *responsible for destroying free superoxide radicals in the body*; *converts naturally occurring but harmful superoxide radicals to molecular oxygen and hydrogen peroxide*. isozyme 2 *contains an antimicrobial peptide that displays antibacterial, antifungal, and anti-MRSA activity* against E. coli, E. faecalis, S. aureus, S. aureus MRSA LPV+, S. agalactiae, and yeast C. krusei. (National Library of Medicine: Gene 6647,2020).

SOD3 (superoxide dismutase 3) effects

SOD3 is an extracellular isoform of superoxide dismutase and uses copper and zinc as cofactors. Regarding tumors and cancer, it *induces vascular normalization in endothelial cells and regulates TIL density in primary colorectal cancers, affecting relapse and patient survival* (Martinez-Rey,2020). It protects the integrity of HS (heparinase sulfate) by reducing heparanase expression and by *preventing ROS-mediated HS degradation*, *thus reducing cancer cell proliferation and invasion* (Teoh,2009). SOD3 is also the primary method of disposal of reactive oxygen species (ROS) which cause serious damage to cells and cell organelles and convert superoxide radicals to hydrogen peroxide and water (Jewison SMP0000468,2014).

GPx (glutathione peroxidase) effects

GPx is an enzyme which *protects the hemoglobin in erythrocytes from oxidative breakdown*. In platelets, glutathione peroxidase plays a crucial role in the arachidonic acid metabolism (Sutherland,2001). It also acts in coordination with other signaling molecules to exert its own *antioxidant role and as a neuromodulator in neurodegenerative disorders* (i.e. Parkinson's disease, Alzheimer's disease, cerebral ischemia, and convulsive disorders) *and neuropsychiatric conditions*, such as, stress, bipolar disorder, schizophrenia, and drug intoxication. (Sharpa,2020).

Anti-inflammatory

Additionally, NER shows potent anti-inflammatory activity through the suppression of IL-1β and tumor necrosis factor-alpha (TNF-α) levels in an experimental mouse model of pain (Fonsêca,2016). NER interacts with the Nrf2 and NF-κB pathway in the treatment of oxidative stress (Iqubal,2020) and neuroinflammation (Qu,2020). Specifically, NER significantly increased antioxidant enzymes activity (Superoxide dismutase (SOD) and Catalase (CAT)), Hemeoxygenase-1 (HO-1), and SOD3 mRNA levels. NER treatment in TNF-α-challenged HT-29 cells significantly decreased proinflammatory (CXCL1, IL-8, CCL2) and COX-2 mRNA levels. NER supplementation attenuates colon inflammation through its potent antioxidant and anti-inflammatory activity both in in vivo and in vitro models of colonic inflammation. (Raj,2020). As mentioned above, NER also remediates ALI and AKI. It also attenuates cyclophosphamide-induced cardiac inflammation, apoptosis and fibrosis and reduces the side effects of this drug which is used for cancer treatment and as an immunosuppressant (Iqubal,2019).

Nerolidol attenuated the *Aspergillus fumigatus* keratitis inflammatory response by inhibiting the growth of A. fumigatus, reducing the recruitment of the neutrophils and the macrophages, and inhibiting the LOX-1/ IL-1β signaling.

Extracellular signal-regulated kinase-1/2 (ERK1/2), peroxisome proliferator-activated receptor-gamma (PPARc) and hemeoxy-genase-1 (HO-1), which play important roles in inflammation, were identified as

the potential targets of Nerolidol (Li,2014).

Aging

Symptoms of the human aging process and resulting frailty in old age are often caused by oxidative stress. Oxidative stress disrupts the balance of both proteins and enzymes in the body, affects the ability to replace mutated genes, increases susceptibility to viral and microbial infections, and leads to cell death. Nrf2 increases genes that combat oxidative stress (McCord,2020). Nerolidol modulates Nrf2 to reduce oxidative stress (Iqubal,2019), (Iqubal,2020), (Ni,2019), (Raj,2020), (Zhou,2018). NER may therefore ease the aging process and possibly prolong life by activating Nrf2.

Protective

Cardioprotective:

NER also provided effective cardioprotection through reduction of hypertension associated inflammation and oxidative stress by targeting TLR4/NF-κB signalling in rats(Lin,2020)

Gonadoprotective:

In a recent study of mice, CP (Cyclophosphamide) -used as an antineoplastic and immunosuppressant drug- caused reduced sperm count, sperm motility and testosterone level which were reversed upon treatment with nerolidol in a dose-dependent manner. Nerolidol thus acted as a gonadoprotective molecule and prevented the gonadotoxicity of CP (Iqubal,2020).

