IJPSR (2020), Volume 11, Issue 4 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 June 2019; received in revised form, 11 November 2019; accepted, 06 February 2020; published 01 April 2020

GUGGULSTERONE: MECHANISM OF ACTION AND PROSPECTS OF CHEMO PREVENTION IN PROSTATE CANCER

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Keywords:

Guggulsterone (GS), Prostate cancer (PCa), Castration-resistant prostate cancer (CRPCa), Cancer therapeutics, Plant sterol, Chemoprevention

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ABSTRACT: Remarkable pharmacological effects exhibited by Guggulsterone (GS) establish its potential as an effective anticancer agent. GS is widely known for its anti-inflammatory and hypolipidemic activities. At the molecular level, GS is known to modulate the activity of various nuclear receptors, growth factors and transcription factors. However, the complete sequence of the underlying anticancer mechanism of GS is not completely understood. Prostate cancer (PCa) initiation is marked with inflammatory events and disease advances towards castration-resistant prostate cancer (CRPCa) through constitutively activated androgen receptor and hyperactivated survival signaling such as NF-kB; therefore PCa cell lines offer a good model system to study the anticancer activities of GS. Considering the fact that GS is both anti-inflammatory and androgen receptor (AR) antagonist, it can be widely employed to achieve two objectives simultaneously - chemoprevention as well as an anti-cancer agent. Evidence from preclinical trials and prescribed usage in Ayurvedic medicine has promoted the use of guggul as a health supplement. However, the use of GS is not completely free of side effects and exploring GS's mode of action is required to safeguard the fast-growing success of guggul as a health supplement, which would be a key to the safety and efficacy of GS as a drug. This review presents a brief synopsis about the anticancer activity of GS with a special focus on prostate cancer, features of GS which promote it as a potential Chemopreventive agent, and account of potential GS molecular targets which are involved in PCa pathogenesis.

INTRODUCTION: Guggulsterone (GS), a plant sterol, is earning high attention in the field of pharmacology and alternative cancer treatment due to its known pharmacological effects which target multiple pathways and molecules as observed in diverse cancer cell lines [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(4).1527-36

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(4).1527-36

Guggulipid, a gum resin, is also fast becoming a popular choice as a health supplement to manage weight loss and to check various malignancies such as arthritis and cancer due to major role played by GS in hypolipidemic effects, anti-inflammatory pathways, apoptotic signaling and anti-proliferative properties [2].

In addition, GS is known to modulate the activity of nuclear receptors, growth factors, and transcription factors. The underlying mechanisms and key events leading to the anti-cancer effects of GS are not yet completely understood. Prostate cancer is a highly heterogeneous disease with high

morbidity and mortality rates worldwide. Prostate cancer initiation is marked with inflammatory events and oxidative DNA damage, which progresses towards adenocarcinoma, and finally due to failure of castration therapy prostate cancer recurs as fatal castration-resistant PCa (CRPCa). Therefore, it has become increasingly important to study new therapeutic targets, drugs and chemopreventive strategies for prostate cancer prevention and management of CRPCa.

1.1 Prevalence and Complexity of Prostate Cancer: Prostate cancer (PCa) is one of the most common health hazards in man. Although, the incidences of new PCa cases are falling over the last 10 years, PCa remains to be the leading cause of morbidity and mortality for males in the western world (SEER stat fact sheets). However, the molecular mechanisms underlying its initiation and progression of disease remains poorly understood due to a unique set of pathways employed at different stage.

PCa displays the wide variety of heterogeneity regarding genetic alteration, gene fusion events, versatile expression profiles, cell surface markers, patient ethnicity, *etc.* [3] Highly heterogeneous nature of PCa and involvement of multiple signaling pathways poses genuine challenges for prognosis and clinical management of the disease. The Androgen receptor singling axis, however, remains to be the most important signaling pathway in PCa initiation and progression. It is perhaps the reason that Androgen deprivation therapy (ADT) remains the major treatment option for disseminated tumor while prostatectomy is used to treat organ-confined PCa. ADT, however, yields transient efficacy and most patients with metastatic PCa eventually die of hormone-refractory or castration-resistant PCa (CRPCa) [4]. Lack of survival benefits in ADT and highly heterogeneous nature of the disease have underscored the need to develop new therapeutic measures that implicate the use of inhibitor and/or cocktail of inhibitors that target multiple molecules and pathways. To mention, CRPCa is generally featured by the presence of constitutively activated androgen receptor and hyperactivated survival signaling such as NF-kB [5, 6]. Since, GS is known to exhibit both anti-inflammatory and AR antagonistic activities; it is tempting to assume if GS can be used as a bifocal strategy as both chemopreventive and anti-cancer agents for the management of prostate cancer. The fact that environmental elements and age are the major risk factors associated with the etiology of prostate cancer suggests that the population at the risk of developing PCa could easily be identified. Consequently, easy identification of risk-prone population, slow progression and late diagnosis of disease represent a wide scope to employ the benefits of chemopreventive agents.

Plant-based sterols and polyphenolic compounds represent the reliable class to search for a chemopreventive agent and new therapeutic compounds to treat advanced PCa, due to their properties like - multiple molecular targets, non-toxic effects on normal cells and least side effects. Many medicinal plants are known for their chemopreventive properties such as turmeric, tinospora and withania [7, 8]. The introduction of such plant products into diet might decrease the risk of cancer, while usage of the active component from these plants could be conveniently used for multiple molecular targeting as well as for disease management. One such compound is Guggulsterone (GS). GS has received a great deal of attention recently as an anti-cancer agent.

