Rauwolfia (Rauwolfia serpentine/vomitera) extract

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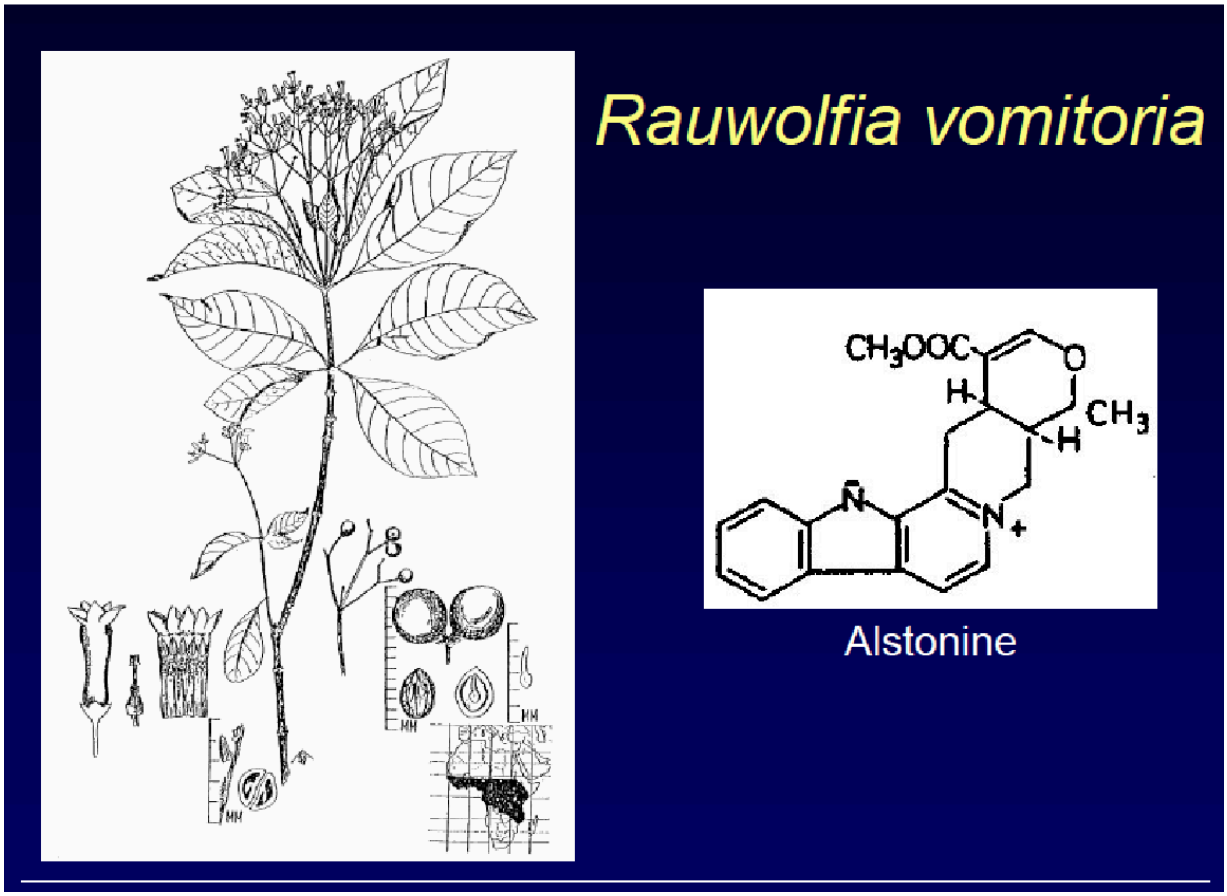
Rauwolfia serpentina root has been used for centuries in India, and *Rauwolfia vomitera* in Africa, for the treatment of a variety of disorders including snake bites, insect bites and stings, insomnia and insanity. In India and Nepal, it is a common treatment for hypertension and insomnia. Gandhi took it frequently at night for its calming actions.[1]

Rustom Jal Vakil was a famous Indian cardiologist who, in 1949, “published a watershed paper on the antihypertensive properties of *Rauwolfia serpentina* and affected a paradigm shift in the management of hypertension”. “Rauwolfia was the world’s 1st successful blood-pressure-lowering agent.”[2]

Some of these alkaloids act similar to beta blockers. Reserpine inhibits the reuptake of norepinephrine, causing its depletion by enzymes. Another alkaloid, ajmaline, has antiarrhythmic action and inhibits ventricular arrhythmias. Rauwolfia has tranquilizing

effects because, like beta-blockers, it reduces the effects of the stress hormone norepinephrine.[3]

Although reserpine remains very useful in the treatment of hypertension, whole rauwolfia extract standardized to 1% reserpine is far more effective and has less side effects. Cardio-Tonic <BP contains the whole plant extract of Rauwolfia, and therefore contains only a tiny amount of reserpine. In low dose, reserpine acts synergistically with the other cardiovascular tonic herbs and nutrients in the formula, which is far different from using it as a specific compound to lower blood pressure.



