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REVIEW ARTICLE

Exploring the Mechanisms of Cytotoxic and Anti-inflammatory Property of Andrographolide and its Derivatives

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ABSTRACT

This review explores the mechanisms of cytotoxic and anti-inflammatory properties of andrographolide and derivatives of andrographolide in various cell lines. *In vitro* and *in vivo* studies that shed light on the molecular mechanisms of cytotoxicity and anti-inflammatory property of andrographolide and its derivatives are reviewed here. Cytotoxic effect of andrographolide on cancer cell lines are mainly due to the induction of reactive oxygen species, activation of c-Jun N-terminal kinase, inhibition of autophagy, and induction of apoptosis. Anti-inflammatory effect of andrographolide is predominantly due to the covalent inhibition of nuclear factor kappa B transcription factor and thereby inhibition of various targets genes such as tumor necrosis factor-alpha, interleukin-6, macrophage inflammatory protein-2, and nitric oxide synthase. Andrographolide is known to directly bind Ras protein; therefore, inhibition of growth factor-activated downstream pathways such as ERK1/2 pathway might be due to the direct inhibition of Ras functions. Inhibition of Ras pathway by antagonizing Ras protein might be a key event contributing to both cytotoxicity and anti-inflammatory functions of andrographolide. Evidence from literature study showed that derivatives of andrographolide such as neoandrographolide and 14-deoxy-11,12-didehydroandrographolides are potent anti-inflammatory agents with less cytotoxicity. Unfavorable chemical modifications such as sulfation and glucuronidation inside the body and fast removal from plasma are the major factors known to hinder the bioavailability of andrographolide.

Key words: Andrographolide, cytotoxic, inflammation, reactive oxygen species

INTRODUCTION

Andrographis paniculata is a medicinal plant commonly found in India and China. It belongs to the family Acanthaceae and is well known for its spectrum of pharmacological properties. Therefore, *A. paniculata* was actively focused by a large number of investigators over the past few decades. The constituents of *A. paniculata* showed bioactivities such as cytotoxicity, immunomodulation, hepatoprotectivity, apoptosis induction, and activates calcium-permeable protein channel called transient receptor potential channels (TRPV4).[1-6] Andrographolide, a diterpenoid lactone is one of the potent bioactive compounds isolated from *A. paniculata*.[7,8] Investigators had studied the cytotoxic effect of andrographolide on various cell lines, but the exact target proteins of andrographolide remain obscure. Informations on cytotoxic and anti-inflammatory mechanisms of andrographolide and its derivatives are scattered in the literature. Therefore, it is an immediate need to compile these informations. A wide variety of diterpenoids with andrographolide like carbon skeleton has been isolated from *A. paniculata*. Table 1 shows the list of derivatives of andrographolide isolated from *A. paniculata*. Chemical structures of important derivatives of andrographolide are given in Figure 1. The present review explores the cytotoxic and anti-inflammatory mechanism of andrographolide and its derivatives. Although experimental conditions are different, compiled data from various studies showed that andrographolide has the lowest IC_{50} values against the proliferation of leukemia, cervical and colon cancer cell lines. The cytotoxicity of andrographolide is mainly due to the generation of reactive oxygen species, c-Jun N-terminal kinase activation (JNK) activation, inhibition of autophagy and induction of apoptosis. Anti-inflammatory effect of andrographolide is mainly due to the inhibition of nuclear factor kappa B (NF-κB) and ERK1/2 pathways. Reason for the low bioavailability of andrographolide was investigated and methods adopted to improve the bioavailability of andrographolide were also discussed here.

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CYTOTOXIC AND ANTI-INFLAMMATORY MECHANISM OF ANDROGRAPHOLIDE

Mechanism of cytotoxicity of andrographolide against cancer cell lines

Andrographolide is cytotoxic against a broad range of cancer cell lines,[1] and IC_{50} values of andrographolide against the proliferation of various cancer cell lines are listed in Table 2. Interestingly, it was showed that andrographolide inhibited the proliferation of MCF7 cells and HL-60 cells at G0-G1 phase, but inhibited the proliferation of MDA-MB-231 and HepG2 cell lines at G2/M phase of the cell cycle.[1,20-22,31] In the case of colon cancer cell line (Lovo cell line),

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Table 1: Chemical formula of andrographolide and its derivatives are listed here