1.2 Guggulsterone (GS): Guggulsterone [4, 17 (20)-pregnadiene-3, 16-dione], is a plant sterol derived from the gum resin (guggulu) of the tree *Commiphora mukul*. The gum resin of the tree is a complex mixture, which gets purified sequentially by using ethyl acetate extraction, pH gradient fractionation, and ketonic fractionation. Ethyl acetate extract of the resin is known as guggulipid (GL). GL has been traditionally used as highly valued and safe Ayurvedic medicine for the treatment of various ailments such as epilepsy and obesity. Further, purified ketonic fraction contains a number of steroids, including two isomers of GS - E (cis) and Z (trans) **Fig. 1**. These isomers are known to have pronounced hypolipidemic activity [9]. Initially, In India GL and its isomers were granted approval for marketing as lipid-lowering / cholesterol-lowering agents in 1986, as evident from Indian Pharmacopeia 2007 in pgs. 2038-2040. Since, then much research, several preclinical and a few clinical trials have been carried out which largely support the therapeutic claims of guggul as described in ancient Indian medicine and

established its pharmacological effects. The key pharmacological activities such as hypolipidemic, anti-oxidant, and anti-inflammatory effects have been attributed to its bioactive component - Z-GS [10].

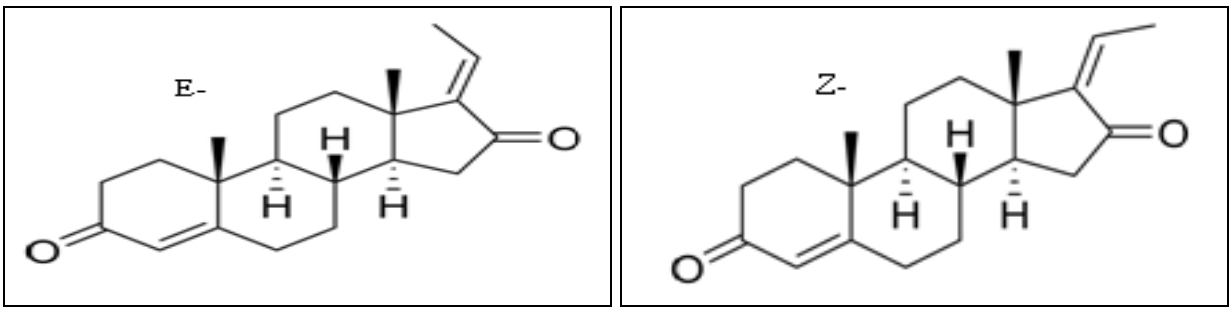

FIG. 1: STRUCTURE OF GS. (EFORM) 4, 17(20)-(CIS)-PREGNADIENE-3, 16-DIONE AND (Z-FORM) 4, 17(20)-(TRANS)-PREGNADIENE-3, 16-DIONE

1.3 Biological Properties of GS:

1.3.1 Hypolipidemic Activity: GS has been widely known to be a hypolipidemic agent. This property has been consistently shown in various animal models of rabbits, mice, and rats where it has been shown to reduce cholesterol levels, serum triglycerides and phospholipids. Various clinical trials have also been conducted to evaluate the efficacy of GS as a hypolipidemic agent. Most of these clinical trials were conducted in India. Surprisingly results from such trials show disparity as compare to a trial conducted in the US [11], where GS was not successful in controlling serum cholesterol levels and thus, this discrepancy shows the vital impact of dietary habits, regional, environmental or genetic factors and ethnic background on GS mediated effects. Although it is preliminary evidence against the efficacy of guggul, definitive deductions can only be concluded after further research [2].

1.3.2 Anti-oxidant and Anti-inflammatory Effects: GS has been shown to inhibit LDL oxidation and accumulation of LDL-derived cholesterol in macrophages [12]. GS has also been described to be therapeutically beneficial for the diseases related to oxidative stresses such as myocardial ischemia and neurodegenerative disease as suggested by preclinical studies reported in animal models.

Indeed, GS is known to inhibit the production of nitric oxide that is associated with oxidative stress [13]. Clinical trials regarding anti-inflammatory activities are lacking, however, a study showed the beneficial effects of guggul therapy on arthritis patient [14].

1.3.3 Anti-cancer and Chemopreventive Activities: Anti-carcinogenic activities of GS have been widely studied in recent times. Substantial literature advocating the use of GS in different types of cancer exists, which exemplified its anti-proliferative, anti-metastatic, anti-angiogenic and pro-apoptotic properties in many cell lines and animal models. Studies by Tripathi and Tripathi (1984 & 1988) were amongst the earliest research work, which showed the thyroid stimulatory action of GS [15]. A study showed that GS is an extremely efficient antagonist of the farnesoid X receptor (FXR), bile acids activated nuclear hormone receptor [16]. Such research out-comes opened up the new avenue of specific biologic effects of GS on relatively promiscuous nuclear hormone receptors. Further, GS was proved to be the antagonist of AR, GR and MR, except PR and ER where it is an agonist [17].