Rauwolfia alkaloids

Rauwolfia alkaloids (the plant contains more than 40 known indole alkaloids) work by controlling nerve impulses along certain nerve pathways. As a result, they act on the heart and blood vessels to lower blood pressure.

Reserpine

The antihypertensive actions of reserpine are a result of its ability to deplete catecholamines from peripheral sympathetic nerve endings. These substances are normally involved in controlling heart rate, and regulating the force of cardiac contraction and peripheral resistance. Reserpine's primary mechanism of action is through inhibition of the ATP/Mg++ pump responsible for the sequestering of neurotransmitters in storage vesicles located in the presynaptic neuron. Neurotransmitters that are not sequestered in storage vesicles are readily metabolized by monoamine oxidase (MAO) causing a reduction in catecholamines. Reserpine served as the lead compound in the search for antihypertensive agents that interfere with the function of the sympathetic nervous system.

Rauwolfia, is very specific for type A sympathetic types for it tones down the sympathetic nervous and can also act as a mild tranquilizing agent. Rauwolfia is a “sympatholytic’ agent. As a result, it increases the level of calm and decreases stress responses. This can be associated with an emotional-cognitive feeling of tranquility. Some people find this a little disturbing and experience it as “depression” or “emptiness”, others find it a positive and beneficial experience conducive to meditation – Gandhi used to drink Rauwolfia tea daily for its calming and pro-meditative influence.

A recent review of all compiled studies done on reserpine found it to be as effective as other first-line antihypertensive drugs.[4]

Antihypertensive and Antihyperlipidemic Activity of Aqueous Methanolic Extract of *Rauwolfia Serpentina* in Albino Rats

Rauwolfia serpentina has a wide range of therapeutic effects so this study was planned to explore the antihypertensive and antihyperlipidemic therapeutic responses of *R serpentina* doses using albino rats by measuring the blood pressure, biochemical parameters, and histological architecture of liver and kidney tissues. Thirty albino rats were divided into 5 groups (n = 6) as G1 (normal Control) received normal diet, G2 (positive control) received only 8% NaCl added diet (high salt diet); G3 was given atenolol (standard drug control) 50 mg/kg body weight, G4 and G5 groups were given methanolic plant extract as low dose (100 mg/kg body weight) and high dose (200 mg/Kg body weight) daily along with high salt diet for 4 weeks, respectively. *Rauwolfia serpentina* significantly ($P < .05$) decreased the blood pressure in G4 and G5 groups as compared to G2 and G3. Significant ($P < .05$) impact was reported, on serum lipid profile and serum proteins as well as hepatoprotective and renoprotective potential on studying tissues sections under microscope, in animal groups given herbal extract as compared to control groups. It could be concluded that *R serpentina* has therapeutic effect to manage the hypertension and hypercholesterolemia most probably via protecting the liver and renal architectures.[5]

