Review

The Guggul for Chronic Diseases: Ancient Medicine, Modern Targets

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Abstract. *Identification of active principles and their molecular targets from traditional medicine is an enormous opportunity for modern drug development. Gum resin from Commiphora wightii (syn C. mukul) has been used for centuries in Ayurveda to treat internal tumors, obesity, liver disorders, malignant sores and ulcers, urinary complaints, intestinal worms, leucoderma (vitiligo), sinuses, edema and sudden paralytic seizures. Guggulsterone has been identified as one of the major active components of this gum resin. This steroid has been shown to bind to the farnesoid X receptor and modulate expression of proteins with antiapoptotic (IAP1, XIAP, Bfl-1/A1, Bcl-2, cFLIP, survivin), cell survival, cell proliferation (cyclin D1, c-Myc), angiogenic, and metastatic (MMP-9, COX-2, VEGF) activities in tumor cells. Guggulsterone mediates gene expression through regulation of various transcription factors, including NF-κB, STAT-3 and C/EBPα, and various steroid receptors such as androgen receptor and glucocorticoid receptors. Modulation of gene expression by guggulsterone leads to inhibition of cell proliferation, induction of apoptosis, suppression of invasion and abrogation of angiogenesis. Evidence has been presented to suggest that guggulsterone can suppress tumor initiation, promotion and metastasis. This review describes the identification of molecular targets of guggulsterone, cellular responses to guggulsterone, and animal studies and clinical trials of guggulsterone in cancer and other diseases.*

Extensive research over the last half century has revealed that most chronic illnesses, including cancer, are caused by dysregulation of multiple genes. Most modern medicines, however, tend to target only a single gene product or pathway at any given time. This is perhaps one of the major reasons that some of the recently discovered medicines are ineffective. Most allopathic medicines, furthermore, exhibit

Abbreviations: ABC, ATP-binding cassette; ABCB1, ATP-binding cassette subfamily B; AP-1, activator protein-1; BSEP, bile salt export pump; CAR, constitutive androstane receptor; CDK, cyclin-dependent kinase; COX, cyclo-oxygenase; DSS, dextran sulfate sodium; EKG, electrocardiogram; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; FXR, farnesoid X receptor; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; H_2O_2, hydrogen peroxide; HPLC, high-performance liquid chromatography; HPTLC, high-performance TLC; HUVEC, human umbilical vein endothelial cells; HVEC, human vascular endothelial cells; HVSMC, human vascular smooth muscle cells; IAP, inhibitor of apoptosis; I-BABP, ileum bile acid binding protein; IEC, intestinal epithelial cells; IFN-γ, interferon gamma; IκBα, inhibitory subunit of NF-κB; IL, interleukin; iNOS, inducible nitric oxide synthase; JAK, janus kinase; JNK, cJun *N*-terminal kinase; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; MKK4, MAP kinase kinase; MMP, matrix metalloproteinase; MRP1, multidrug-resistance protein 1; NF-κB, nuclear factor-κB; PARP, polyadenosine ribose polymerase; PBMC, peripheral blood mononuclear cells; PCNA, proliferating cell nuclear antigen; PGE, prostaglandin E; PMA, phorbol myristate acetate; PXR, pregnane X receptor; RANKL, receptor activator of NF-κB ligand; SCC, squamous cell carcinoma; SHP, small heterodimer partner; SMCS, *S*-methyl cysteine sulfoxide; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TLC, thin-layer chromatography; TNF, tumor necrosis factor; VDR, vitamin D receptor; VEGF, vascular endothelial growth factor; VLDL, very low-density lipoprotein; WOMAC, Western Ontario and McMaster Osteoarthritis Index.

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numerous side-effects and thus are not well tolerated by either healthy or sick people. These limitations, combined with the enormous cost of such medicines, have forced people to turn to traditional medicine. Although numerous health claims have been made for traditional medicines, neither the active principles nor the mechanisms are well understood in most cases. This information, if available, has the potential to make traditional medicine highly useful.

Guggul is one of the very ancient Ayurvedic drugs, having been first described in *Atharva Veda* (2000 B.C). According to *Sushrut Samhita*, guggul is, when taken orally, curative of obesity, liver dysfunction, internal tumors, malignant sores and ulcers, urinary complaints, fistula-in-ano, intestinal worms, leucoderma, sinus, edema and sudden paralytic seizures (Figure 1A). It is also considered a cardiac tonic (1, 2).

Guggul is the dry gum resin obtained from the bark of the guggul tree. It is a mixture of diterpenes, sterols, steroids, esters and higher alcohols. The active components of the plant are the guggulsterones, specifically the stereoisomers, guggulsterone E and guggulsterone Z. These are plant sterols with a high degree of human bioactivity, which have been shown to affect many biological processes. This review describes the active principle in guggul, its mechanism of action, and the findings of animal studies and clinical trials on this agent.

Sources and Chemistry

Gum guggul is obtained from the tree *Commiphora wightii* (Arnott.) Bhandari (syn. *Commiphora mukul*), which belongs to the family *Burseraceae* and is found extensively in the dry regions of the Indian subcontinent, mainly India, Pakistan and Bangladesh (3). The tree is small and shrub like, with thorny branches (Figure 1B).

In the guggul tree, the balsam or guggul (oleo-gum-resin) is present in "balsam canals" in the phloem of the larger veins of the leaf and in the soft base of the stem (4). The development and widening of the gum-resin canal in the young stem occurs schizogenously (4). Gum is tapped by incision from February to June in plants over 5 years old with a basal diameter greater than 7.5 cm (5). The fragrant yellow latex oozes out through the incisions and slowly solidifies into vermicular or stalactitic pieces, which are collected manually. About 200 to 500 g of dry guggul is obtained from a typical plant in one season. That guggul production is highest with the onset of summer (stress-induced secondary product) is supported by observations with bright field and fluorescence microscopy. Application of ethrel to the incisions enhances guggul production 22 times over that obtained without ethrel. In the long term, however, excessive production through ethrel application exhausts and eventually kills the plant (5). Ethrel did not increase the guggulsterone content in cell cultures of *C. wightii*, indicating a senescence effect on the plant *in vivo*, and a visible increase in gum resin may not essentially enhance guggulsterone production (unpublished findings). The components of guggul are known to occur in some other organisms, including some from the animal kingdom; examples include Z-guggulsterol in *Acitus sulcatus*, *Cybister tripuncatus*, *Dytiscus marginalis*, *Ilybius fenestratus*, and the bark of *Khaya grandifolia*, and guggulsterones in *Ailanthus grandiflora* (6, 7).

Research on *C. wightii* has been supported by the Ministry of Science and Technology, India, for the past 30 years. These programs resulted in development of a method for cloning (8), propagation through somatic embryogenesis in callus cultures (9, 10), development of resin canal during somatic embryogenesis (11) and guggulsterone production in callus tissue (12, 13), cell suspension cultures (14) and during embryogenesis (10). These methods need further improvement towards development of an efficient micropropagation system, as only a few shoots could be produced from explants and their rate of establishment in the field was low (8). Somatic embryogenesis was asynchronous and the conversion rate was low (9). Since the molecule of interest, guggulsterone, is present in the resin canal, cytodifferentiation may be a prerequisite for a high yield of guggulsterones. As yet, undifferentiated cell suspension cultures have only been able to produce 200 μg/l guggulsterone (Table I) in a 2l stirred-tank bioreactor system (unpublished data); the needed 10- to 50-fold increase in this yield can be generated by manipulation of the biosynthetic pathway through addition of precursors and elicitors.

Field-grown plant populations from Rajasthan and Gujarat states of India showed high genetic variability by RAPD marker and also in guggulsterone accumulation (unpublished data). Plants from the Udaipur region, which had high guggulsterone content, were selected for further studies on genetic diversity and guggulsterone production. Though apomixis is known in the plant, the variability in field-grown plants demonstrated that apomixis is not the main method of reproduction, and the seeds are produced by cross-pollination, resulting in heterozygous populations (15). Therefore, selection of superior germplasm, followed by cloning, should produce a high-yielding plant population.