These data took the attention of the scientific community towards the effects of GS on steroid hormones dependent anomalies. Anticancer and anti-inflammatory effects of GS are the result of suppression of constitutive NF-kB activation through inhibition of IKK by GS [18]. Due to the anti-oxidant and anti-inflammatory properties of GS, it's been thoroughly and successfully assessed for its anticancer and chemopreventive activities. All type of cancers is marked by six distinctions: autonomous growth signals, resistance to growth inhibitory signals and anti-cancer therapy, evasion of programmed cell death, indefinite replication ability, persistence in angiogenesis, and invasion and metastasis. Research data on GS confirm that GS interferes with multiple biological events

simultaneously, which includes dysfunctional proliferation pathways, inducing apoptosis, inhibition of invasion and angiogenesis.

1.3.3.1 Proliferation: GS inhibits cell growth dose-dependently by inhibiting DNA synthesis in a variety of tumor cells including leukemia, head and neck cancer, multiple myeloma, lung carcinoma, melanoma, breast cancer and ovarian cancer. The 25 mm concentration of GS is capable of arresting the cell cycle in S-phase through the down-regulation of much-required proteins such as cyclinD1 and cdc219.

1.3.3.2 Apoptosis: In addition to inhibiting cell division, inducing apoptosis is another approach by which GS hinders cancer cell growth. GS efficiently induces apoptosis in various cancer cells while keeping its effects minimal on healthy cell viability. Both Mitochondrial/intrinsic pathways and mitochondria independent/extrinsic pathway mediates GS dependent apoptosis. A common step in both pathways is Caspase activation. GS-induced Caspase-dependent apoptosis pathway in PCa is facilitated by Bax and Bak proteins [20]. Additionally, GS inhibits the growth of various tumor cells and induces apoptosis through down-regulation of anti-apoptotic gene products (IAP1, xIAP, Bfl-1/A1, Bcl-2, cFLIP, and survivin), activation of caspases, inhibition of Akt and activation of JNK [18, 21, 22].

1.3.3.3 Invasion: The next step of cancer progression is the invasion. When chemotherapy fails, cancer cells invade into nearby normal tissues causing recurrence of the tumor. Z-GS mediates the inhibition of cell migration via various pathways such as Akt, JAK/STAT and Src/FAK [23, 24]. GS is also known to inhibit angiogenesis *in-vivo* in various types of cancers such as colon cancer and PCa [25, 26].

2. Method: Based on an electronic literature search we produced a brief synopsis about published research and scientific progress that had been accomplished regarding the anticancer activity of GS, its mechanism and signaling molecules involved with special focus on prostate cancer.

3. RESULTS:

3.1 Guggulsterone and Prostate Cancer: The very fact that GS is both anti-inflammatory and targets steroid receptors, projects its plausible role in anomalies which are hormone-dependent such as PCa and breast cancer. PCa cell lines became an early model to study GS mediated apoptosis and therefore, have been studied extensively in recent years. Apart from PCa, the anti-cancer activity of GS has also been described in other cancers such as skin cancer, colon cancer, lung cancer, head and neck cancer, and leukemia [25, 27-29]. Few of the early studies added the insights about the molecular mechanism of GS mediated apoptosis by testing PC-3, a human PCa cell line, as a model system [20]. GS-mediated cell growth arrest and apoptosis are known to be mechanized by activation of the c-Jun N-terminal kinase, suppression of Akt pathway, and down-regulation of anti-apoptotic gene products [20, 24, 26, 30]. Molecular targets of GS includes the whole array of protein families such as nuclear receptors (FXR, AR, ER), growth factors (VEGF), transcription factors (NF-kB), enzymes (Cyclooxygenase), and Kinases (MAPK, JNK). Interestingly, GS has been reported to have selective apoptotic effects, as it induces apoptosis in cancer cells only while normal prostate epithelial cells remains unaffected [20]. All the data available about various singling molecules modulated by GS lacks in the sequence of events leading to cell death, however, it establishes GS as a multi-target chemotherapeutic agent. In addition, its pharmacological activities tempt investigators to consider the possibility of projecting GS as a potential chemopreventive agent. Therefore, one might envision the use of GS as a potent antitumor-promoting agent.

3.2 Molecular Mechanisms for Guggulsterone's Chemo Preventive and Anti-cancer Activity Against Prostate Cancer: GS is known to exert anti-cancer effects by activation pro-apoptotic pathways (JNK) and inhibiting anti-apoptotic factor (NF-KB). Well, known molecular targets of GS and mechanisms of regulating apoptosis specifically studied in PCa cell lines and models are discussed below.

3.2.1 NF-KB: Constitutive activity of NF-kB is a major survival mechanism in castration-resistant PCa, in the absence of active androgen receptor, which is a natural survival signal for prostatic epithelium. Active NF-kB leads to malignancy *via* positively regulating the expression of many anti-

apoptotic genes. GS is shown to suppress the DNA binding of NF-kappaB induced by various factors like tumor necrosis factor (TNF), phorbol ester, and interleukin-1. Additionally, GS has been verified to suppress constitutive NF-kB activation in most tumor cells however none of the reports confirm the comparable outcome in PCa samples and/or cell lines. The mechanism of this inhibition commences with inhibition of IkappaB kinase activation followed by preventing IkappaB alpha phosphorylation and its degradation, which in turn suppresses p65 phosphorylation and its nuclear translocation. As a result, GS decreased the expression of NF-kB-dependent genes and gene products involved in anti-apoptotic activities (IAP1, xIAP, Bfl-1/A1, Bcl-2, cFLIP, and survivin), proliferation (cyclin D1 and c-Myc), and metastasis (MMP-9, COX-2, and VEGF) [18] **Fig. 2**.