Rauwolfia Serpentina extract Antihypertensive and Antihyperlipidemic Activity

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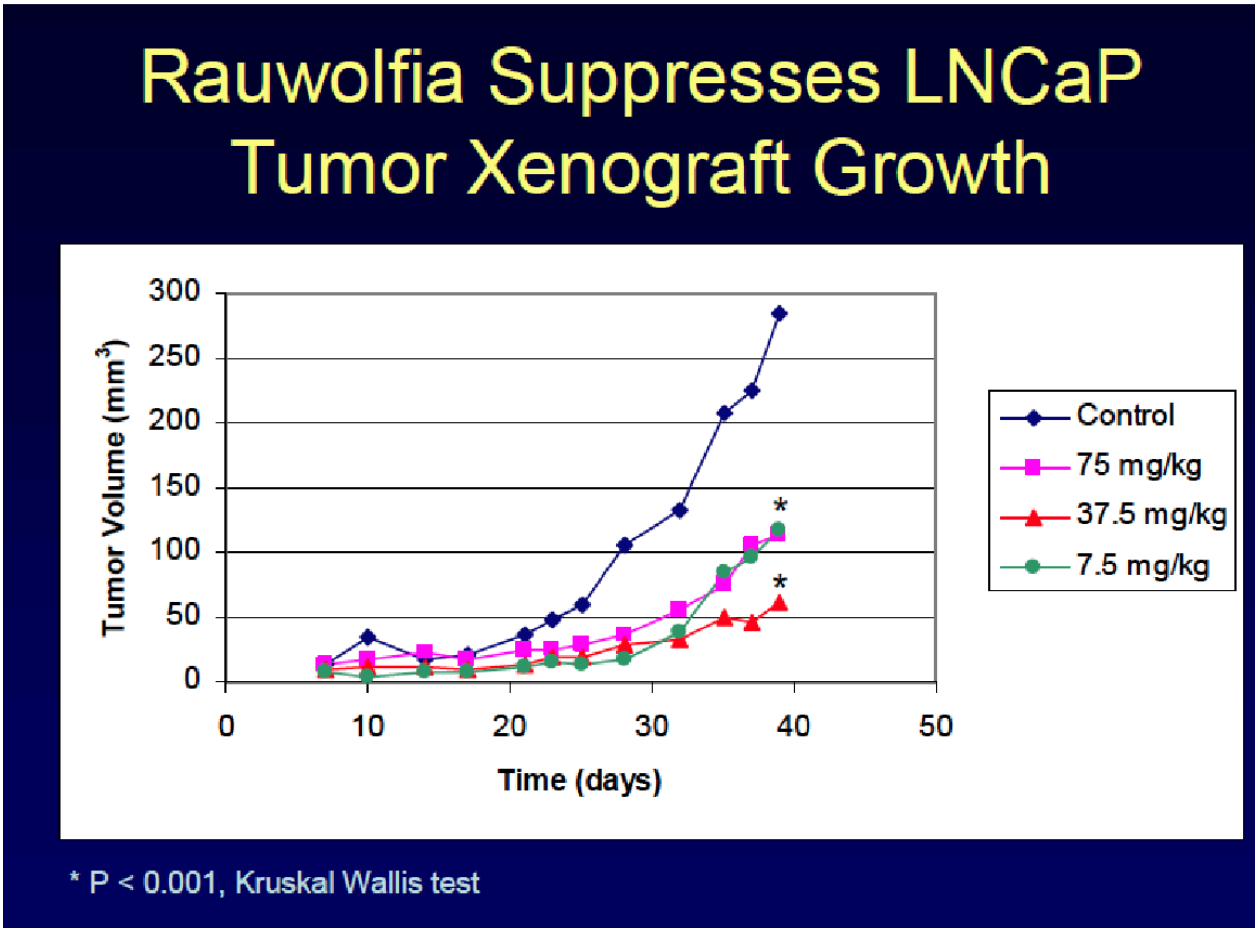
Anti-prostate cancer activity of a beta-carboline alkaloid enriched extract from *Rauwolfia vomitoria*

The tropical shrub, *Rauwolfia vomitoria*, is a medicinal plant used traditionally to treat a variety of ailments. A bioactive beta-carboline alkaloid, alstonine, present in this extract was previously shown to have anti-cancer activity against cancer cell lines. This study considers the potential anti-prostate cancer activity of this extract in vitro and in vivo. *Rauwolfia vomitoria* extract standardized for beta carboline alkaloids was tested for ability to influence the growth and survival of the human LNCaP prostate cancer cell line. A WST-1 assay was used to measure cell growth, and cell cycle analyses were conducted with flow cytometry. Western blot detection of PARP cleavage and accumulation of cells containing sub-genomic DNA indicated induction of apoptosis. Pathway specific microarray analyses were utilized to identify the effect of Rauwolfia extract on the expression of 225 genes. Mice xenografted with LNCaP cells were treated with the extract or placebo control, and tumor growth was measured for 5 weeks. The effects of the extract on xenografted tumor cell proliferation and apoptosis were measured by in situ BrdU incorporation and TUNEL staining. Rauwolfia extract decreased in vitro cell growth in a dose-dependent manner ($p<0.001$) and induced the accumulation of G1 phase cells. PARP cleavage demonstrated that apoptosis was induced only at the highest concentration tested (500 microg/ml) which was confirmed by detection of cells containing sub-genomic DNA. The expression of genes associated with DNA damage signaling pathway was up-regulated by Rauwolfia treatment, including that of GADD153 and MDG. The expression of a few cell cycle genes (p21, cyclin D1 and E2F1) was also modulated. These alterations were confirmed by RT-PCR. Tumor volumes were decreased by 60%, 70% and 58% in the groups fed the 75, 37.5 or 7.5 mg/kg Rauwolfia, respectively (Kruskal-Wallis test, $p<0.001$). The *Rauwolfia vomitoria* extract significantly suppressed the growth and cell cycle progression of LNCaP cells, in vitro and in vivo.[7]



Rauwolfia serpentina **compound, Reserpine Induces Apoptosis and Cell Cycle Arrest in Hormone Independent Prostate Cancer Cells through Mitochondrial Membrane Potential Failure**

Background: Reserpine, an indole alkaloid commonly used for hypertension, is found in the roots of Rauwolfia serpentina. Although the root extract has been used for the treatment of cancer, the molecular mechanism of its anti-cancer activity on hormonal independent prostate cancer remains elusive.