The oleo-gum-resin of the guggul tree is a very complex mixture of gum, minerals, essential oils, terpenes, sterols, ferrulates, flavanones, and sterones (Figure 2 A and B); several other unknown compounds have also been isolated (16). The resin yields two fractions upon ethyl acetate extraction. The ethyl acetate-soluble fraction contains 45% of the gum resin. The insoluble fraction consists of the carbohydrate gum, which is about 55% of the gum resin. The bioactive components have been found in the ethyl acetate-soluble fraction, whereas the insoluble carbohydrate fraction is devoid of any hypolipidemic effects (17). The

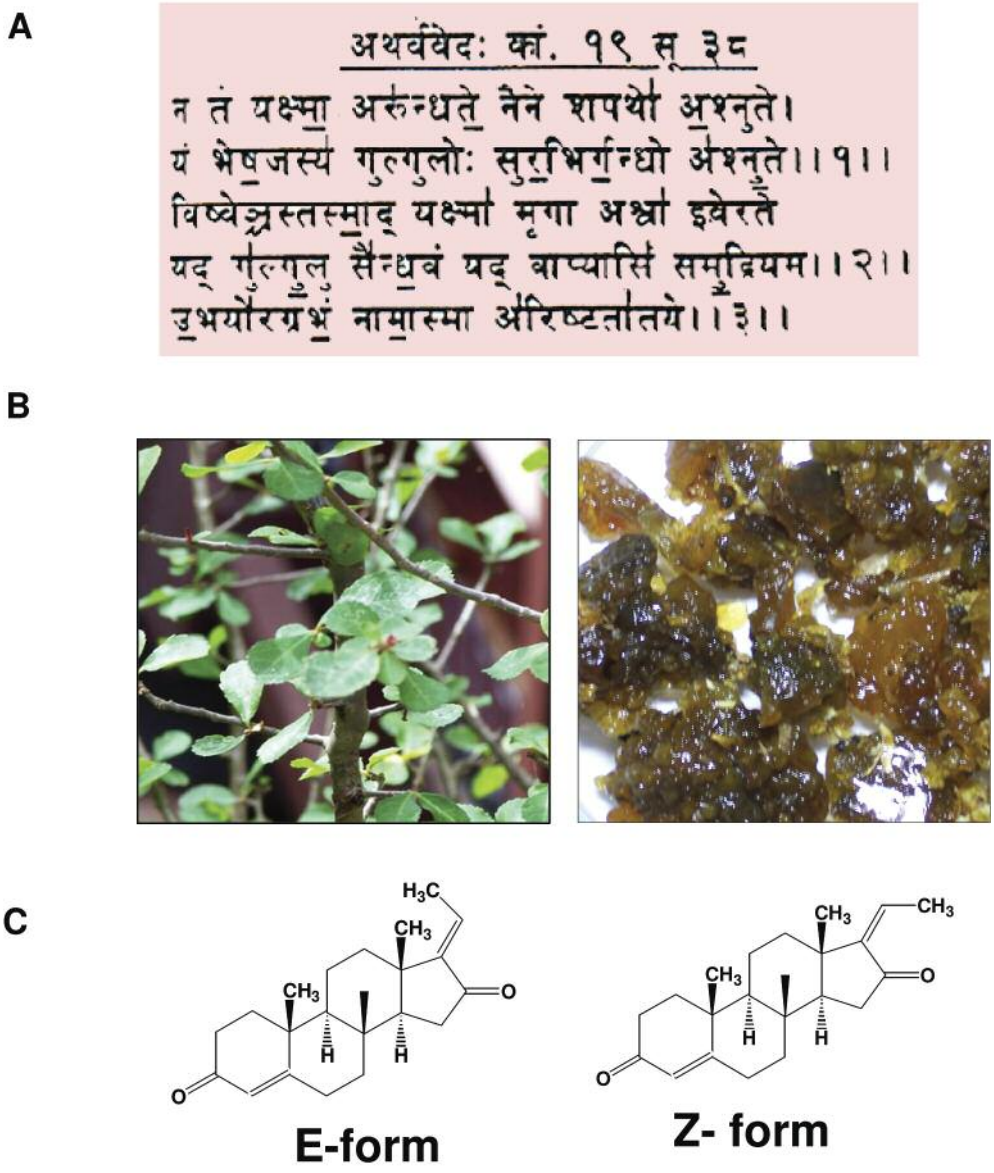

Figure 1. *A, Guggul is one of the most ancient medicines described in Ayurveda. The Veda says, "Yakshma (disease), it cannot appear in sunlight. Guggulu is the best medicine, because it develops through the rays of hot sun on specific circumstances. Guggulu has an aromatic odor. It removes the disease, like a deer that runs away on seeing the horse. A mixture of Guggulu and common salt remove the disease along with their complications" (www.gugulipid.com/trad.htm). B, (i) Commiphora wightii; (ii) gum guggul. C, Structure of guggulsterone [E form] 4,17(20)-(cis)-pregnadien-3,16-dione and [Z form] 4,17(20)-(trans)-pregnadien-3,16-dione.*

ethyl acetate-soluble fraction consists of diterpenoids, triterpenoids, steroids, lignans and fatty tetrol esters (6, 16).

Further pH gradient fractionation of the ethyl acetate-soluble fraction of guggul yields 4% acidic fraction, 1% basic fraction and 95% neutral fraction. The neutral fraction contains the bioactive components of guggul. Additional fractionation of the neutral fraction led to isolation of a major nonketonic fraction (88%) and a small ketonic fraction (12%). The hypolipidemic activity was found to be associated with the ketonic preparation, which contains a number of steroids, including the two isomers E- and Z-guggulsterone (*cis*- and *trans*-4,17(20)-pregnadien-3,16-dione) (Figure 1C). These compounds, which constitute approximately 2% of gum guggul and 5% of guggulipid, by weight, are pregnane derivatives. They are reportedly devoid of any estrogenic, antiestrogenic, or progestational activity, and recent studies confirm that they do not activate the conventional steroid receptors (18). Pharmacological studies

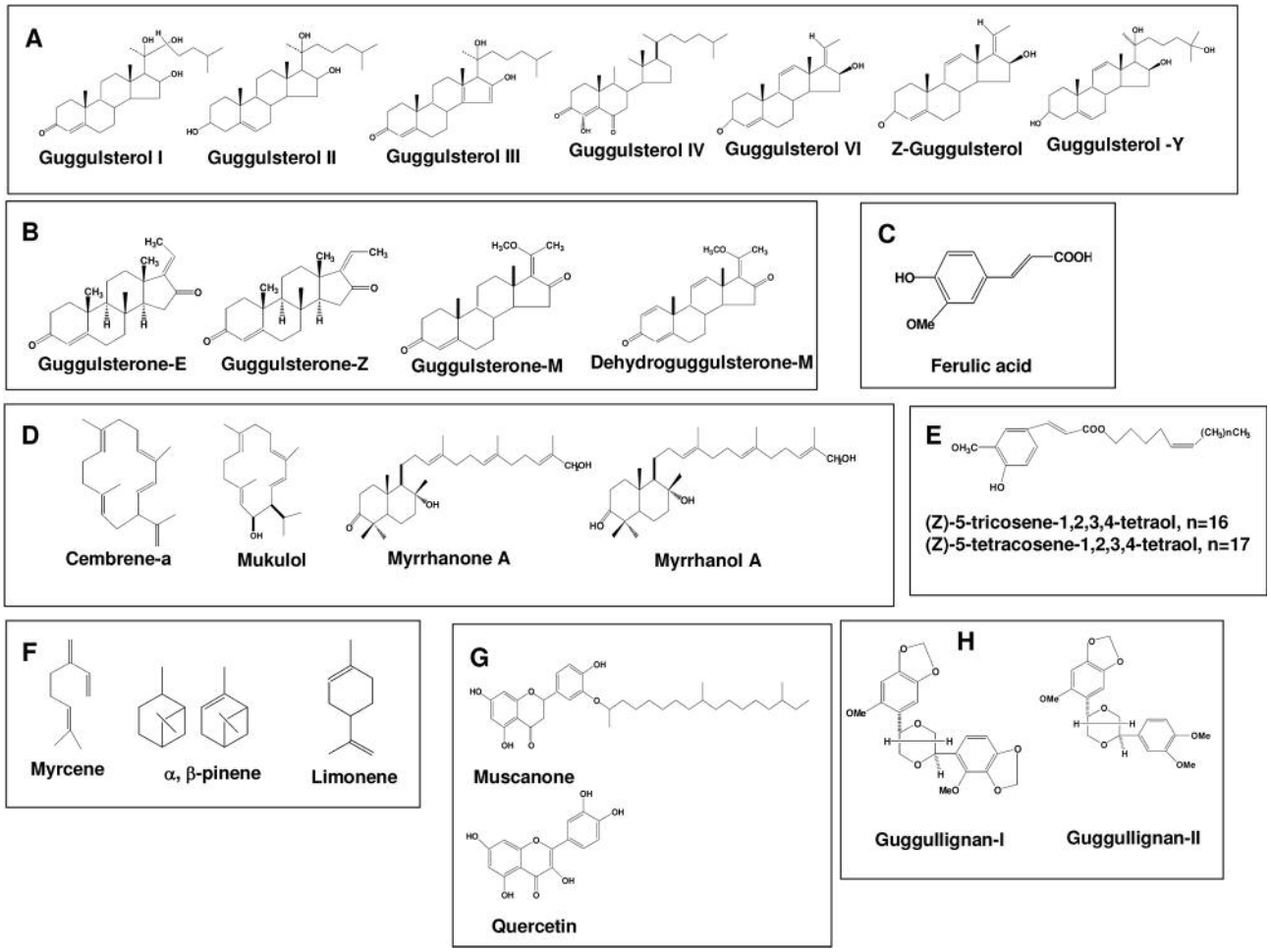

Figure 2. *Chemical constituents of Commiphora wightii/mukul gum resin (6, 16). A: sterols; B: steroids; C: acids; D: terpenes and alcohol; E: ester mixture; F: essential oils; G: flavanones; H: lignans.*

revealed that the pure guggulsterone isomers had pronounced hypolipidemic activity (3).