3.2.2 BCL2 Family Protein: Apoptosis apparently an important mechanism of anti-cancer activity for many natural compounds. BCl2 protein family is the main regulator of the mitochondrial apoptotic pathway. The expression of BCL2 family proteins is directly under control of NF-kB transcription factor. As described earlier, GS intervenes the expression of NF-kB target gene by inhibiting P65 nuclear translocation, resulting in low expression of anti-apoptotic proteins (BCL2) and increased activity of pro-apoptotic factors (BAK,BAX). BAK and BAX are directly responsible for breaching the mitochondrial outer membrane which in turn initiates the release of cytochrome-C and other mitochondrial inter-membrane proteins and facilitates the activation of multi-domain proteins leading to cleavage (activation) of caspase-9, caspase-8 and caspase-3 to facilitate GS-dependent apoptosis. There is evidence that shows cells becoming comparatively more resistant towards GS mediated apoptosis after double knockout of Bax-Bak. This observation manifests that GS-induced apoptosis is in-fact directly associated with Bcl-2 family members Bax and Bak [20] **Fig. 2**.

3.2.3 JNK: Induction of oxidative stress in tumor cells is a powerful strategy to induce apoptosis. In fact many natural anti-cancer compounds are known to exhibit their effects *via* ROS-dependent activation of apoptosis. Similarly, GL-induced cell deaths in androgen-responsive PCa cell line, LNCaP and androgen-in responsive cell line - C-81 is caused by reactive oxygen intermediate (ROI) species. This process is regulated by ROI-dependent activation of JNK. The same study also confirms that GL-induced generation of ROI and activation of JNK is a selective process that occurs only in PCa cells but not in a normal prostate epithelial cell line (PrEC) [24] **Fig. 2**.

3.2.4 VEGF: Vascular targeting agents, that selectively inhibit the growth of blood vessels in and around tumors are appealing agents for the treatment of solid tumors. z-GS have been shown to decrease angiogenesis in tumor-induced by DU-145 implanted cells in mice by inhibiting vascular endothelial growth factor (VEGF) with its receptors (VEGFR) [25, 26]. Akt singling axis is shown to be involved in the process which is further in accordance with the previous observation that showed a decrease in anti-apoptotic genes upon GS treatment. z-GS works as a vascular targeting agent *in-vitro* also which suppresses the secretion of pro-angiogenic growth factors (VEGF), down-regulation of VEGF receptor 2, and inactivation of Akt.

Although, the exact mechanism by which GS induces apoptosis and selectively eliminates prostate cancer cells is still not clear. Yet, all the research work carried out on GS's effects on prostate cancer both *in-vitro* and *in-vivo* provides reasonable evidence to supports the fact that GS has the potential to be a potent chemopreventive as well as an anti-cancer agent. Most of the data on anti-cancer potentials of GS have been obtained on range of cancer cell lines or animal models and there is a lack of data specifically tested on PCa cell lines or PCa models. Data about GS's mode of action derived from other cancer cell lines might or might not correspond to PCa cells; therefore, additional studies directed towards target identification and pathway analysis specific to prostate cancer are needed for considering the use of GS in PCa management and anticancer therapy.

The following section covers the major molecular pathways that could be or have been tested for being potential targets of various anticancer agents and have the potential to be the target of GS as well. Analyzing the effects of GS on these pathways will provide an ample opportunity for research as well as strengthen the potential of GS as chemopreventive and anticancer agent.

3.3 Molecular Pathways and Genetic Modification in Prostate Cancer: PCa is a heterogeneous and multifaceted disease as it operates the unique set of pathways during initiation and progression. Recently, genetic information about PCa was intensely explored through NGS, and thus collected genetic and epigenetic portfolio of PCa revealed number of features that are not encountered in other cancers; such as- non-random copy number variation in various oncogenes and tumor suppressor, chromosomal rearrangement frequently involving ERG, involvement of developmental pathways, alteration of transcriptional programs particularly those which are governed by AR, and continuous somatic and genetic changes from PIN to CRPCa.

This section gives a brief overview of remarkable variation both at the genetic level and in molecular pathways that are exhibited by prostate cancer. Such variations that are commonly involved in PCa pathogenesis could be targeted either for prevention or therapy. Either GS or GL has already been assessed against few of these pathways and molecular targets (*e.g*. NF-kB, PI3K/AKT, JAK/STAT, c-Myc, MAPK, VEGFR and Wnt/b-catenin) in various cancers (Eg. Head and neck, colon, breast, prostate, pancreatic and hepatocellular carcinoma); yet it would be interesting to evaluate the effect of GS or GL on pathways, molecules and genetic modification that are unique to PCa, such as - ERG-TMPRSS2 chromosomal rearrangement, loss of expression of NKX3.1, AR synthesis enzymes, AR cofactors expression and stability, EZH2 over-expression, activity of growth factor receptors and activity of protein chaperons like HSP90.