Women's health

In addition to breast cancer treatment and cervical cancer/HPV treatment discussed previously, NER also reverses endometriosis including increasing glutathione and SOD levels, improving histological parameters such as hemorrhage, vascular congestion, necrosis, and inflammatory cell infiltration in the endometriotic foci (Rauf,2018)

Docking studies and computer matching studies indicate that Nerolidol has the potential to interact with androgen, HER2 (human epidermal growth factor receptor 2), and dopamine receptors. Lipinski's rule of 5 is a rule of thumb to determine if a chemical compound with a certain pharmacological or biological activity has chemical and physical properties that would make it a likely orally active drug in humans (drug likeliness). NER has an acceptable drug likeliness and can pass the blood-brain barrier but further in vitro, in vivo, and clinical investigations must be conducted to consider whether NER can be considered as a lead compound for drug development (Sakhteman,2020).

HER2 effects

HER2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family and is also called HER2/neu, receptor tyrosine-protein kinase erbB-2, CD340, and ERBB2. Amplification or overexpression of this oncogene receptor plays an important role in the development and progression of some aggressive types of breast cancer. HER2 has become an important target of therapy for approximately 30% of breast cancer patients (Nixon,2017) and overexpression is also known to occur in ovarian and aggressive forms of uterine cancers, such as uterine serous endometrial carcinoma and lung adenocarcinoma (Harbeck,2018), (Santin,2008), (Halle,2017). It is the target of the monoclonal antibody

trastuzumab (marketed as Herceptin). In theory, any molecules that compete with trastuzumab for binding to HER2 may alter its effects. Spathulenol is a minor constituent in DC EO and also interacted with the HER2 receptor (3rcd) with the binding energy of −7.7, which was 77% of the binding energy of the co-crystallized ligand." (Sakhteman,2020). The significance of the interaction of NER and HER2 is that while cancer cells prosper by causing an increase of transcription by various receptors, NER has the potential for binding with these receptors and preventing an increase in cancer activity. Similarly, cancer treatment medications use the same receptors for their actions and the simultaneous use of DC EO could be detrimental.

Neurological/Brain protectant

Research on rats shows the potential neuroprotective benefits of NER in treating Parkinson's disease which results from exposure to pesticide components. NER protects the brain by preventing the loss of DA neurons in the SNc and DA nerve fibers in the brain striatum, suppressing excessive lipid peroxidation, ameliorating GSH levels and reversing the decrease in the activity of antioxidant enzymes caused by mitochondrial inhibitors rotenone (ROT) found in pesticides. It also attenuates the release of proinflammatory cytokines and reduces COX-2 and iNOS expression induced by ROT. The result is the attenuating of dopaminergic neurodegeneration (loss of dopaminergic neurons and nerve fibers), improving antioxidant enzymes, and inhibiting inflammatory mediators and lipid peroxidation in the rat's brain (Javed,2016).

Nerolidol also demonstrated potential anxiolytic activities and treatment for Alzheimer's disease and epilepsy treatment (De Carvalho,2018). It Increased noradenaline and dopamine, mediated by 5-HT1A receptors in the cortex and hippocampus which reduced oxidative stress and reduced seizure severity in mice (da Fonsêca,2019). According to Kaur (Kuar,2016), nerolidol (12.5, 25, and 50 mg/kg, i.p.), in a rat model of epileptogenesis induced by pentylenetetrazole, presented protective effects since it *reduced seizure severity*. These results may be explained by an observed *decrease in oxidative stress* and *favorable neurochemical changes including increased levels of noradrenaline, dopamine, and serotonin in both the cortex and the hippocampus* of the treated animals. Nerolidol also *improved depression and memory loss* in the PTZ-kindled animals (psychiatric comorbidities associated with epilepsy.

In addition, administration of nerolidol (50 mg/kg, i.p.) to rats reversed neuroinflammation and cerebral oxidative stress by *increasing levels of the antioxidant enzymes SOD, CAT, GSH and decreasing lipid peroxidation and MDA levels, besides reducing glial cell activation and dopaminergic neuron loss* (Javed,2016). Nogueira-Neto and colleagues (Nogueira,2013) found that treatment with nerolidol (75 mg/kg, i.p.) decreased oxidative stress in the mouse hippocampus, resulting in increased SOD and CAT activity, as well as reducing levels of nitrite and lipid peroxidation. The results reveal the therapeutic potential of nerolidol to treat and prevent brain diseases associated with oxidative stress.