Methods: we evaluated the cytotoxicity of reserpine and other indole alkaloids, yohimbine and ajmaline on Prostate Cancer cells (PC3) using MTT assay. We investigated the mechanism of apoptosis using a combination of techniques including acridine orange/ethidium bromide staining, high content imaging of Annexin V-FITC staining, flow cytometric quantification of the mitochondrial membrane potential and Reactive Oxygen Species (ROS) and cell cycle analysis.

Results: Our results indicate that reserpine inhibits DNA synthesis by arresting the cells at the G2 phase and showed all standard sequential features of apoptosis including, destabilization of mitochondrial membrane potential, reduced production of reactive

oxygen species and DNA ladder formation. Our in silico analysis further confirmed that indeed reserpine docks to the catalytic cleft of anti-apoptotic proteins substantiating our results.

Conclusion: Collectively, our findings suggest that reserpine can be a novel therapeutic agent for the treatment of androgen-independent prostate cancer.[8]

Antitumor Activities of *Rauwolfia vomitoria* Extract and Potentiation of Gemcitabine Effects Against Pancreatic Cancer

Pancreatic cancer is one of the most lethal malignancies with very limited treatment option. In the effort of enhancing the effect of the conventional chemotherapeutic drug gemcitabine against pancreatic cancer, we investigated in vitro and in vivo the anticancer effect of a β-carboline-enriched extract from the plant *Rauwolfia vomitoria* (Rau), either alone or in combination with gemcitabine, in preclinical pancreatic cancer models. Rau induced apoptosis in pancreatic cancer cells in a concentration-dependent manner, and completely inhibited colony formation of PANC-1 cells in soft agar. The combination of Rau and gemcitabine had a synergistic effect in inhibiting cell growth with dose reduction effect for gemcitabine. In an orthotopic pancreatic cancer mouse model, PANC-1 tumor growth was significantly suppressed by Rau treatment. Metastasis was inhibited by Rau.

Adding Rau to gemcitabine treatment reduced tumor burden and metastatic potential in the gemcitabine non-responsive tumor. These data suggest that Rau possesses anti-pancreatic cancer activity and could improve the effect of gemcitabine.[9]

Antitumor Activities of *Rauwolfia vomitoria* Extract and Potentiation of Carboplatin Effects Against Ovarian Cancer

Background: Tumor resistance to platinum-based drugs has been an obstacle to the treatment of ovarian cancer. Extract of the plant *Rauwolfia vomitoria* has long been used by cancer patients. However, there have not been systematic studies of its anticancer activity.

Objective: In an effort to enhance the effectiveness of platinum-based drugs, we investigated the anticancer effect of a *Rauwolfia vomitoria* extract (Rau), both alone and in combination with carboplatin (Cp).

Methods: In vitro cytotoxicity and colony formation were evaluated in several ovarian cancer cell lines. In vivo effects were evaluated in an intraperitoneal ovarian cancer mouse model. The combination of Rau and Cp was assessed using Chou-Talalay's constant ratio design and median effect analysis based on the isobologram principle to determine the combination index values.

Results: Rau decreased cell growth in all three tested ovarian cancer cell lines dose dependently and completely inhibited formation of colonies in soft agar. Apoptosis was induced in a time- and dose-dependent manner and was the predominant form of Rau-induced cell death. Synergy of Rau with Cp was detected, with combination index values <1 and dose reduction index values for Cp ranging from 1.7- to 7-fold. Tumor growth in mice was significantly suppressed by 36% or 66% with Rau treatment alone at a low (20 mg/kg) or a high dose (50 mg/kg), respectively, an effect comparable to that of Cp alone. The volume of ascitic fluid and the number of non-blood cells in ascites were also significantly decreased. Combining Rau with Cp remarkably enhanced the effect of Cp and reduced tumor burden by 87% to 90% and ascites volume by 89% to 97%.[10]

Cytotoxicity of the indole alkaloid reserpine from *Rauwolfia serpentina* against drug-resistant tumor cells.

Background: The antihypertensive reserpine is an indole alkaloid from *Rauwolfia serpentina* and also exerts profound activity against cancer cells in vitro and in vivo. The present investigation was undertaken to investigate possible modes of action to explain its activity toward drug-resistant tumor cells.

Material and Methods: Sensitive and drug-resistant tumor cell lines overexpressing P-glycoprotein (ABCB1/MDR1), breast cancer resistance protein (ABCG2/BCRP), mutation-activated epidermal growth factor receptor (EGFR), wild-type and p53-knockout cells as well as the NCI panel of cell lines from different tumor origin were analyzed. Reserpine's cytotoxicity was investigated by resazurin and sulforhodamine assays, flow cytometry, and COMPARE and hierarchical cluster analyses of transcriptome-wide microarray-based RNA expressions.