The synthesis and stereochemistry of these compounds were reported long before their actual isolation (19). The compounds isolated from gum guggul were found to be identical to the synthesized compounds in all respects. The chemical synthesis method was used to produce E and Z form of guggulsterone so that the crystal structures of these steroids could be determined (20, 21). Besides the guggulsterones, sterols have also been isolated from guggul and evaluated for their pharmacological properties. Some of these properties, such as the antidiabetic and anti-inflammatory activities of specific fractions, have been patented (22, 23). Several methods such as thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and high-performance liquid chromatography (HPLC) have been reported for the separation and estimation of guggulsterones in oleo-gum-resin and small tissue samples (7).

Several metabolites of guggulsterone have been identified in fungi, such as *Aspergillus niger* and *Cephalosporium aphidicola*. The hydroxylated metabolites, *cis*- and *trans*-7β-hydroxyl-4,17(20)-pregnadien-3,16-dione and 6β,11α-dihydroxyl-4,17(20)-pregnadien-3,16-dione, have antibacterial activity, whereas the 11α-hydroxylated metabolite has free radical-scavenging activity (24, 25).

Molecular Targets

Several reports have shown that guggulsterone modulates numerous targets (Figure 3). These include growth factors, growth factor receptors, transcription factors, cytokines, enzymes, and genes regulating apoptosis.

Farnesoid X receptor. Guggulsterone has been described as a farnesoid X receptor (FXR) antagonist (18). FXR is a member of the nuclear hormone receptor superfamily and is

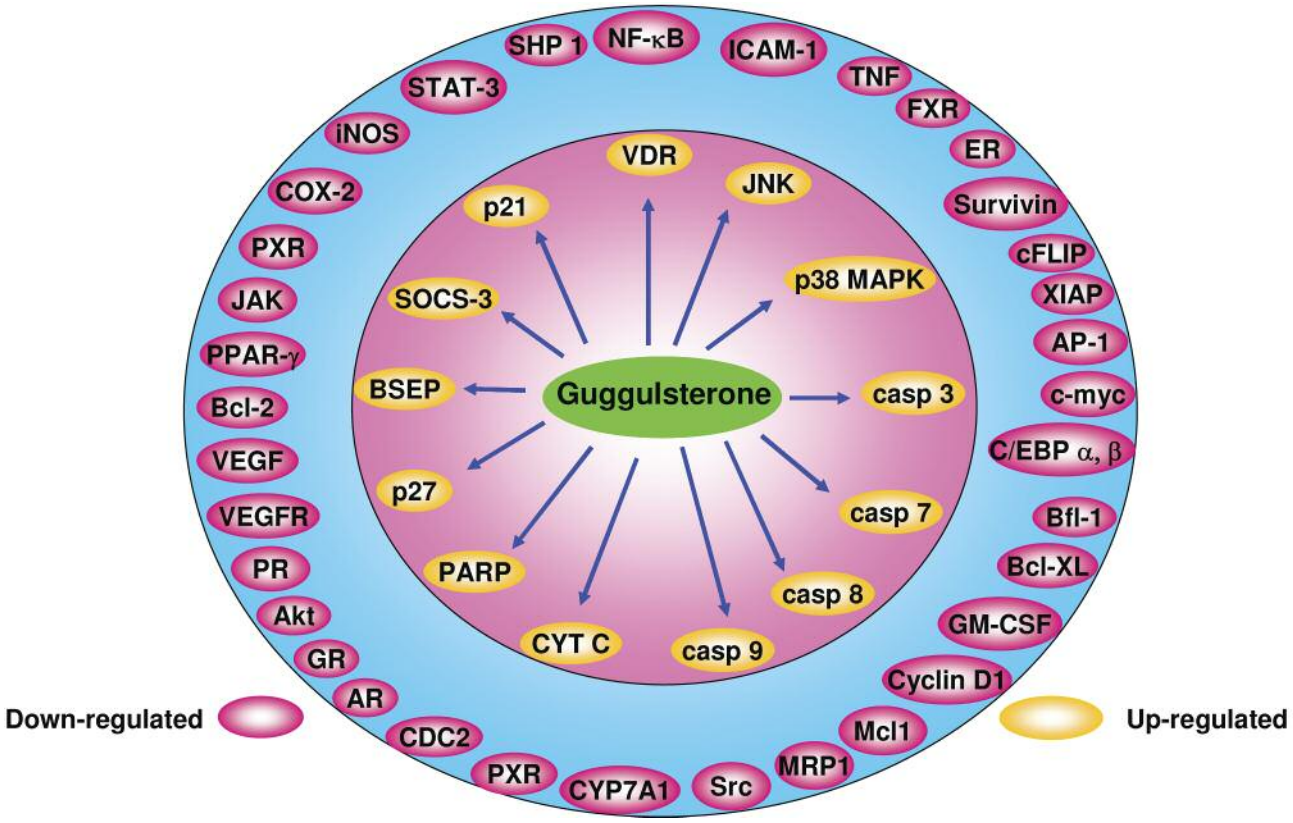

Figure 3. *Molecular targets of guggulsterone. These include AP-1, activator protein-1; AR, androsterone receptor; BSEP, bile salt export pump; casp, caspase; CDC 2, cell division cycle kinase 2; C/EBP,CCAAT/enhancer binding protein; c-FLIP, cellular caspase-8 (FLICE)-like inhibitory protein; COX, cyclo-oxygenase; CYT C, cytochrome C; ER, estrogen receptor; FXR, farnesoid X receptor; GM-CSF, granulocyte monocyte colony-stimulating factor; GR, glucocorticoid receptor; ICAM, intracellular adhesion molecule; iNOS, inducible nitric oxide synthase; JAK, janus kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; Mcl1, myeloid leukemia cell 1; MRP1, multidrug resistance protein 1; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; PARP, polyadenosine ribose polymerase; PPAR-γ, peroxisome proliferators-activated receptor-gamma; PR, progesterone receptor; PXR, pregnane X receptor; SHP, small heterodimer partner; STAT, signal transducer and activator of transcription; SOCS3, suppressor of cytokine signaling; TNF, tumor necrosis factor; VDR, vitamin D receptor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; XIAP, x-linked inhibitor of apoptosis protein.*

expressed mainly in the liver, kidney and small intestine (26). It is involved in the regulation of bile acid (27), cholesterol (27), triglyceride (28) and glucose (29) metabolism. Guggulsterone treatment reduced hepatic cholesterol in wild-type mice fed with a high-cholesterol diet, but was not effective in *FXR*-null mice, suggesting that inhibition of FXR activation may be the basis for the cholesterol-lowering activity of guggulsterones (18) (Figure 4).

Barrett's esophagus is a risk factor for esophageal adenocarcinoma and is associated with reflux disease. FXR is significantly overexpressed in Barrett's esophagus compared to normal mucosa, mucosa affected by esophagitis, and esophageal adenocarcinoma (30). *In vitro* treatment with guggulsterone induced apoptosis in Barrett's esophagus-derived cells, suggesting that FXR contributes to regulation of apoptosis in Barrett's esophagus (30).

The reports described here suggest that FXR can be a potential target for cancer therapy. As guggulsterone has been shown to modulate FXR, its potential as an anticancer agent needs to be explored.

Nuclear receptors. Nuclear receptors are ligand-modulated transcription factors (31). The constitutive androstane receptor (CAR) is a central regulator of xenobiotic metabolism. Guggulsterone represses expression of the cytochrome P450 2b10 (*Cyp2b10*) gene by inhibiting CAR activity in hepatocytes lacking a functional pregnane X receptor (32).

Both E and Z forms of guggulsterone display a high affinity for steroid receptors, including androgen, glucocorticoid, and progesterone receptors; with K_i values ranging from 224 to 315 nM, this binding alters the functions of these steroid receptors (33).

Bile salt export pump. Conversion of cholesterol to bile acids in the liver is initiated by the rate-limiting enzyme CYP7A1,

and excretion of bile acids from the liver is mediated by the bile salt export pump (BSEP) (34, 35).

Deng *et al*. demonstrated that guggulsterone synergistically induced expression of BSEP in cells treated with FXR-agonist bile acids, possibly through the AP-1 activation pathway, and that such transactivation was dominant over its FXR antagonism (36). The up-regulation of BSEP expression by guggulsterone without activating FXR to suppress CYP7A1 favors metabolism of cholesterol to bile acids by removing the trigger (*i.e*. bile acids) of the negative-feedback suppression of CYP7A1. Thus, enhanced BSEP expression by guggulsterone represents a possible mechanism by which guggulsterone exerts its anticancer and hypolipidemic effects.

Nuclear factor-κB. Guggulsterone may operate through suppression of nuclear factor kappaB (NF-κB) activation (37). NF-κB is a nuclear transcription factor required for expression of genes involved in cell proliferation, cell invasion, metastasis, angiogenesis and resistance to chemotherapy (38, 39). Several groups, including ours, have shown that activated NF-κB suppresses apoptosis in a wide variety of tumor cells and it has been implicated in chemoresistance. We have shown that guggulsterone down-regulates NF-κB activation induced by various tumor promoters, including phorbol ester, cigarette smoke, tumor necrosis factor (TNF) and hydrogen peroxide (37). Guggulsterone-induced down-regulation of NF-κB is mediated through suppression of IκBα kinase activation. Guggulsterone also suppresses the constitutively active NF-κB in tumor cells. Suppression of NF-κB by guggulsterone decreased expression of gene products involved in antiapoptosis (IAP1, xIAP, Bfl-1/A1, Bcl-2, cFLIP, survivin), proliferation (cyclin D1, c-Myc) and metastasis (MMP-9, COX-2, VEGF) (37).