3.3.1 ETS/TMPRSS2 Fusion: Gene fusion is an outcome of chromosomal rearrangement, which brings the coding sequence of one gene and promoter sequence of another gene together. In the case of PCa, the coding sequence from the ETS family of transcription factors is brought under control of the androgen-responsive promoter of TMPRSS2 [31]. EGS, a member of the ETS family, is the most frequently reported fusion gene, occurring in 40-60% of PCa cases and 90% of early-onset PCa [32]. Thus, fusion appears to be an early event sufficient to generate PIN [33]. The Consequence of this fusion event is the up-regulation of oncogenic genes such as Myc, EZH2, SOX9 and repression of NKX3.1, a tumor suppressor gene [34-36]. These events prevent differentiation of prostate epithelium, which is usually an AR-dependent process and promote EZH2 mediated de-differentiation. However, the presence of ETS negative PCa makes the obligatory role of this fusion in PCa initiation questionable. In such ETS negative tumors, EZH2 mediated repression of epigenetic programs is achieved more directly [37]. Although, AR facilitates the fusion by inducing double-stranded breaks *via* recruiting enzymes like deaminase and endo-nucleases; however, TMPRSS2-ERG expression is persistent in CRPCa too indicates that expression is not exclusively controlled by AR [38]. Currently, there are no drugs targeting ETS transcription factors or fusion events. It would be interesting to study if GS poses interference infusion process and/or EZH2 mediated epigenetic program.

3.3.2 NKX3.1: It is a homeobox gene, expression of which is prostate-specific and androgen-regulated. It is a marker of prostate stem cells and required for prostate development [39]. During PCa initiation and progression it shows deregulation in expression pattern due to mutation (5% of PCa cases) or deletion (20% of PCa cases) of a single allele, and frequently due to drop in protein levels in 80% of CRPCa cases. Single copy loss of NKX3.1 gene is one of the initiating events in prostate carcinogenesis. Mutation in this gene is mostly seen to be associated with hereditary patterns of PCa. Reduced expression of NKX3.1 is proposed to be the result of epigenetic silencing. These data are supported by a mouse model, which develops prostatic tumor under influence of monoallelic inactivation of NKX3.1 [40]. NKX3.1 has a cross talk and loop networking with various other molecules such as AR, FOXA1, PTEN, and ETS. Early involvement of NKX3.1 in PCa initiation marks it as a strong target in the field of chemoprevention. However, very less is known about the role of NKX3.1 in cancer prevention. Additionally, no such molecule is known which can stabilize the NKX3.1 expression for therapeutic purposes.

3.3.3 AR Pathway Alteration: AR is a key molecule that governs alteration of transcriptional programs, drive DNA rearrangement, activate

survival pathways such as JAK/STAT, regulate developmental pathways during PCa. Although AR has a limited role in PCa initiation and is rarely altered during primary PCa, it is a driving force in castration-resistant PCa (CRPCa). Altered AR has been reported in 60% of CRPCa cases [41], which harbors transcriptionally active AR in CRPCa despite the castrated levels of testosterone in circulation. The Role of AR in CRPCa is mainly to acquire and execute a distinct program required to attain androgen-independent growth in CRPCa [42]. Alteration in AR is achieved by various means in CRPC such as - amplification of AR, alternating splicing leading to ligand-independent activation, genomic rearrangement to produce truncated AR version that is constitutively activated, post-translation modifications like phosphorylation / sumoylation / methylation/acetylation to increase AR stability and activity, somatic genetic changes in AR associated factors and co-factors, and intramural androgen synthesis.

Therefore, AR remains to be the major target for PCa treatment which is reflected by the fact that most of the drugs currently in use are either AR antagonists or blocks androgen synthesis or hinder the interaction of AR with its co-factors [43]. However, benefits from such treatments are limited [34] and strongly advocates the involvement of investigational therapies which not only targets AR but other deregulated signaling pathways as well. GS is a known AR-antagonist yet there is no evidence relating the effect of GS on the interaction of AR with its cofactors. Further research in this concern might establish GS as an effective targeted therapy that blocks key signaling cascade -AR, as well as restrain other aberrant pathways during PCa progression.

3.3.4 EZH2: EZH2 (enhancer of zeste homolog 2) has a conserved function of epigenetic gene suppression and it is an essential component of polycomb repressive complex 2 (PRC2) which is responsible for conducting histone methylation. Since its discovery in the past decade, EZH2 has become a key molecule to investigate its potential as a robust biomarker for metastatic prostate cancer and novel therapeutic approaches. EZH2 is tightly linked to aggressive prostate cancer, yet we lack understanding about EZH2′s molecular involvement in prostate cancer pathogenesis.

However, it underscores the importance of downstream targets of the histone methyl-transferase. In addition, Drugs targeting histone acetylation *via* EZH2 and HDAC are under phase I trial.