Number	Compound	Calculated molecular weight	Molecular formula	Reference
1	Andrographolide	350	$C_{20}H_{30}O_5$	[8,9]
2	Neoandrographolide	481	$C_{26}H_{40}O_8$	[9,10]
3	Isoandrographolide	351	$C_{20}H_{30}O_5$	[9]
4	14-deoxy-12-methoxyandrographolide	365	$C_{21}H_{32}O_5$	[9]
5	12-*epi*-14-Deoxy-12-methoxyandrographolide	365	$C_{21}H_{32}O_5$	[9]
6	14-*epi*-andrographolide	351	$C_{20}H_{30}O_5$	[9]
7	14-deoxy-11-hydroxyandrographolide	351	$C_{20}H_{30}O_5$	[9]
8	14-deoxy-11,12-dihydroandrographiside	495	$C_{26}H_{38}O_9$	[9]
9	6-acetylneoandrographolide	523	$C_{28}H_{42}O_9$	[9]
10	Bisandrographolide (A, B, C)	665	$C_{40}H_{56}O_8$	[9]
11	Bisandrographolide D	697	$C_{41}H_{60}O_9$	[9]
12	Bisandrographolide ether	829	$C_{46}H_{68}O_{13}$	[11]
13	Andrograpanin	318	$C_{20}H_{30}O_3$	[11,12]
14	14-deoxy-15-isopropylidene-11,12-didehydroxyandrographolide	373	$C_{23}H_{33}O_4$	[13]
15	Andrographiside	513	$C_{26}H_{40}O_{10}$	[9,14]
16	14-deoxyandrographolide	335	$C_{20}H_{30}O_4$	[9,11,15]
17	Deoxyandrographiside	497	$C_{26}H_{41}$	[9]
18	Andrographone	465	$C_{32}H_{64}O$	[16]
19	Andrographane	579	$C_{40}H_{82}$	[16]
20	Andrographosterol	331	$C_{23}H_{38}O$	[16]
21	Homoandrographolide	441	$C_{22}H_{32}O_9$	[17]
22	14-deoxy-11,12-didehydroandrographolide	333	$C_{20}H_{29}O_4$	[15]
23	14-deoxy-11-oxoandrographolide	348	$C_{20}H_{28}O_5$	[15]
24	3-O-β-D-Glucopyranosyl-14,19-dideoxyandrographolide	480	$C_{26}H_{39}O_8$	[18]
25	14-Deoxy-17β-hydroxyandrographolide	353	$C_{20}H_{32}O_5$	[18]
26	19-O-[β-D-apiofuranosyl (1-2)-β-D-glucopyranoyl]-3,14-dideoxyandrographolide	612	$C_{31}H_{47}O_{12}$	[18]
27	3-O-β-D-Glucopyranosyandrographolide	512	$C_{26}H_{39}O_{10}$	[18]
28	12S-Hydroxyandrographolide	369	$C_{20}H_{32}O_6$	[18]
29	Andrographatoside	498	$C_{26}H_{41}O_9$	[18]
30	8,17-Epoxy-14-deoxyandrographolide	351	$C_{20}H_{30}O_5$	[18]
31	19-hydroxy-3-oxo-ent-labda-8 (17),11,13-trien-16,15-olide	331	$C_{20}H_{26}O_4$	[19]
32	3,18,19-trihydroxy-entlabda-8 (17),13-dien-16,15-olide	351	$C_{20}H_{30}O_5$	[19]
33	3,19-dihydroxy-ent-labda-8 (17),12-dien-16,15-Olide	335	$C_{20}H_{30}O_4$	[19]
34	19-[(b-D-glucopyranosyl) oxy]- 19-oxo-ent-labda-8 (17),13-dien-16,15-olide	495	$C_{26}H_{38}O_9$	[19]
35	ent-labda-8 (17),13-diene-15,16,19-triol	323	$C_{20}H_{34}O_3$	[19]
36	3,15,19-trihydroxy-ent-labda-8 (17),13-dien-16-oicacid	353	$C_{20}H_{32}O_5$	[19]
37	3,19-dihydroxy-14,15,16-trinor-ent-labda-8 (17),11-dien-13-oic acid	295	$C_{17}H_{26}O_4$	[19]
38	13,14,15,16-tetranor-ent-labd-8 (17)-ene-3,12,19-triol	267	$C_{16}H_{28}O_3$	[19]