Signal transducer and activator of transcription. Signal transducer and activator of transcription (STAT) proteins are signaling molecules with dual functions that were discovered during studies on interferon (IFN)-γ-dependent gene expression (40). Recently, STAT3 was shown to be a direct activator of the vascular endothelial growth factor (*VEGF*) gene, which is responsible for increased angiogenesis. Ahn *et al*. found that the Z- but not the E-stereoisomer of guggulsterone inhibited both constitutive and interleukin (IL)-6-induced STAT3 activation in human multiple myeloma cells (41). The suppression of STAT3 was mediated through inhibition of activation of protein tyrosine kinases JAK2 and c-Src. Guggulsterone induced expression of both the protein and mRNA for tyrosine protein phosphatase SHP-1, but this activity was not due to demethylation of the SHP-1 promoter previously implicated in the epigenetic silencing of SHP-1. Guggulsterone also down-regulated expression of STAT3-regulated antiapoptotic (Bcl-2, Bcl-xl, Mcl-1), proliferative (cyclin D1), and angiogenic (VEGF) gene products, and this correlated with suppression of proliferation, accumulation of cells in the sub-G_1-phase of the cell cycle and the induction of apoptosis (41). Overall, guggulsterone is a novel blocker of STAT3 activation and may have potential in the regulation of growth and metastasis of tumor cells.

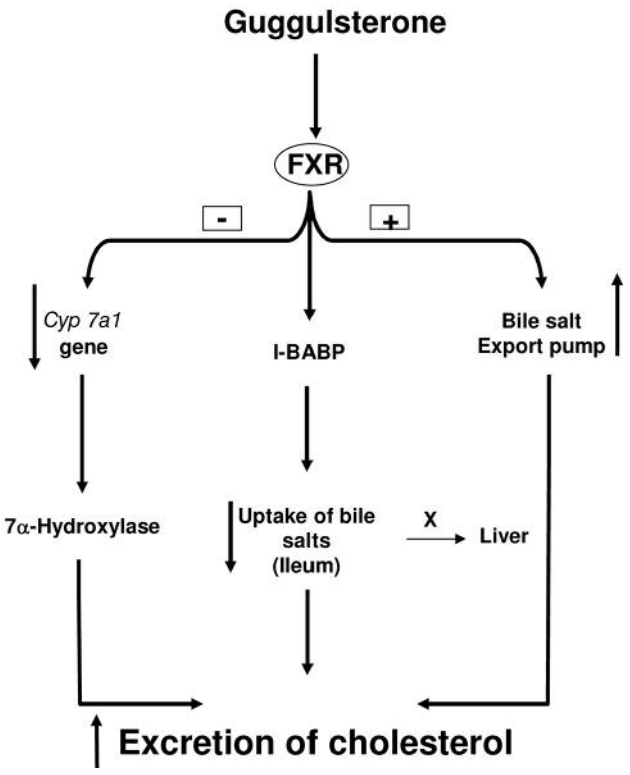

Figure 4. *Guggulsterone eliminates cholesterol by binding to FXR. Guggulsterone acts as an agonist that activates bile salt export pump (BSEP), which eliminates cholesterol. Ileum-bile acid-binding protein (I-BABP) inhibits uptake of bile salt from the ileum, resulting in higher excretion of cholesterol. Guggulsterone also acts as antagonist by inhibiting cytochrome P450 7a1 gene resulting in higher cholesterol excretion.*

Vascular endothelial growth factor. For most solid tumors, including breast cancer, angiogenesis is essential for tumor growth and metastasis (42).

Z-Guggulsterone has been shown to inhibit angiogenesis both *in vitro* and *in vivo* (43). Treatment with Z-guggulsterone inhibited capillary-like tube formation by HUVEC and migration by HUVEC and DU145 human prostate cancer cells in a concentration- and time-dependent manner. The Z- and E-isomers of guggulsterone seemed equipotent as inhibitors of HUVEC tube formation. The Z-guggulsterone-mediated inhibition of angiogenesis *in vitro* correlated with suppression of secretion of proangiogenic growth factors VEGF and granulocyte colony-stimulating factor, VEGF-R2 proteins, and inactivation of Akt. Oral gavage of 1 mg/day Z-guggulsterone

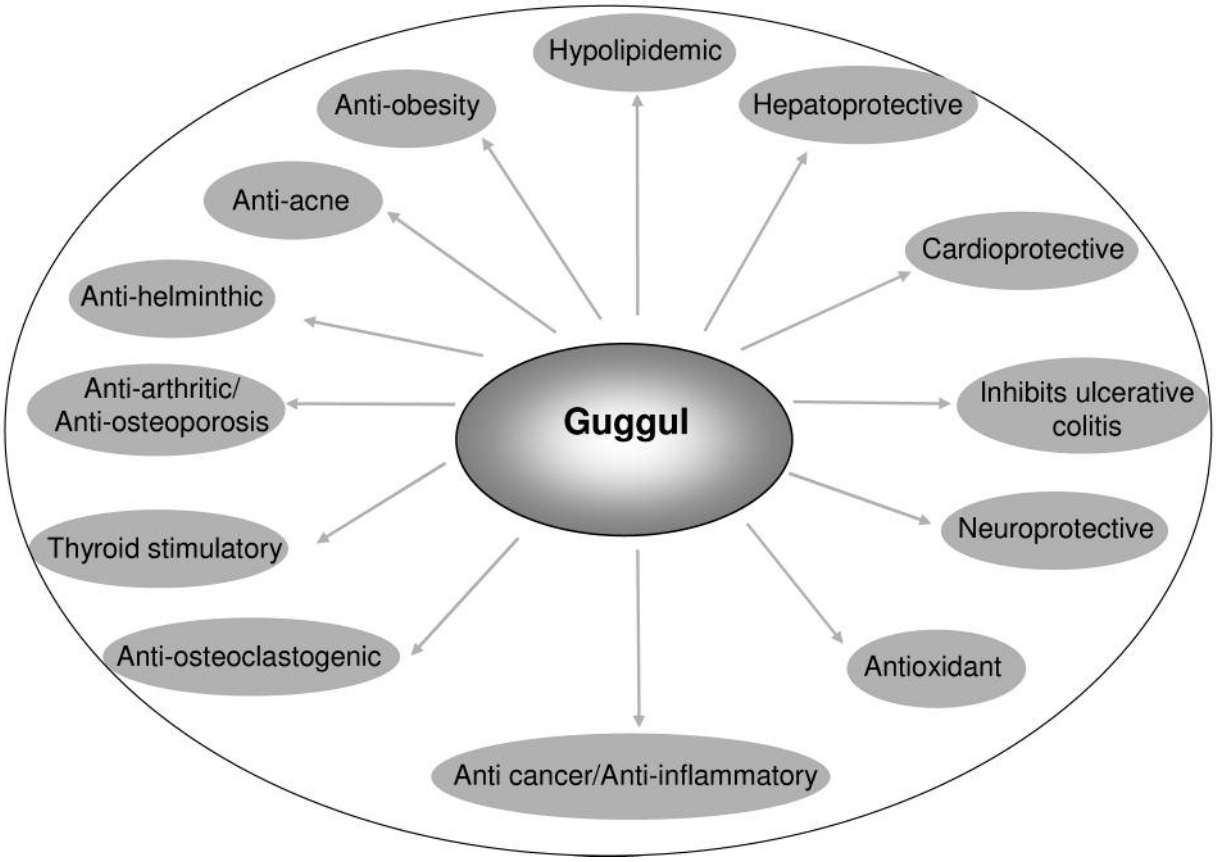

Figure 5. *Use of guggul for chronic proinflammatory diseases*.

(five times/week) to male nude mice inhibited *in vivo* angiogenesis in a DU145-Matrigel plug assay as evinced by a statistically significant decrease in tumor burden, microvessel area, and VEGF-R2 protein expression (43).

Inflammatory cytokines. Several reports have demonstrated that guggulsterone exerts its antiinflammatory effects through suppression of cytokines. The crude ethyl acetate extract of gum guggul suppressed inflammatory mediators such as IFN-γ, IL-12, TNF-α, and IL-1β, whereas no inhibition was observed in the case of antiinflammatory cytokine IL-10 (44). Receptor activator of NF-κB ligand (RANKL), a member of the TNF superfamily, has been implicated as a major mediator of bone resorption that is commonly associated with aging and certain types of cancer, including multiple myeloma and breast cancer. Guggulsterone modulated signaling and osteoclastogenesis induced by RANKL and tumor cells. Guggulsterone suppressed RANKL and tumor cell-induced osteoclastogenesis by suppressing activation of NF-κB (45).

To better understand the role of guggulsterone on cytokine-induced inflammation, Lv *et al*. studied the effect of guggulsterone on IL-1β- and IFN-γ-induced beta-cell damage in the islets of Langerhans (46). Treatment of rat insulinoma cells with IL-1β and IFN-γ induced cell damage, which correlated with nitric oxide (NO) and prostaglandin E2 (PGE2) production. Guggulsterone completely prevented cytokine-mediated cytotoxicity, as well as NO and PGE2 production. Guggulsterone suppressed levels of inducible nitric oxide synthase (iNOS) and cyclo-oxygenase (COX)-2 mRNA and protein expression, most likely through suppression of NF-κB. The cytoprotective effects of guggulsterone were also mediated through suppression of the JAK/STAT pathway. Levels of the protein SOCS-3 were down-regulated in cells treated with the cytokines. Collectively, these results suggest that guggulsterone prevented cytokine-induced cell damage (46).