Results: P-glycoprotein- or BCRP overexpressing tumor cells did not reveal cross-resistance to reserpine. EGFR-overexpressing cells were collateral sensitive and p53-Knockout cells cross-resistant to this drug compared to their wild-type parental cell lines. Reserpine increased the uptake of doxorubicin in P-glycoprotein-overexpressing cells, indicating that reserpine inhibited the efflux function of P-glycoprotein. Using molecular docking, we found that reserpine bound with even higher binding energy to P-glycoprotein and EGFR than the control drugs verapamil (P-glycoprotein inhibitor) and erlotinib (EGFR inhibitor). COMPARE and cluster analyses of microarray data showed that the mRNA expression of a panel of genes predicted the sensitivity or resistance of the NCI tumor cell line panel with statistical significance. The genes belonged to diverse pathways and biological functions, e.g. cell survival and apoptosis, EGFR activation, regulation of angiogenesis, cell mobility, cell adhesion, immunological functions, mTOR signaling, and Wnt signaling.

Conclusion: The lack of cross-resistance to most resistance mechanisms and the collateral sensitivity in EGFR-transfectants compared to wild-type cells speak for a promising role of reserpine in cancer chemotherapy. Reserpine deserves further consideration for cancer therapy in the clinical setting.[11]

Reserpine inhibits DNA repair, cell proliferation, invasion and induces apoptosis in oral carcinogenesis via modulation of TGF-β signaling

Reserpine is a natural indole alkaloid isolated from Rauwolfia serpentina and has potent antioxidant, antimicrobial, and anti-mutagenic properties. Accordingly, this study aimed to investigate the effect of reserpine on DNA repair, cell proliferation, invasion and apoptosis in 7,12-dimethylbenz[a]anthracene(DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. Transforming growth factor-β (TGF-β) was found to induce Smad2, 3 and 4 phosphorylation triggering Smad3/Snail mediated DNA repair proteins and Smad2/4 nuclear translocation. In contrast, reserpine inhibits TGF-β dependent Smad2/3/4 phosphorylation, thereby blocking Smad3/Snail activation and Smad2/4 nuclear translocation. Interruption of these oncogenic signaling pathways leads to downregulating ERCC1, XPF, Ku70, DNA-PKcs, PCNA, cyclin D1, HIF-1α, IL-6, Mcl-1 and stimulates Bax, cytochrome C, Apaf-1, caspase-9, caspase-3 and PARP protein expressions.

This study provides therapeutic potential of reserpine in inhibiting DNA repair, cell proliferation, and invasion while simultaneously inducing apoptosis via modulation TGF-β signals.[12]

Rauwolfia serpentina improves altered glucose and lipid homeostasis in fructose-induced type 2 diabetic mice

Rauwolfia serpentina is well-reported in traditional medicines for the treatment of hypertensive and neurological disorders. However, its antidiabetic potential has been currently described in both alloxan-treated and normoglycemic mice. Present effort was carried out to investigate the effect of methanol root extract (MREt) of *R. serpentina* in fructose-induced type 2 diabetic mice. Experimental mice were grouped into normal control (distilled water 1 ml/kg) and fructose-induced type 2 diabetic groups (10% fructose 1 ml/kg).The second group sub-divided into negative (0.05% DMSO 1ml/kg) control, positive (pioglitazone 15 mg/kg) control and three test groups (MREt 10, 30 & 60 mg/kg). Each treatment was given orally for 14 days consecutively then mice were sacrificed in order to collect serum and liver samples to analyze physical, biochemical as well as hematological markers. MREt significantly improved percent body weight and glycemic change along with serum insulin, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-c), very low-density lipoprotein (VLDL-c), high-density

lipoprotein-cholesterols (HDL-c), total hemoglobin, glycosylated hemoglobin, hepatic glycogen, coronary risk and fasting insulin resistance indices while suppressed down the activity of 3-hydroxy-3-methylglutaryl Coenzyme A reductase enzyme in test groups when compared with diabetic controls. The present findings conclude that MREt of *R. serpentina* can effectively better the carbohydrate and lipid homeostasis by either inhibiting fructose absorption in intestine or decreasing insulin resistance in fructose-induced type 2 diabetic mice.[13]

Rauwolfia serpentina's Ajmalicine and Reserpine: as Multi-Target Directed Ligands Towards Factors Implicated in Alzheimer's Disease