In-vivo study showed protective effect of NER against CP-induced neuroinflammation, oxidative stress, cognitive impairment and structural abnormalities in the hippocampus and cortex regions in mice (Iqubal,2019)

Anti-cancer effects on cell lines

NER has been shown to have some inhibitive effects on human breast cancer MCF-7 and colon cancer HCT-116 and SW-480 cell lines. The inductive effects on cancer cell apoptosis were more significant than the arrested effects on cell cycle. (Sun,2010) At low concentrations, it induces cell death by apoptosis, while at high concentrations, it kills ovarian cancer cells by necrosis (Tai, 2010). Anti-proliferative action of DC extracts has been effective against several cancer cell lines including HL60 (leukemia cells) (Tai,2006). DC stem extract showed significant potent antiproliferative effect on cancer cell lines (SW-480 (colon), HCT-116 (colon), HT-29 (colon), MCF-7 (breast) and NSCLC (lung)) by arresting them in S- and G2/M-phases. At 0.1 mg/ml, stem extract inhibited SW-480 cell growth by 85.1%, and almost completely inhibited HCT-116 cell growth. Compared to berry extract, the stem extract showed much stronger antiproliferative effects on human cancer cells (Wang,2009).

Antiemetic

NER suppressed side effects of cytotoxic CINV and inhibit current in neurons in animal testing by acting as a noncompetitive agonist against 5-HT3R (serotonin) receptors. (Lee,2018) 5-HT3R (5-hydroxytryptamine receptor 3A) is one of the several different receptors for 5-hydroxytryptamine (serotonin), a biogenic hormone that functions as a neurotransmitter, a hormone, and a mitogen (UniProtKB P46098,2020).

Antiparasitic

In addition to findings that NER has strong anti-leishmanial activity (Chan,2016), it is also effective and as an antiparasitic (da Silva,2015). NER was tested in the treatment of malaria in mice with a 1000mg/kg/dose in 2 doses delivered by oral or inhalation. In mice treated with nerolidol, parasitemia was inhibited by >99% (oral) and >80% (inhalation) until 14 days after infection ($P < 0.0001$). On Day 30 post-infection, the survival rate of orally treated mice was 90% compared with 16% in controls ($P < 0.0001$). In contrast, inhalation-treated mice showed a survival rate of 50% vs. 42% in controls ($P > 0.05$). The toxicity of nerolidol administered by either route was not significant, while genotoxicity was observed only at the highest dose tested (Saito,2016)

Antiviral

SARS-CoV-2

NER can inhibit viral replication of SARS-CoV-2 by binding with SARS-CoV-2M proteins and can bind with Nsp15 (an endoribonuclease of SARS-CoV) to prevent viral infection. The SARS-CoV-2 spike protein helps in the attachment of the viral cell to human cell via interaction with angiotensin-converting enzyme 2 (ACE2) proteins present on host cells, making this interface a promising target to prevent binding of SARS-CoV-2 rS to human respiratory cells (Zhang,2020). Binding with human ACE2 *in vitro* was observed with (E)-nerolidol. These phytochemicals are present in variable quantities in essential oils obtained from different plants which can be used to treat COVID-19 but data from well-established preclinical and clinical studies is required. (Asif,2020)

Further research by McCord (et al) details the significance of activating NRF2 in combatting and defeating viruses and: “Numerous published studies have implicated Nrf2 as a regulator of susceptibility to respiratory viral infections. A recent review by Lee points out that virus-induced modulation of the host antioxidative response has turned out to be a crucial determinant for the progression of several

viral diseases. A virus needs to keep oxidative stress at a level optimal for viral reproduction, which is higher than normal, to support the viral metabolism but should not be so high as to kill off the host cell. *Viruses have* evolved *mechanisms for manipulating the Nrf2 pathway in both directions*, depending on the needs of the virus, but *importantly taking control away from the host cell*. Among the types of viruses studied are influenza virus, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV). The phenomenon is also seen in non-respiratory viruses including dengue virus (DENV), rotavirus, herpes simplex virus, Zika virus, and HIV, suggesting that *regulation of oxidative stress may be a need common to most, if not all, viruses* and that *Nrf2 activators may offer multiple ways to regain control of important pathways* to increase resistance and slow viral replication." (McCord,2020)
These recent discoveries indicate that the methods of viral control and replication are more understood and that nerolidol (and DC EO) has the potential for therapeutic benefit. However, successful in vivo and clinical trials are mandatory before DC EO can be listed as a valid treatment.

HPV

Antiviral AV2® is a mixture of natural essential oils (eugenol, carvone, nerolidol, geraniol) in olive oil, and has a broad spectrum anti-viral activity. In a phase II randomized controlled trial (RCT), AV2® proved effective in reducing the size of cervical lesions associated with human papillomavirus (HPV) (Mutombo,2019).

Summary:
Based on recent in vitro and in vivo (mice and rats) research, nerolidol has the potential to reduce the effects and damage of chemotherapy drugs by acting as a protectant to various organs, to interrupt virus command/control processes and viral metabolism, to support the auto-immune system and suppress excesses of the natural auto-immune response. A significant means of doing so is by preventing the interruption of critical regulatory biological processes by toxins, viruses, and parasites. Additional potential benefits involves the primary actions reduction of inflammation and oxidative stress.

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