Chemical structures of important compounds listed in this table are given in Figure 1 with corresponding number shown in the table

andrographolide inhibited the cell cycle at G1-S phase.[23] These studies clearly indicated that andrographolide does not have any selectivity towards a specific cell cycle phase. Andrographolide inhibited the proliferation of MDA-MB-231, HepG2 and lymphoma cells by inducing reactive oxygen species.[21,31,32] In HepG2 cells, generation of reactive oxygen species caused a reduction in reduced glutathione (GSH) by the accumulation of hydrogen peroxide.[31] Similarly, it was showed that depleting GSH using buthionine sulfoxamine has sensitized the lymphoma cell lines to andrographolide induced cytotoxicity.[32] This showed that cytotoxic activities of andrographolide have a close association with intracellular levels of GSH. In breast cancer cell line (MCF7), andrographolide treatment increased the level of cell cycle-dependent kinase (CDK4) inhibitory protein, p27.[20] In colon cancer cell line (Lovo), the andrographolide treatment has increased the levels of p16, p21 and phosphorylated p53 proteins; and reduced the expression of the cell cycle associated protein such as CDK2 and CDK4.[23] Studies on MCF and colon cancer cell lines consistently showed a reduction in CDK4 proteins which is required for the G1 phase to S phase transition.

In different cancer cell lines, andrographolide exhibited contrasting effects, but analysis of downstream proteins activated following andrographolide treatment indicated the role of reactive oxygen species in causing cytotoxic effect. Andrographolide inhibited the proliferation and migration of human nonsmall cell lung cancers H3255, A549, and human colorectal carcinoma-Lovo cells.[33-35] Andrographolide reduced the proliferation of VEGF-induced lung carcinoma in mccsp-hVegf-A_{165}-sv40 transgenic mice by reducing the levels of VEGF, cyclin A and cyclin B proteins.[36] In small cell lung carcinoma cell line (NCI-H358), andrographolide induced the expression of tumor-suppressor protein HLJ1 and inhibited the proliferation and invasion.[37] Andrographolide triggered apoptosis in cancer cell lines HepG2, HeLa, and MDA-MB-231 through the activation of caspase 8; the release of cytochrome c from mitochondria and activation of caspase cascade by ROS-mediated activation of tumor suppressor p53 by JNK.[38-40] Cytochrome C release from mitochondria following andrographolide treatment is consistently observed in HepG2, MDA-MB-231, HL-60, and rheumatoid arthritis fibroblast-like synoviocytes (RAFLSs).[21,22,38,41] Effect of andrographolide on the activation of ERK1/2, P38, NF-κB, and JNK proteins in cancer cells are shown in Table 3.

Andrographolide inhibits autophagy in the liver cancer cells. Andrographolide disrupted the mitochondrial membrane permeability transition pore cyclophilin D and showed increased levels of ROS and autophagy marker, LC3II.[45] It was showed that cyclophilin D is required for the cytotoxicity of andrographolide. In another study, it was identified