Alzheimer's disease (AD) is a multifactorial disorder characterized by exponential loss of memory and cognitive deficit involving several disease modifying targets (amyloid beta, beta-secretase, monoaminoxidase-B, and cholinesterase). The present study explores multi-target directed ligand approach using secondary metabolite reserpine (RES) and ajmalicine (AJM) obtained from *Rauwolfia serpentina* roots. Novel LCMS and HPLC methods were developed for identification and quantification of reserpine and ajmalicine. In vitro enzyme inhibition assays were performed to evaluate anti-cholinesterase, β-site amyloid cleaving enzyme (BACE-1) inhibition and monoamine oxidase-B (MAO-B) inhibition, further analyzed with in silico analysis. Anti-amyloidogenic potential was studied using anti-aggregation studies along with TEM and circular dichroism (CD) analysis. In vitro neuroprotective potential against Aβ toxicity and anti-oxidative stress was demonstrated using PC12 cell cultures. Reserpine is a more potent dual cholinesterase inhibitor than ajmalicine (IC_{50} values of 1.7 μM (AChE) and 2.8 μM (BuChE)). The anti-aggregation activity of reserpine (68%) was more than ajmalicine (56%). Both compounds demonstrated neuroprotective activity against Aβ42 (92%) and H_2O_2 (93%) induced toxicity in PC12 cells against controls. Phytocompounds also inhibited MAO-B and BACE-1 enzymes in concentration dependent manner. Molecular docking studies indicated the strong binding of compounds to the catalytic site of targets. This novel study demonstrated that reserpine and ajmalicine as a multi-target directed ligand that have disease modifying potential for amelioration of AD.[14]

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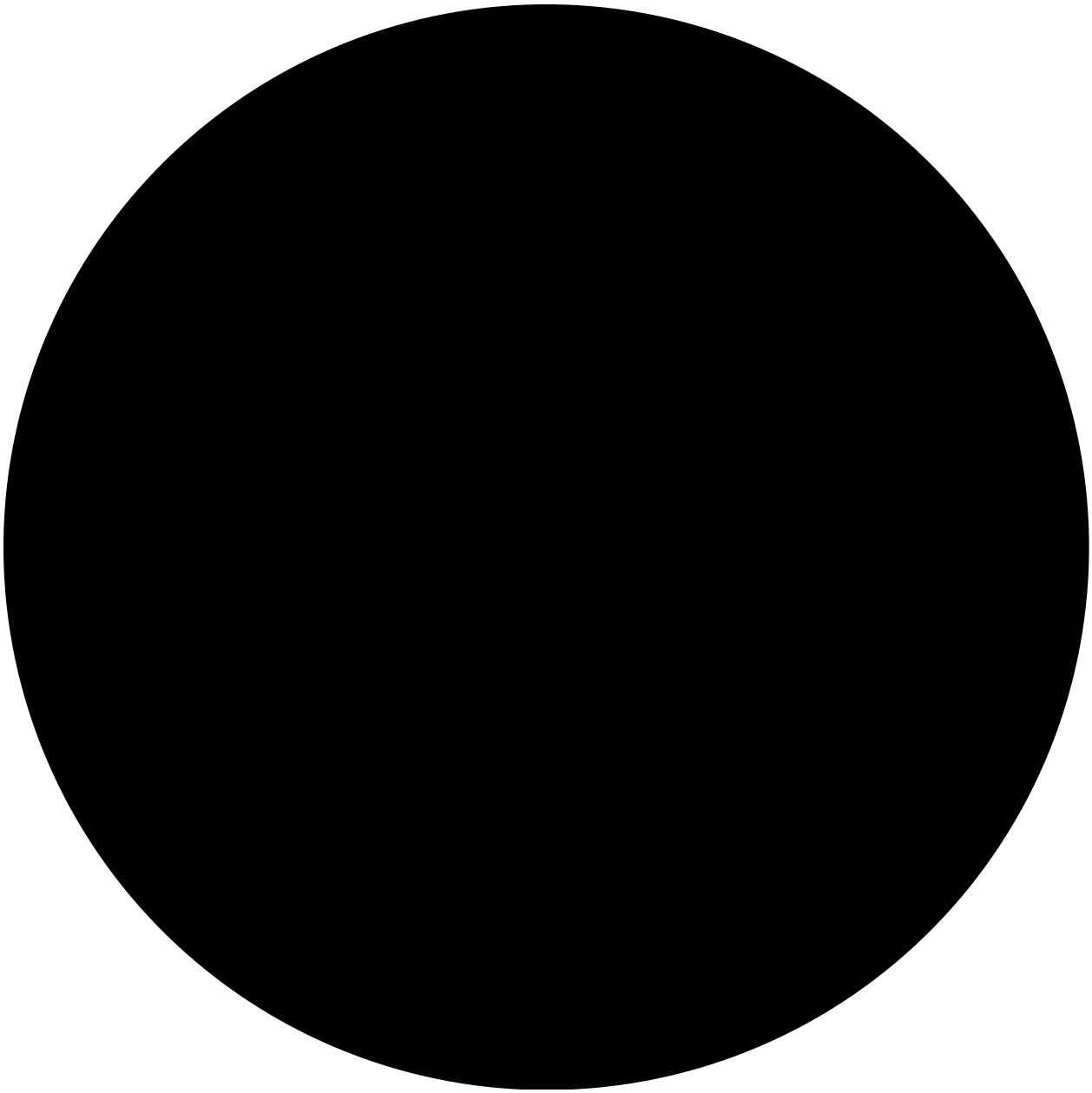