Cyclo-oxygenase 2. COX-2 converts arachidonic acid into prostaglandins and prostanoids. COX-2 induction is responsible for inflammation and pain. We have demonstrated that guggulsterone suppressed TNF-induced *COX-2* promoter activity and protein expression *in vitro* in a dose-dependent fashion (37). As already noted, guggulsterone suppressed cytokine-induced expression of *COX-2* mRNA and protein in rat insulinoma cells (46).

In another study, bioassay-guided isolation of compounds from the hexane-soluble portion of the methanol extract of water-boiled gum guggul yielded 14 compounds that were assayed for lipid peroxidation and COX-inhibitory activities. At a concentration of 100 ppm, the fraction containing cembrenoids and lignan inhibited lipid peroxidation by 50%; the rest of the isolated compounds showed 20% to 40% inhibitory activity with respect to the controls. At the same concentration, cembrenoids inhibited COX-1 and COX-2 by 79% and 83%, and guggulsterone by 67% and 54%, respectively. All 14 compounds inhibited COX-1 at this concentration (47). The COX-2–inhibitory activity of guggul may explain in part its antiinflammatory activity and anticancer effects.

Matrix metalloproteinase 9. The MMP family of extracellular proteinases has been associated with cancer invasion and metastasis by virtue of their ability to collectively degrade all components of the ECM (48). We have demonstrated that guggulsterone suppresses activation of MMP-9 in tumor cells through suppression of NF-κB (37).

Nitric oxide synthase (iNOS). iNOS is one of three key enzymes generating NO from the amino acid L-arginine. Guggulsterone inhibited lipopolysaccharide-induced NO production in macrophages (49). Lv *et al*. recently reported that guggulsterone suppressed cytokine-induced expression of *iNOS* mRNA and protein. These observations suggest that guggulsterone may have antimetastatic activity through suppression of iNOS (46).

Mitogen-activated protein kinases. Most inflammatory stimuli are known to activate three independent mitogen-activated protein kinase (MAPK) pathways, p44/42 MAPK (also called ERK1/ERK2), JNK and p38 MAPK. We have found that guggulsterone activates JNK in an *in vitro* kinase assay. Guggulsterone induced JNK activation without altering levels of JNK protein expression. JNK activation is required for guggulsterone-induced apoptosis (50). Singh *et al*. have shown, furthermore, that guggulsterone-induced cell death in human prostate cancer cells is caused by reactive oxygen intermediate (ROI)–dependent activation of JNK (51). Exposure of PC-3 and LNCaP prostate cancer cells to apoptosis-inducing concentrations of guggulsterone resulted in activation of JNK and p38 MAPK in both cell lines and activation of ERK1/2 in LNCaP cells. Pharmacological or genetic suppression of JNK reduced guggulsterone-induced apoptosis in PC-3/LNCaP cells. Suppression of p38 MAPK activation in PC-3 or LNCaP cells and ERK1/2 activation in LNCaP cells did not protect against guggulsterone-induced cell death. The guggulsterone treatment caused generation of ROI in prostate cancer cells but not in a normal prostate epithelial cell line, which was also resistant to guggulsterone-mediated JNK activation (51).

In contrast to this observation, however, Manjula *et al*. showed that a crude ethyl acetate extract of gum guggul inhibited phosphorylation of all three MAPK in peripheral blood mononuclear cells (PBMCs). This crude extract also suppressed *c-fos* and *c-jun* mRNA levels in phorbol myristate acetate (PMA)-stimulated PBMC (44). Most likely, the difference in results can be attributed to the cell type and the crude nature of the extract.

Cell cycle proteins. Guggulsterone has been shown to modulate several proteins involved in regulation of the cell cycle. Cyclin D1 has been shown to be overexpressed in many types of cancer, including those of the breast, esophagus, head and neck, and prostate (52-56). It is possible that the antiproliferative effects of guggulsterone are due to inhibition of cyclin D1 expression. Guggulsterone inhibited expression of both cyclin D1 and cdc2 in U937 leukemia cells (50). It induced expression of both p21 and p27 in a time-dependent manner (50). Both p21 and p27 modulate cell cycle progression by inhibiting the activity of cyclin/cyclin-dependent kinase (CDK)-2 complexes.

Apoptosis-inducing proteins. Guggulsterone inhibits the growth of a wide variety of tumor cells and induces apoptosis through down-regulation of antiapoptotic gene products, modulation of cell cycle proteins, activation of caspases, inhibition of Akt, and activation of JNK (50). Guggulsterone down-regulates the expression of antiapoptotic gene products Bfl-1, xIAP, cFLIP, cMyc, Bcl-2, Bcl-X_L, survivin, c-myc and COX-2 (50, 57). It induces apoptosis in part through activation of caspase-8, cleaving BID, which then translocates to the mitochondria and stimulates release of cytochrome *c*, which in turn activates caspase-9. Cytochrome *c* release and activation of caspase-3 and polyadenosine ribose polymerase (PARP) cleavage follow guggulsterone-induced BID cleavage. Thus, one possible mechanism by which guggulsterone induces apoptosis is through changes in the mitochondrial membrane potential, which would lead to release of cytochrome *c* from the mitochondria, and thus to sequential activation of caspase-9 and caspase-3 (50, 58).

Besides down-regulating antiapoptotic gene products, guggulsterone also mediates its apoptotic effects through down-regulation of the Akt pathway (50, 59). Guggulsterone activated JNK and that suppression of JNK by its specific inhibitor abolished activation of caspase-3, PARP cleavage and cell proliferation. The activation of JNK requires activation of an upstream kinase, MKK4 (60). We have shown that guggulsterone activated JNK in wild-type murine embryonic fibroblast cells but not in *MKK4*-deficient mutant cells, suggesting that activation of JNK by guggulsterone requires MKK4. The lack of JNK activation in mutated cells correlated with suppression of guggulsterone-induced apoptosis, suggesting the critical role of JNK (50).

Multidrug transporters. The effects of guggulsterone on human drug efflux transporters P-glycoprotein (P-gp, ABCB1) and multidrug-resistance protein 1 (MRP1, ABCC1) were investigated using P-gp-overexpressing human KB-C2 carcinoma cells and human *MRP1* gene-transfected KB/MRP cells (61). Presence of guggulsterone led to accumulation of daunorubicin or rhodamine 123, fluorescent substrates of P-gp, in KB-C2 cells. The efflux of rhodamine 123 from KB-C2 cells was inhibited by guggulsterone. Guggulsterone also increased accumulation of calcein, a fluorescent substrate of MRP1, in KB/MRP cells. The ATPase activities of P-gp and MRP1 were stimulated by guggulsterone. These results suggest that guggulsterone may have dual inhibitory effects on P-gp and MRP1 and the potential to interact with drugs.

Anticancer Activities

Guggulsterone has been shown to induce apoptosis and suppress proliferation, invasion, angiogenesis and metastasis of tumor cells. Various mechanisms have been suggested to explain the anticarcinogenic effects of guggulsterone, including inhibition of ROI, suppression of inflammation and inhibition of nuclear receptors, transcription factors, inflammatory cytokines, antiapoptotic proteins, cell survival pathways, COX-2, MMP-9, iNOS and cell cycle–related- proteins.

Proliferation. Guggulsterone suppresses the growth and proliferation of a wide variety of tumor cells, including leukemia, head and neck carcinoma, multiple myeloma, lung carcinoma, melanoma, breast carcinoma and ovarian carcinoma (50). It also inhibited proliferation of imatinib mesylate resistant leukemia, dexamethasone-resistant multiple myeloma and doxorubicin-resistant breast cancer cells. Guggulsterone suppressed proliferation of tumor cells through inhibition of DNA synthesis, producing cell cycle arrest in the S phase, and this arrest correlated with decreases in the levels of cyclin D1 and cdc2 and concomitant increases in the levels of CDK inhibitors p21 and p27 (50). Guggulsterone-mediated suppression of PC-3 cell proliferation was characterized by the appearance of subdiploid cells and cytoplasmic histone-associated DNA fragmentation (51).

Apoptosis. Besides suppressing proliferation, guggulsterone induces apoptosis in a wide variety of cells. The mechanism of apoptosis induced by guggulsterone was both mitochondria dependent as well as mitochondria-independent (50, 51). The growth-inhibitory effects correlated with externalization of phosphatidylserine and loss of mitochondrial membrane potential (58). Guggulsterone induced apoptosis in a dose-dependent manner in PC-3 cells, but not in normal prostate epithelial cells. Guggulsterone-induced apoptosis in PC-3 cells was associated with induction of multidomain proapoptotic Bcl-2 family members Bax and Bak (51). The apoptotic effects of guggulsterone were preceded by activation of JNK and down-regulation of Akt activity (50, 51).

As already noted, guggulsterone has been found to induce apoptosis in a Barrett's esophagus derived cell line in which FXR is significantly overexpressed. The induction of apoptosis by guggulsterone in this cell line suggests that FXR may contribute to regulation of apoptosis (30).