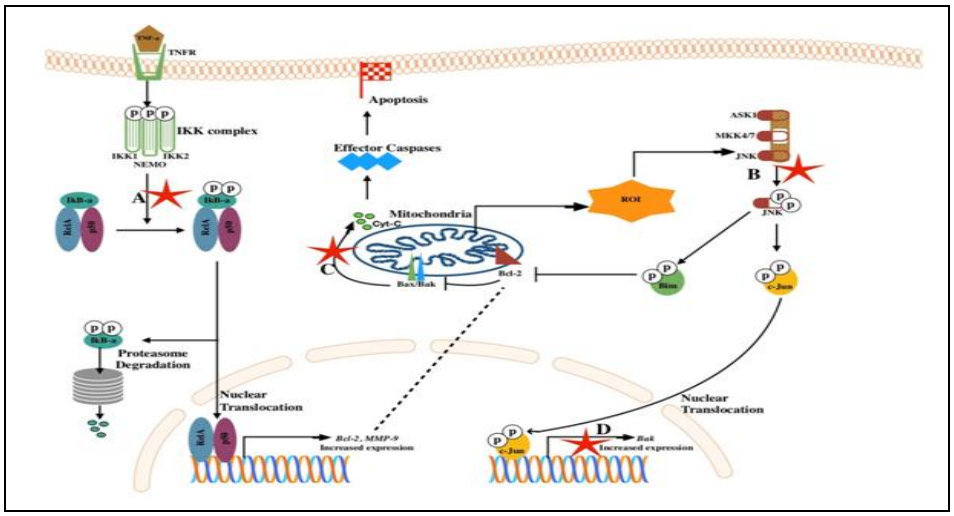

FIG. 2: MECHANISM OF ACTION OF GS MEDIATED APOPTOSIS IN PCA CELLS. RED STARS DEPICTS KNOWN MOLECULAR TARGETS OF GS, BLUNT END LINES SHOWS INHIBITORY EFFECT, DOTTED LINE SHOWS POSSIBLE CROSS TALK BETWEEN TWO PATHWAYS. A) GS IS KNOWN TO INHIBIT NF-KB SURVIVAL PATHWAY BY DIRECT INHIBITOR OF IKK COMPLEX WHICH IN TURN PREVENTS NUCLEAR TRANSLOCATION OF RELA/P50 DIMER. INTERFERENCE OF GS WITH IKKS TRANSLATES INTO BLOCKED EXPRESSION OF GENES (BCL-2) RESPONSIBLE FOR ANTI-APOPTOTIC EFFECTS. B) GS HAS BEEN PROVED TO INDUCE APOPTOSIS THROUGH ROI INDUCED ACTIVATION OF JNK. THIS RESULTS INTO ELEVATED LEVELS OF PRO-APOPTOTIC PROTEINS. C) APOPTOSIS BY GS IS CASPASE DEPENDENT WHICH IS MEDIATED BI BAX AND BAK PROTEINS LOCATED AT MITOCHONDRIAL MEMBRANE. D) GS IS INVOLVED IN UP REGULATING THE EXPRESSION OF PRO-APOPTOTIC GENES LIKE BAK TO PERFORM APOPTOTIC AND ANTI-CANCER ACTIONS

CONCLUSION AND FUTURE PERSPECTIVE: Numerous strategies are being employed to limit prostate cancer growth such as Immunotherapeutic agents, selective adrenal inhibitors, anti-angiogenic molecules, and newly engineered androgen receptor inhibitors. Among all, GS seems to be a multi-target pharmacological compound that can be complemented with an existing therapeutic paradigm for improving beneficial outcomes. In spite of the continuous flow of data about the mechanism of action of GS, an unambiguous claim about its molecular mechanism still waits which could fill in the gaps of present knowledge. Therefore, deeper knowledge about the mechanism of action of GS is required to address its potential benefit mainly in the castration-resistant PCa forms. Additionally, little scientific information exists on the potential toxicity, side effects and dosage of guggul extract and GS. To manage such issues, it is essential to gain profound knowledge concerning the effects of GS on various signaling pathways with specific mention to newly emerging targets for PCa. Thus, exploring the potential effects of GS on diverse molecular targets may drive us towards a more tailored approach regarding the usage of GS.

There have been incidences when plant extracts were widely used for the management of various health issues as supplements. However, later on, clinical studies revealed that the use of such herbs was not free of side effects. Likewise, Guggul is now fast becoming a promising agent for its hypo-lipidemic and anti-cancer activity. Consumption of Guggul extracts has increased in Indian markets too. The question now before the scientific community is to scrutinize the extensive popularity of GS. Therefore, a definitive mode of action should positively defend the fast-growing success of GS.

Besides, PCa is becoming a popular model to study effects of GS as it offers a system with an anomalous hormonal receptor (AR), active inflammatory response pathways (NF-kB), aberrantly active kinases (PI3K/Akt), deregulated developmental genes (NKX3.1), genetic rearrange-ments (ETS/TMPRSS2) and epigenetic changes. Out of all these anomalies, few are known to be directly affected by GS such as – AR, NF-kB and its kinases, Akt pathway and JNK; while remaining anomalies have not yet been explored for possible effects of GS on these particular signaling molecules or transcription factors. Since GS is known to be the antagonist of AR, it would be interesting to see the effect of GS on various AR variants from advanced PCa (CRPCa) which includes the constitutively active form and the truncated form of AR. This knowledge could extrapolate GS's role in advanced CRPCa. In addition, many questions remain unanswered regarding the mechanism of GS, such as how GS is able to generate reactive oxygen species and how GS interacts with IKK complex to inhibit its action? **Fig. 2** Inferring the action of GS that translates into its apoptotic effect will contribute to uncovering the mechanism for the chemopreventive activity of GS. Besides, the lack of information in these segments provides ample opportunity for research and evaluation.

Although advanced screening efforts helped to improve early detection of PCa, it is also supporting in investigating prospects for chemoprevention and combinatorial therapy. The imminent efficacy of the prevention and robust therapeutics of PCa is impingent upon new agents and strategies. Therefore, investigating the complete mode of action for GS could establish GS as an ideal chemoprevention agent and open new opportunities for therapeutic intervention.