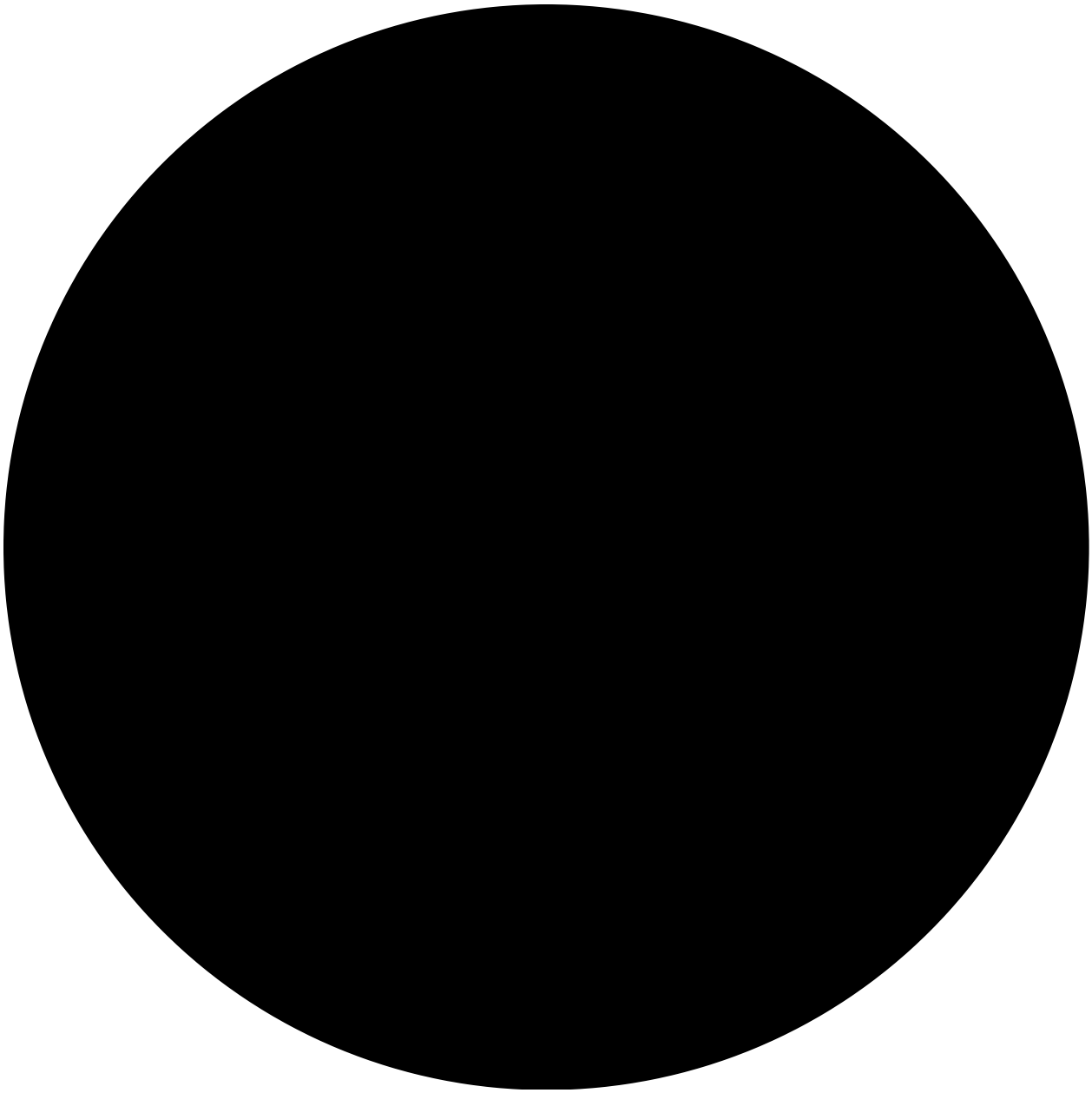

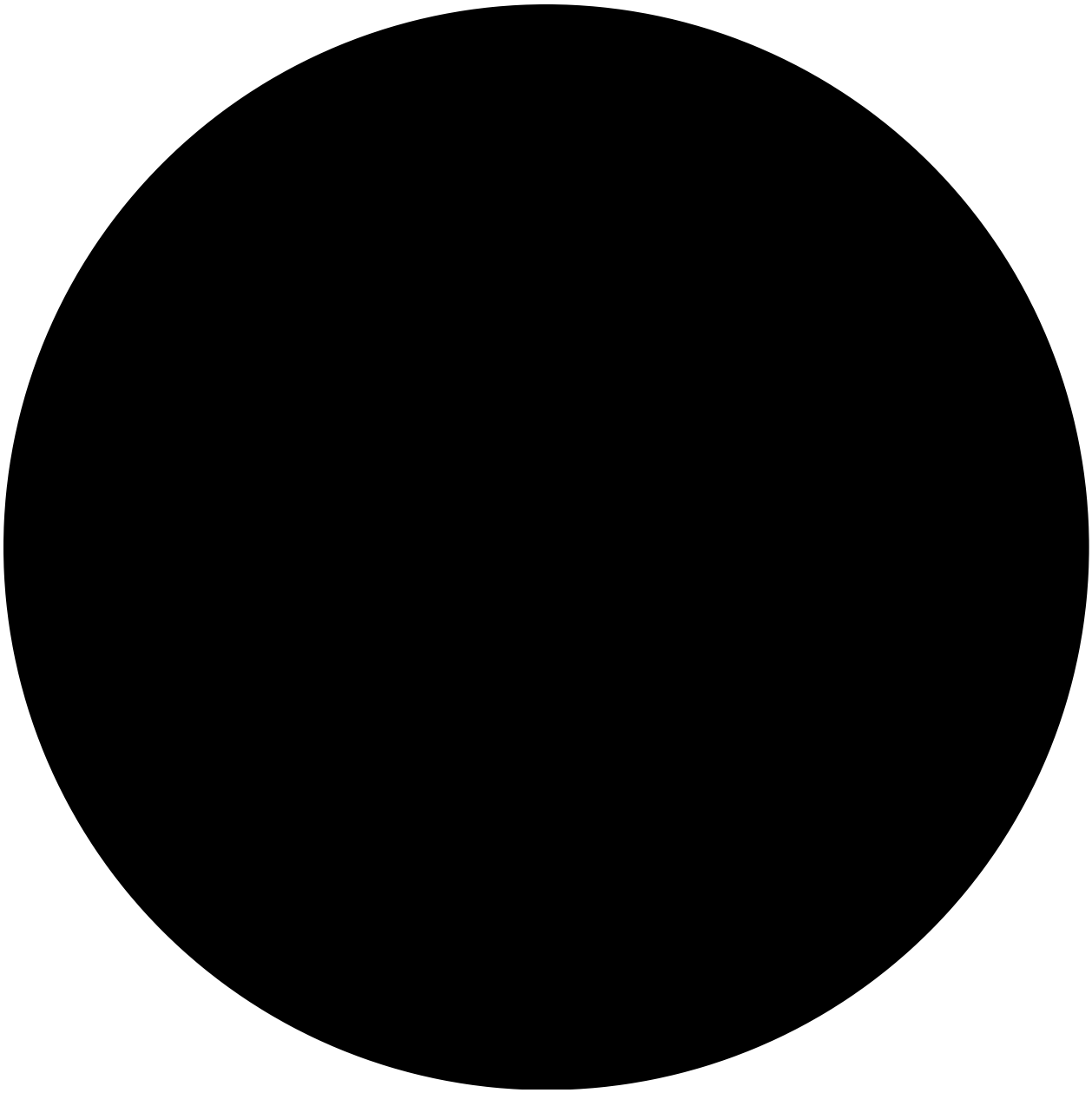

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