Invasion. When treatment is ineffective, the remaining tumor cells inevitably infiltrate the surrounding normal tissue, which leads to tumor recurrence. Guggulsterone suppresses the invasion through down-regulation of the expression of MMP-9 (37).

Angiogenesis. Angiogenesis is a crucial step in the growth and metastasis of many cancers (42). As described earlier guggulstrone was found to inhibit the process of angiogenesis both *in vitro* as well as *in vivo*. (43).

Metastasis. The metastasis of cancer requires migration of cancerous cells both into and out of the walls of vessels that transport them to other parts of the body. The ability to penetrate through vessel walls is mediated by specific molecules that are expressed on the endothelial cells of the blood vessels in response to a number of signals from inflammatory cells and tumor cells. The NF-κB-regulated gene products MMP-9, COX-2, and VEGF have been implicated in tumor cell metastasis, and guggulsterone has been shown to suppress these proteins (37).

Silva *et al*., while investigating the role of bone lipids in breast cancer migration to bone, showed that sodium deoxycholate released from osteoblast-like cells MG63 and bone tissue promotes cell survival and induces migration of metastatic human breast cancer MDA-MB-231 cells. The FXR antagonist Z-guggulsterone prevented migration of these cells and induced apoptosis in breast cancer cells (62).

Other Proinflammatory Chronic Diseases

Inflammation is caused by activation of inflammatory signal pathways such as the NF-κB signal pathway and release of inflammatory mediators such as the proinflammatory cytokines (*e.g.* TNF and IL-1β) and proinflammatory enzymes that mediate production of prostaglandins (*e.g.* COX-2) and leukotrienes (*e.g.* lipo-oxygenase), together with expression of adhesion molecules and MMPs (63). This combination of events leads to chronic inflammatory diseases such as atherosclerosis, arthritis, colitis, or even cancer. The first documented evidence of the anti-inflammatory activity of guggul was reported in 1960 by Gujral *et al*. (64); a

confirmatory report was published in 1977 by Sharma and Sharma (65), but it was not until 2004 that guggulsterone was demonstrated for the first time to suppress activation of the proinflammatory transcription factor NF-κB and the genes regulated by NF-κB (37).

Oxidative stress plays an important part in many human diseases. Although it is unknown whether oxidative stress is the cause or a consequence of disease, antioxidants are widely used for maintaining health and preventing diseases. Guggulipid suppresses formation of lipid peroxides (66) and prevents oxidation of low-density lipoproteins (LDL) *in vitro* (67, 68). In more recent studies, guggulsterone at concentrations of 5 to 20 μM effectively inhibited LDL peroxidation and generation of free oxygen radicals (69, 70). This finding indicates that guggulsterone may be of therapeutic benefit in diseases associated with oxidative stress, such as myocardial ischemia and neurodegenerative diseases (Figure 5).

Hypolipidemic activity. Herbal extracts from guggul have been widely used in Asia as cholesterol-lowering agents, and their popularity is increasing in the United States. Guggul extract was approved by the United States Food and Drug Administration in 1994 as a dietary supplement. Guggulsterones -E and -Z are responsible for the lipid-lowering properties of guggul in human blood and at least four mechanisms have been proposed to explain their activity (7). First, guggulsterones might interfere with formation of lipoproteins by inhibiting biosynthesis of cholesterol in the liver (71). Second, guggulsterones have been shown to enhance the uptake of LDLs by the liver through stimulation of LDL receptor–binding activity in the membranes of hepatic cells (72). Third, guggulsterones increase fecal excretion of bile acids and cholesterol, substantially decreasing the rate of absorption of fat and cholesterol in the intestine (71). Finally, guggulsterones directly stimulate the thyroid gland (73). Several animal studies and clinical trials have been performed to evaluate the hypolipidemic effects of guggul.

To evaluate the effects of guggul on disorders of lipid metabolism, with special reference to atherosclerosis and obesity, Satyavati *et al.* conducted the first animal study on rabbits, from 1964 through 1966 (74). In this study, rabbits were fed hydrogenated vegetable oil to raise their cholesterol levels. One group of rabbits was fed guggul, whereas the other group served as a control. Satyavati *et al.* demonstrated that administration of gum guggul significantly lowered the serum cholesterol levels of hyperlipidemic rabbits, prevented cholesterol-induced arteriosclerosis and decreased the body weight of the animals (74). These data provided the first experimental evidence to support claims in the Ayurvedic text that guggul may be effective in the treatment of hypercholesterolemia and atherosclerosis. In another study, hypercholesterolemia was induced in male albino rabbits by administration of cholesterol (500 mg/kg body weight). The experimental animals were then fed gum guggul at the dose of 2 g/kg body weight daily for 6 weeks. Both the control and experimental cholesterol-fed animals showed elevated levels of serum and tissue cholesterol, but guggul-fed animals had significant decreases in the level of cholesterol and body weight (2).

The study by Satyavati *et al.* did not examine the effect of guggul on triglyceride levels; however, another study by Singh *et al.* examined the effect of guggulsterone on cholesterol and triglyceride levels in rats. Guggulsterone (25 mg/kg body weight for 10 days) lowered serum cholesterol and triglyceride levels by 27% and 30%, respectively (72). LDL binding to hepatic cell membranes was significantly increased in guggulsterone-treated rats (72). Chander *et al.* examined the effect of guggulsterone on serum lipid levels in triton- and cholesterol-fed rats. In triton-fed rats, guggulsterone (50 mg/kg body weight) significantly reduced serum lipid levels. In cholesterol-fed rats, guggulsterone (5 mg/kg body weight) decreased serum levels of LDL and very low-density lipoprotein (VLDL). Moreover, guggulsterone treatment was found to increase the activity of lipolytic enzymes as well as receptor-mediated catabolism of LDL (75). The effect of dietary guggulipid on serum lipid levels was also evaluated in Fisher rats, which were fed a diet containing 1% to 5.6% guggulipid for 10 days. Guggulipid-induced decreases in serum levels of triglycerides (22%-70%), LDL, and VLDL and increases in serum levels of high-density lipoprotein (HDL) were dose dependent (76).

In another study, the lipid-lowering action of guggulipid was compared with that of *S*-methyl cysteine sulfoxide isolated from *Allium cepa* in Sprague-Dawley rats fed a 1% cholesterol diet (77). Animals that received guggulipid at 50 mg/kg body weight for 45 days had significantly reduced serum cholesterol, triglyceride, and phospholipids levels and atherogenic index. Free fatty acid levels in serum, liver and heart were also significantly decreased, whereas lipolytic activity was increased in liver and heart. The study also found that fecal excretion of bile acids and sterols was significantly increased by 57% and 75%, respectively (77). Administration of Z-guggulsterone at a dose of 100 mg/kg body weight has been shown to decrease liver cholesterol levels in mice fed a high-cholesterol diet for 7 days (18).

The hypolipidemic effect of guggul has been studied in several other animal models, including chickens (78), pigs (79), dogs and monkeys (80). Guggul has been shown to accelerate the decrease in lipid levels after a high fat diet. When leghorn chicks were fed a high-fat diet for 1 month to induce hyperlipidemia, followed by either a normal diet or a normal diet plus gum guggul at a dose of 3 g/kg body weight, serum cholesterol and triglyceride levels fell at a significantly faster rate in the group treated with gum guggul. Furthermore, administration of gum guggul partially reversed the atherosclerosis in the aorta that was induced by the high-fat diet (78).

High-sensitivity C-reactive protein is an indicator of inflammation. It is an acute-phase protein synthesized in response to cytokine stimulation in the liver and is believed to promote all stages of atherosclerosis (81). High-sensitivity C-reactive protein decreased in hyperlipidemic subjects receiving guggulipid at a dose of 2,000 mg daily (82), suggesting an anti-inflammatory role for guggul.

Cardioprotective effects. Several studies have reported the cardioprotective activity of guggulsterone. Guggulsterone has been shown to reverse isoproterenol-induced cardiac damage and the associated metabolic changes in rats. Isoproterenol-treated rats were shown to have marked increases in creatine phosphokinase, phospholipase and xanthine oxidase activities, increased levels of lipid peroxides and lowered superoxide dismutase levels, all indicative of oxidative stress. Guggulsterone treatment reversed these metabolic changes (83). In more recent studies, the cardioprotective activity of guggulsterone was compared with that of a hypolipidemic drug, gemfibrozil. Both isomers of guggulsterone, at doses of 50 mg/kg body weight, exhibited significant cardioprotective effect against isoproterenol-induced cardiac damage (69, 70). In another study, the cardioprotective effect of guggulipid was evaluated in a rat model. Rats received guggulipid orally at a dose of 50 mg/kg for 30 days. Guggulipid significantly reversed the cardiac damage and biochemical changes induced by isoproterenol (84). Moreover, guggulipid significantly decreased total cholesterol and lipid peroxide levels in the serum.