ACKNOWLEDGEMENT: This work was supported by the Ministry of Science and Technology, India [Women Scientist Scheme A].

CONFLICTS OF INTEREST: The author of the manuscript has no conflict of interest with a financial or non-financial interest in the subject matter or materials discussed in this manuscript.

REFERENCES:

1. Xiao M and Xiao D: Medicinal chemistry gugulipid, an extract of ayurveda medicine plant *Commiphora mukul* as a potent agent for cancer chemoprevention and cancer chemotherapy 2012; 2.
2. Ulbricht C, Basch E and Szapary P: Guggul for hyperlipidemia: a review by the natural standard research collaboration. Complement Ther Med 2005 doi: 10. 1016/j.ctim.2005.08.003
3. Barqawi AB, Krughoff KJ and Eid K: Current challenges in prostate cancer management and the rationale behind targeted focal therapy. Adv Urol 2012. doi: 10. 1155/2012/862639

4. Ramalingam S, Ramamurthy VP and Njar VCO: Dissecting major signalling pathways in prostate cancer development and progression: mechanisms and novel therapeutic targets. J Steroid Biochem Mol Biol 2017. doi:10.1016/j.jsbmb.2016.07.006
5. Shukla S, MacLennan GT and Fu P: Nuclear factor-kappaB/p65 (Rel A) is constitu-tively activated in human prostate adenocarcinoma and correlates with disease pro-gression. Neoplasia 2004. doi:10.1593/neo.04112
6. Jain G, Voogdt C and Tobias A: IκB kinases modulate the activity of the androgen receptor in prostate carcinoma cell lines 1, 2. Neoplasia 2012; 14: 178-89.
7. Chen S, Nimick M, Cridge AG, Hawkins BC and Rosengren RJ: Anti-cancer potential of novel curcumin analogs towards castrate-resistant prostate cancer. Int J Oncol 2018; 52(2): 579-88.
8. Pigozzi PB; LDL; NM; F: Herbal supplements. J Sport Sci Med 2008; 7(4): 562.
9. Shishodia S, Harikumar KB, Dass S, Ramawat KG and Aggarwal BB: The guggul for chronic diseases: ancient medicine, modern targets. Anti-cancer Res 2008; 28(6A): 3647-64.
10. Shah R, Gulati V and Palombo EA: Pharmacological properties of guggulsterones, the major active components of gum guggul. Phyther Res 2012. doi:10.1002/ptr.4647
11. Szapary PO, Wolfe ML and Bloedon LAT: Guggulipid for the treatment of hypercholesterolemia: a randomized controlled trial. J Am Med Assoc 2003; 290(6): 765-72.
12. Wang X, Greilberger J, Ledinski G, Kager G, Paigen B and Jürgens G: The hypolipidemic natural product *Commiphora mukul* and its component guggulsterone inhibit oxidative modification of LDL. Atherosclerosis 2004. doi:10.1016/j.atherosclerosis.2003.10.008
13. Meselhy MR: Inhibition of LPS-induced no production by the oleogum resin of *Commiphora mukul* and its constituents. Phytochemistry 2003. doi: 10.1016/S0031-9422(02)00388-6
14. Singh BB, Mishra LC, Vinjamury SP, Aquilina N, Singh VJ and Shepard N: The effectiveness of *Commiphora mukul* for osteoarthritis of the knee: an outcomes study. Altern Ther Health Med 2003.
15. Tripathi YB, Malhotra OP and Tripathi SN: Thyroid stimulating action of z-guggulsterone obtained from *C. mukul*. Planta Med 1984. doi:10.1055/s-2007-969626
16. Urizar NL, Liverman AB and Dodds DT: A natural product that lowers cholesterol as an antagonist ligand for FXR. Science (80-). 2002. doi:10.1126/science.1072891
17. Burris TP, Montrose C and Houck KA: The hypolipidemic natural product gugguls-terone is a promiscuous steroid receptor ligand. Mol Pharmacol 2005. doi:10.1124/mol.104.007054
18. Shishodia S and Aggarwal BB: Guggulsterone inhibits NF-??B and I??B?? kinase activation, suppresses expression of anti-apoptotic gene products, and enhances apoptosis. J Biol Chem 2004. doi:10.1074/jbc.M408093200
19. Shishodia S, Sethi G, Ahn KS and Aggarwal BB: Guggulsterone inhibits tumour cell prolife-ration, induces S-phase arrest and promotes apoptosis through activation of c-Jun N-terminal kinase, suppression of Akt pathway and down regulation of anti-apoptotic gene products. Biochem Pharmacol 2007. doi:10.1016/j.bcp.2007.03.026
20. Singh S V, Zeng Y and Xiao D: Caspase-dependent apoptosis induction by guggulsterone, a constituent of ayurvedic medicinal plant *Commiphora mukul*, in PC-3 human prostate cancer cells is mediated by Bax and Bak. Mol Cancer Ther 2005; 4(11): 1747-54.
21. Macha MA, Matta A, Chauhan SS, Siu KWM and Ralhan R: Guggulsterone targets smoke-less tobacco induced PI3K/Akt pathway in head and neck cancer cells. Plos One 2011. doi:10.1371/journal.pone.0014728
22. Samudio I, Konopleva M, Safe S, McQueen T and Andreeff M: Guggulsterones induce apoptosis and differentiation in acute myeloid leukemia: identification of isomer-specific anti-leukemic activities of the pregnadienedione structure. Mol Cancer Ther 2005. doi:10.1158/1535-7163.MCT-05-0247
23. Macha MA, Rachagani S and Gupta S: Guggulsterone decreases proliferation and metastatic behaviour of pancreatic cancer cells by modulating JAK/STAT and Src/FAK signalling. Cancer Lett 2013. doi:10.1016/j.canlet.2013.07.037
24. Singh SV, Choi S, Zeng Y, Hahm ER and Xiao D: Guggulsterone-induced apoptosis in hu-man prostate cancer cells is caused by reactive oxygen intermediate dependent activation of c-Jun NH2-terminal kinase. Cancer Res 2007. doi:10.1158/0008-5472.CAN-07-0120
25. Kim ES, Hong SY and Lee HK: Guggulsterone inhibits angiogenesis by blocking STAT3 and VEGF expression in colon cancer cells. Oncol Rep 2008. doi:10.3892/or_00000147
26. Xiao D and Singh SV: z-Guggulsterone, a constituent of ayurvedic medicinal plant *Commiphora mukul*, inhibits angiogenesis *in-vitro* and *in-vivo*. Mol Cancer Ther 2008; 7(1): 171-80.
27. An MJ, Cheon JH, Kim SW, Kim ES, Kim TI and Kim WH: Guggulsterone induces apoptosis in colon cancer cells and inhibits tumour growth in murine colorectal cancer xeno-grafts. Cancer Lett 2009. doi:10.1016/j.canlet.2009. 01.026
28. Macha MA, Matta A, Chauhan S, Siu KM and Ralhan R: 14-3-3 zeta is a molecular target in guggulsterone induced apoptosis in head and neck cancer cells. BMC Cancer 2010. doi:10.1186/1471-2407-10-655
29. Sarfaraz S, Siddiqui IA, Syed DN, Afaq F and Mukhtar H: Guggulsterone modulates MAPK and NF-??B pathways and inhibits skin tumorigenesis in SENCAR mice. Carcinogenesis 2008. doi:10.1093/carcin/bgn180
30. Xiao D, Zeng Y, Prakash L, Badmaev V, Majeed M and Singh SV: Reactive oxygen species-dependent apoptosis by gugulipid extract of ayurvedic medicine plant *Commiphora mukul* in human prostate cancer cells is regulated by c-Jun N-terminal kinase. Mol Pharmacol 2011; 79(3): 499-07.
31. Tomlins SA, Rhodes DR and Perner S: Recurrent fusion of TMPRSS2 and ETS tran-scription factor genes in prostate cancer. Science 2005. doi:10.1126/science.1117679
32. Weischenfeldt J, Simon R and Feuerbach L: Integrative genomic analyses reveal an androgen-driven somatic alteration landscape in early-onset prostate cancer. Cancer Cell 2013. doi:10.1016/j.ccr.2013.01.002
33. Klezovitch O, Risk M and Coleman I: A causal role for ERG in neoplastic transformation of prostate epithelium. Proc Natl Acad Sci USA. 2008. doi:10.1073/pnas.0711711105
34. Arora VK, Schenkein E and Murali R: Glucocorticoid receptor confers resistance to anti-androgens by bypassing androgen receptor blockade. Cell 2013. doi:10.1016/j.cell.2013.11.012
35. Kunderfranco P, Mello-Grand M and Cangemi R: ETS transcription factors control transcription of EZH2 and epigenetic silencing of the tumour suppressor gene Nkx3.1 in prostate cancer. Plos One. 2010. doi:10.1371/journal.pone.0010547