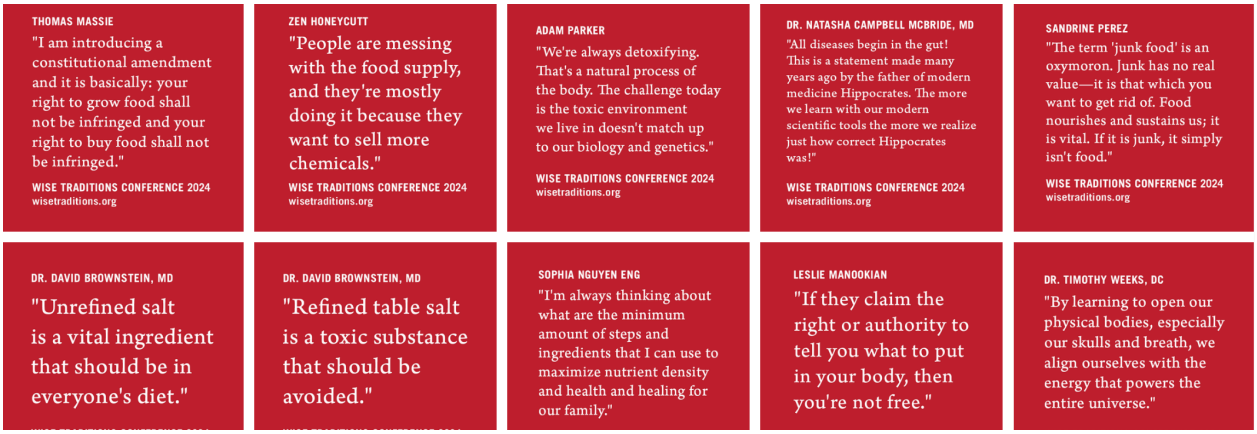
Fri 25



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Fri 1

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