The cardioprotective activity of gum guggul in combination with *Inula racemosa* was examined in 200 patients suffering from ischemic heart disease who had abnormal electrocardiogram (ECG) and chest pain (85, 86). *Inula racemosa* is used in Ayurvedic medicine mainly as an expectorant and bronchodilator. It has been used in the treatment of tuberculosis and topically in the treatment of skin diseases. After treatment with guggul for 6 months, the levels of total cholesterol, triglyceride and total blood lipids were decreased. Moreover, normal ECG was restored in 26% of the patients and another 59% showed improvement in the ECG. Chest pain subsided in 25% of the patients and decreased in the rest of the patients. The results suggest that guggul has cardioprotective benefits in patients with ischemia (85, 86).

Neuroprotective effects. The neuroprotective activity of guggulsterone was demonstrated in a recent study in a mouse model (87). Guggulipid (12.5, 25, or 50 mg/kg per day) caused dose-dependent improvement in scopolamine-induced deficits in a passive avoidance test. Mice were treated with streptozotocin to induce neuronal damage and memory deficits. Guggulipid at 50 mg/kg/day reversed streptozotocin-induced neuronal damage and memory deficits. In parallel with these reversals, levels of glutathione in the brains of guggulipid-treated mice were significantly increased, suggesting that guggulipid inhibits oxidative stress in the brain (87). The study demonstrated that guggulipid has a significant protective effect against streptozotocin-induced memory deficits in this model of dementia that can be attributed to the antioxidant and antiacetylcholine esterase activities of guggulipid. These observations suggest guggulipid as a potential antidementia drug and cognitive enhancer.

Arthritis. Arthritis, an inflammation of the joints, is usually a chronic disease that results from dysregulation of proinflammatory cytokines and proinflammatory enzymes that mediate production of prostaglandins and leukotrienes, together with expression of adhesion molecules and MMPs and hyperproliferation of synovial fibroblasts. All of these factors are regulated by activation of the transcription factor NF-κB. Thus, agents that suppress activation of NF-κB have potential for the treatment of arthritis [for references see (88)].

Sharma *et al*. investigated the role of guggul in an experimental arthritis model resembling rheumatoid arthritis in man. Guggul reduced the thickness of the joint swelling during the course of drug treatment, indicating that gum guggul has a beneficial role in experimental arthritis (65).

The antiinflammatory activity of guggul was evaluated in 30 patients with arthritis in at least one knee (89). The study evaluated the effects of guggul on pain, stiffness and function, and determined the tolerability in older patients with a diagnosis of osteoarthritis of the knee. Gum guggul at 500 mg three times daily for 1 month significantly improved the WOMAC (Western Ontario and McMaster Osteoarthritis Index) total score and continued to improve it at the 2-month marker and follow-up. With secondary measures of pain in the visual analog scales, patients exhibited significant improvement after 2 months of treatment. These results demonstrate the beneficial effect of the therapy in arthritic patients. No side effects were reported during the trial. Thus, gum guggul appears to be a relatively safe and effective supplement to reduce symptoms of osteoarthritis.

Hepatoprotective effects. The hepatoprotective activity of an ethanolic extract of the gum of *Commiphora opobalsamum*, a species closely related to *C. mukul*, was investigated in rats (90). Hepatotoxicity was induced in rats by feeding them carbon tetrachloride and liquid paraffin (1:1). The ethanolic extract lowered the serum levels of transaminases, alkaline phosphatase and bilirubin. Pretreatment with the ethanolic extract prevented prolongation of the barbiturate sleeping time associated with carbon tetrachloride-induced liver damage in mice. In addition, carbon tetrachloride-induced low-level nonprotein sulfhydryl concentration in the liver was replenished by the ethanolic extract. These data suggest that the ethanolic extract of the gum may act as an antioxidant agent and may have a hepatoprotective effect (90).

Table I. *Guggulsterone contents in plant parts, resin and in vitro cultures of Commiphora wightii.*

Sample type	Guggulsterone range (μg/g)		
	-E	-Z	Total
In vivo			
Stem	32-46	88-104	120-151
Leaf	12-23	4-13	15-37
Gum-resin	768-8,743	18,774-24,414	19,542-33,150
In vitro			
Callus	1-2	3-58	4-60
Cell cultures	1-3	3-17	4-19
Embryos	12-37	2-18	13-55

Inflammatory bowel disease. The anti-inflammatory effect of guggulsterone was investigated on intestinal epithelial cells and on experimental murine colitis models (91). Colitis was induced in mice with dextran sulfate sodium in the presence or absence of guggulsterone. Mice receiving guggulsterone exhibited significantly reduced colitis severity as assessed by clinical disease activity score, colon length and histology, indicating the anti-inflammatory activity and possible usefulness of guggulsterone in the treatment of inflammatory bowel disease (91).

Thyroid-stimulatory effects. Several studies have shown that guggulsterone stimulates the thyroid gland. Tripathi *et al*. showed that administration of guggulsterone at a dose of 10 mg/kg body weight increased thyroid function in albino rats (73). In this study, administration of guggulsterone brought about an increase in iodine uptake by the thyroid and enhanced the activities of thyroid peroxidase and protease as well as oxygen consumption (73). In another study, the same group showed that administration of guggulsterone at a dose of 10 mg/kg body weight restored thyroid activity in hypothyroid rats (73). Moreover, administration of guggulsterone at a dose of 5 mg per day restored thyroid activity in neomercazole-treated white leghorn chicks (92). The thyroid-stimulatory effect of guggulsterone may in part be considered a possible mechanism for its lipid-lowering activity, but additional studies are required.

Nodulocystic Acne. Guggulipid has been reported to be effective in the treatment of nodulocystic acne (93). Twenty patients with nodulocystic acne were randomly allocated to one of two groups. Patients in one of the groups received tetracycline (500 mg) and those in the other group received guggulipid (equivalent to 25 mg guggulsterone) twice daily for 3 months; both treatments produced a progressive reduction in lesions in the majority of patients. Guggulipid was as effective as tetracycline in the treatment of this condition. An interesting observation was that, in patients with oily faces the acne responded better to guggulipid (93).

Infectious diseases. The antihelminthic effects of mirazid (from *C. molmol*) have been extensively reported. Treatment with mirazid improved enzyme activities with noticeable reductions in ova count and worm burden (94). The purified oleo-resin extract of mirazid prevented intercellular fibrosis and granulomas in the portal tract of mice infected with schistosoma and had no hepatotoxic effect (95). Exposure of schistosoma worms to mirazid *in vitro* killed the worms in 30 minutes (96).

It has been reported that the essential oil, chloroform extract and seven sesquiterpenoid compounds from the oleo-gum-resin of *C. mukul* showed a wide range of inhibitory activity against both gram-positive and gram-negative bacteria (97).

Pharmacokinetics, Pharmacodynamics and Adverse Effects

The bioavailability, pharmacokinetics and pharmacodynamics of guggulsterone in humans are not well known; it appears, however, that guggulipid can affect the bioavailability of other drugs. The effect of a single oral dose of guggulipid (1 g) on the bioavailability of a single oral dose of propranolol (40 mg) or diltiazem (60 mg) was observed in 10 and 7 healthy male volunteers, respectively (98). Guggulipid significantly reduced peak plasma concentrations of both the drugs in these volunteers. These interactions may be due to activation of pregnane X receptor by guggulsterone, which leads to up-regulation of the enzymes responsible for biotransformation of propranolol and diltiazem (27, 31, 99). It has been shown in rats that administration of guggulsterone significantly increased expression of cytochrome P450 genes, which are responsible for metabolizing most drugs (100).

The safety of long-term use of guggulsterone has not been evaluated in a clinical setting, but guggul appears to be devoid of acute, subacute, or chronic toxicity in rats, dogs and monkeys; no mutagenic or teratogenic effects have been reported (101). Guggul or guggulsterone is generally safe in short-term use, although side-effects such as skin rashes, diarrhea and nausea have been reported with therapeutic doses, which differ among individuals (66). No significant side-effects have been observed on renal function, liver functions, hematological parameters, or electrolytes (82, 102). Allergic contact dermatitis has been reported in anticellulite gel creams containing guggul (103, 104). A case of rhabdomylosis was reported that might have been associated with use of gum guggul (105). Guggulsterone has a long history of safe use in the Ayurvedic system of medicine; however, it should be used cautiously in combination with prescription drugs, as it may modulate the activity of drug metabolizing enzymes.