36. Sun C, Dobi A and Mohamed A: TMPRSS2-ERG fusion, a common genomic alteration in prostate cancer activates C-MYC and abrogates prostate epithelial differentiation. Oncogene 2008. doi:10.1038/onc.2008.183
37. Kim JH, Dhanasekaran SM and Prensner JR: Deep sequencing reveals distinct pat-terns of DNA methylation in prostate cancer. Genome Res 2011. doi:gr.119347.110 [pii] 10.1101/gr.119347.110
38. Casey OM, Fang L and Hynes PG: TMPRSS2- driven ERG expression *in-vivo* increases self-renewal and maintains expression in a castration resistant subpopulation. Plos One 2012. doi:10.1371/journal.pone. 0041668
39. He WW, Sciavolino PJ and Wing J: A novel human prostate-specific, androgen-regulated homeobox gene (nkx3.1) that maps to 8p21, a region frequently deleted in prostate cancer. Genomics 1997. doi:10.1006/geno.1997. 4715
40. Kim MJ, Cardiff RD and Desai N: Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. Proc Natl Acad Sci USA 2002.
41. Grasso CS, Wu YM and Robinson DR: The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012. doi:10.1038/nature11125
42. Wang Q, Li W and Zhang Y: Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell 2009. doi:10.1016/j.cell.2009.04.056
43. Mostaghel EA: Abiraterone in the treatment of metastatic castration-resistant pros-tate cancer. Cancer Manag Res. 2014. doi:10.2147/CMAR.S39318

How to cite this article:
Jain G and Das P: Guggulsterone: mechanism of action and prospects of chemoprevention in prostate cancer. Int J Pharm Sci & Res 2020; 11(4): 1527-36. doi: 10.13040/IJPSR.0975-8232.11(4).1527-36.