Table II. *Clinical studies with gum guggul, its ethyl acetate fraction ether soluble fractions, and guggulsterone.*

Study	Patients	Dose	Response
Double-blind randomized controlled study	60 obese pts 60 non-obese pts	GG 2 g, 2×/day × 3 wks EE 500 mg, 3x/day x 3 wks	Reduced serum lipid levels in HL patients (107)
Double-blind randomized controlled study	48 pts	GE 500 mg, 3×/day × 4 wks	Reduced total cholesterol and TG (110)
Double blind randomized controlled study	40 HL pts	GG 4.5 g/day × 16 wks	Reduced total cholesterol and TG (111)
Double blind randomized controlled study	10[a]	GS, 25 mg, 2×/day × 8 wks	Decreased serum cholesterol levels (109)
Multicenter clinical trial Open trial (double study)	205[b]	GL 500 mg/day	Decreased serum cholesterol and TG in 70-80% subjects (112)
Multicenter clinical trial Open trial (double study)	125	GL 500 mg/day	Average fall in cholesterol and TG was 11% and 16.8% (112)
Multicenter clinical trial Open trial (double study)	108	Clofibrate therapy	Average fall in cholesterol and TG was 10% and 21.6% (112)
Randomized double blind	31 HL pts[c] 30 HL pts[d]	GL 50 mg 2×/day × 24 wks Placebo capsules 2×/day × 24 wks	Decreased the cholesterol (65) No decrease in cholesterol level (65)
Double blind randomized placebo controlled	33 HC pts 34 HC pts 36 HC pts	GL 1 g, 3×/day × 8 wks GL 2 g, 3×/day, × 8 wks Placebo, 3×/day × 8 wks	Increased the level of LDL (82) Increased the level of LDL (82) Decreased the LDL levels (82)
Cardiovascular disease	200[c]	GG with Innula racemosa for 6 mts	Decreased total cholesterol, TG and TBP. Improved EKG and pain (85, 86)
Rheumatoid Arthritis	30	GG, 500 mg 3×/day, 1 month	Improved WOMAC score, improvement after 2 months (89)

GG, gum guggul; EE, ether extract; GE, guggul extract; GL, guggulipid; TG, triglycerides; TBP, total blood lipid, EKG, electrocardiogram; mts, months; pts, patients; WOMAC, Western Ontario MacMaster; HL, hyperlipidemia; HC, hypercholesterolemia. [a]Healthy individuals; [b]after 8 -week diet and placebo therapy; [c]all patients had a low fat diet with fruits and vegetables for 1 week prior to treatment; [d]patients were suffering with ischemic heart disease, abnormal EKG and chest pain.

Clinical Studies

Despite the availability of extensive preclinical data, there has not been any clinical trial of guggulsterone in cancer patients. The hypolipidemic effects of gum guggul and its ethyl acetate– and ether-soluble fractions have been extensively evaluated, however, in human clinical trials (Table II). Most of the clinical studies demonstrated that guggul has hypolipidemic activity, with an average decrease in total cholesterol and triglyceride levels of about 20% . A significant degree of individual variation has been registered in response to guggul treatment, but some response to guggul treatment was observed overall in about 70% to 80% of patients (for references see (106)).

Kuppurajan *et al*. evaluated the effect of guggul on serum lipids in obese patients. Guggul or guggul extract was administered orally to obese patients for 21 days. No significant changes in cholesterol levels were observed after 21 days of treatment with either guggul or ether extract "fraction A" (107). Five years later, however, the same group repeated a randomized controlled double-blind trial on 120 patients with hyperlipidemia (108). The patients were given gum guggul (2 g twice daily) or ether extract (500 mg three times daily) for 21 days. Both guggul and ether extract (fraction A) significantly reduced serum lipid levels in hyperlipidemic non-obese patients; however, the hypolipidemic effects were not observed in obese patients. The reasons for variation in response to guggul treatment between non obese and obese patients are not clear. Part of the findings of that study was supported by another study in which 10 healthy volunteers were administered guggulsterone at a dose of 25 mg twice daily for 8 weeks. The results showed a significant decrease in the serum total cholesterol levels, suggesting that negative findings with guggul in obese patients may be obesity specific (109).

Several other clinical trials conducted between 1975 and 1990 demonstrated that administration of guggulipid, the ethyl acetate extract of gum guggul, significantly lowers LDL cholesterol and triglyceride levels in patients with hyperlipidemia. In one trial, 48 patients were treated with ether extracts of guggul at a dose of 500 mg three times a day for 4 weeks. Both total cholesterol and triglyceride levels were significantly reduced in patients receiving the guggul extracts (110). In another study, 85 patients with hyperlipidemia received ether extract of guggul at a dose of 500 mg three times daily for 12 weeks. Total cholesterol

levels after this treatment were significantly lower than baseline levels. Verma and Bordia tested the effect of gum guggul on 40 patients with hyperlipidemia. The patients received gum guggul at a daily dose of 4.5 g for 16 weeks. At the end of 16 weeks, total cholesterol and triglyceride levels were significantly lower than baseline levels (111).

Nityanand *et al*. conducted the largest multicenter clinical trial to test the efficacy of guggulipid; the agent was administered in both an open trial and a double-blind crossover study with the antihyperlipidemic drug clofibrate. Two hundred and five patients completed the 12-week open trial with guggulipid at a dose of 500 mg daily after 8 weeks of diet and placebo therapy. Serum cholesterol (23.6%) and serum triglyceride (22.6%) levels were significantly lower in 70% to 80% of patients. In the double-blind crossover study, 125 patients received guggulipid therapy and 108 patients received clofibrate therapy. With guggulipid, the average declines in serum cholesterol and triglyceride levels were 11% and 16.8%, respectively; with clofibrate, they were 10% and 21.6%, respectively. The lipid-lowering effect of both drugs became evident 3 to 4 weeks after starting the drug and had no relationship with age, sex, or concomitant drug intake. Hypercholesterolemia responded better to guggulipid therapy than hypertriglyceridemia, which responded better to clofibrate therapy. In patients with mixed hyperlipidemia, responses to the two drugs were comparable. HDL level was increased in 60% of cases that responded to guggulipid therapy, whereas clofibrate had no effect on HDL (112). The study clearly demonstrated the benefits of guggul therapy in reducing cholesterol and lipid levels in hypercholesterolemic patients.

In another randomized, double-blind study, the effects of guggulipid were evaluated in 61 patients with hypercholesterolemia (66). The patients were randomized into two groups (31 in the guggulipid group and 30 in the placebo group). All patients were instructed to eat a low-fat diet with fruits and vegetables for 12 weeks prior to the treatment. The patients received either guggulipid (50 mg) or placebo capsules twice daily for 24 weeks. Guggulipid reduced the total cholesterol level by 11.7%, LDL by 12.5%, triglyceride by 12.0%, and total cholesterol/HDL ratio by 11.1% from the postdiet levels, whereas the levels were unchanged in the placebo group. The HDL cholesterol level showed no changes in either group. The lipid peroxide level, indicating oxidative stress, declined by 33.3% in the guggulipid group but not at all in the placebo group. The combined effect of diet and guggulipid at 36 weeks was as great as the reported lipid-lowering effect of modern drugs. After a washout period of another 12 weeks, changes in blood lipoproteins were reversed in the patients who received guggulipid, whereas no such change was observed in the placebo group (66).

With the increasing popularity of guggul extract in the United States, a study was designed to evaluate the short-term safety and efficacy of guggul extracts in Western populations (82). A double-blind, randomized, placebo-controlled trial was performed with 103 ambulatory, community-dwelling, otherwise healthy adults with hypercholesterolemia. The subjects received standard-dose guggulipid (1,000 mg), high-dose guggulipid (2,000 mg), or matching placebo three times daily by mouth for 8 weeks. Treatment with guggulipid at doses of 1,000 or 2,000 mg resulted in increases in LDL levels by 4% and 5%, respectively, whereas LDL level decreased by 5% in patients who received placebo. Guggul treatment also yielded no significant changes in serum levels of total cholesterol, HDL, VLDL, or triglycerides. Further analysis within the groups revealed that 18% of patients in treatment groups responded favorably to guggulipid treatment, with a decrease in LDL level of more than 5%. However, this response rate is much smaller than the 70% to 80% response rate observed in most previous studies. Forty-five participants with high baseline levels of LDL (160 mg/dl or greater) showed significantly reduced serum triglyceride levels (14% and 10% decreases for the high- and low-dose groups, respectively), whereas triglyceride levels increased by 10% in those receiving placebo. Despite plausible mechanisms of action, guggulipid did not appear to improve levels of serum cholesterol over the short-term in this population of adults with hypercholesterolemia, and actually partially raised the levels of LDL.

Ulbricht *et al*. performed a meta-analysis to evaluate the scientific evidence on guggul for hyperlipidemia, including expert opinion, folkloric precedent, history, pharmacology, kinetics/dynamics, interactions, adverse effects, toxicology, and dosing (113). They observed that most scientific evidence dating from before 2003 that suggested that guggulipid elicits significant reductions in serum total cholesterol, LDL, and/or triglyceride levels, as well as elevations in HDL level, came from studies that were small and methodologically flawed. No significant changes in total cholesterol, HDL, or triglyceride levels were measured in the placebo-controlled study performed by Szapary *et al*. in 2003 (82). The effects of guggulipid in patients with high cholesterol are not clear, some studies finding cholesterol-lowering effects while other research suggests no benefits.

The variations in outcomes of clinical studies may be attributed to differences in ethnic and genetic backgrounds, dietary restraints and lifestyle. Data on bioavailability, pharmacokinetics, and metabolism of guggulipid and guggulsterone are needed to round out the evidence on the efficacy of this therapy. At this juncture, more scientific evidence is required to support the use of guggul for any medical condition.

Conclusion

Although guggulsterone was shown to inhibit proliferation and induce apoptosis in cancer cells, further preclinical and clinical studies are required to establish the anticancer

potential of guggulsterone. The mechanism of action of guggulsterone has begun to reveal new uses of this drug. The bioavailability, pharmacokinetics, pharmacodynamics and metabolism of guggulsterone require further examination. Furthermore, data on drug-drug interactions with guggul are not available. Whether guggul alters the metabolism and bioavailability of other drugs needs to be evaluated before it can be used in combination with other drugs. Overall, guggul has been successfully used for thousands of years as an Ayurvedic medicine and shows great potential for development as an anticancer drug.

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