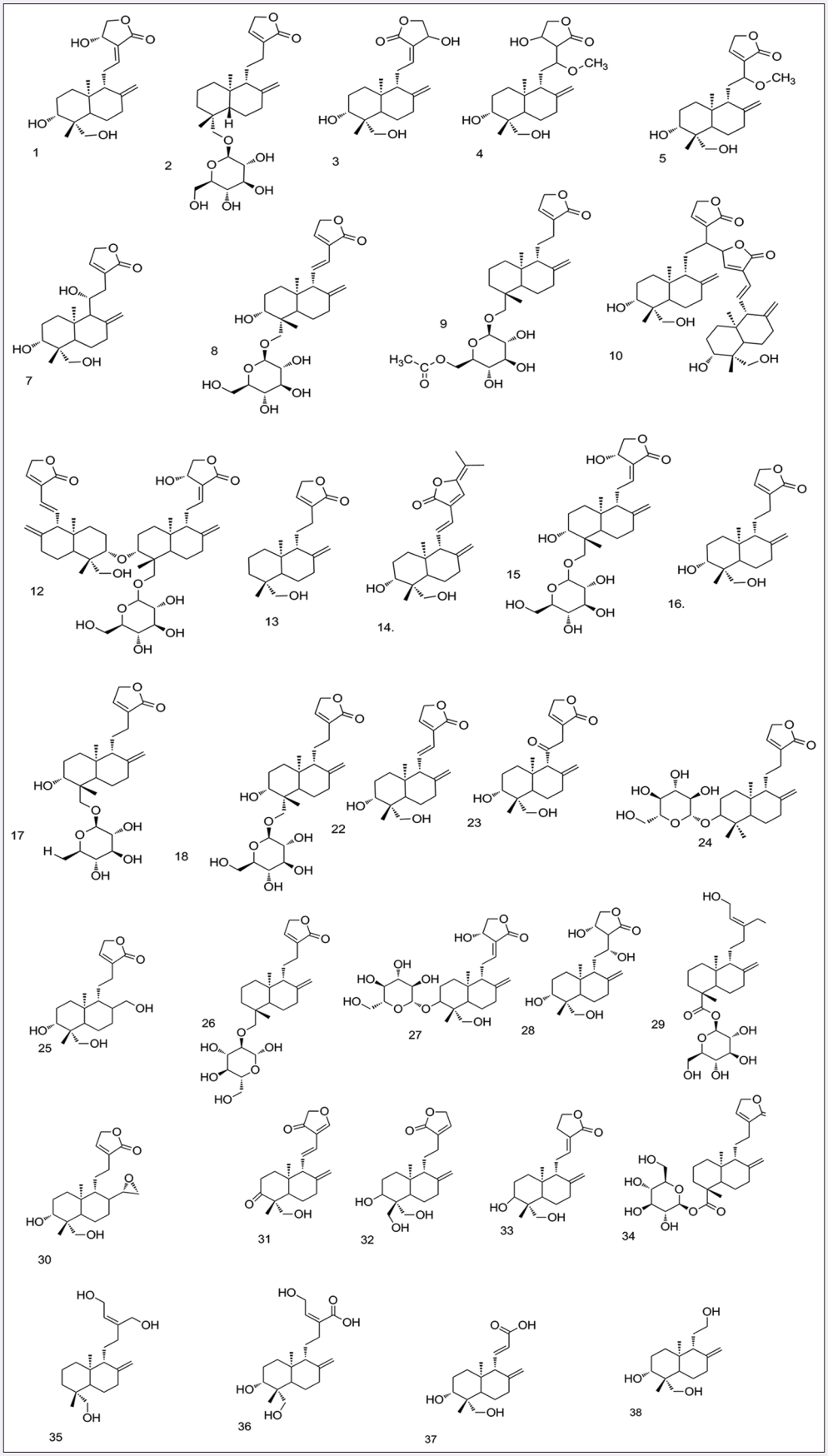

Figure 1: Chemical Structure of andrographolide and its derivatives

Table 2: IC_{50} values of andrographolide against proliferation of various cell lines

Cell line	Cell type	Cell cycle block	IC_{50} reported for Androgarpholide	Reference
PC-3	Prostate cancer		5 μg/ml	[25]
DU145	Prostate cancer		28 μM	[26]
Jurkat cells	Human T-cell leukemia		> 200 μM	[1]
Jurkat E6-1	Human T-cell leukemia		>100 μM	[27]
P388	Leukemia		1.0 μg/ml	[28]
HL-60	Promyelocytic leukemia	G0/G1 phase	2.4 μg/ml	[22]
HepG2	Hepatoma	G2/M phase	40.2 μM	[24]
H-4-II-E	Rat hepatoma		5.98 μg/ml	
Chang liver	Normal liver		>40 μM	[24]
NCI/ADR-RES	Breast cancer		15 μM	[26]
SW620	Colon cancer		11 μM	[26]
COLO205	Colon cancer		<5 μM	[20]
SW620	Colon cancer		10 μM	[29]
HCT-116	Colon cancer		>40 μM	[24]
Lovo	Colon cancer	G1-S phase	8.6 μM	[23]
CT26	*Mus musculus* colon carcinoma		10 μM	[30]
H522	Lung cancer		16 μM	[26]
M-14	Melanoma		14 μM	[26]
SKOV-3	Ovarian cancer		18 μM	[26]
MiaPaCa-2	Pancreas cancer		>40 μM	[24]
Hela	Cervix cancer		>40 μM	[24]
KB cells	Cervix cancer		1.5 μg/ml	[28]
A549	Alveolar cancer		>40 μM	[24]
A375	Skin cancer		>40 μM	[24]
MCF-7/ADR	Breast cancer		15 μM	[29]
MCF	Breast cancer	G1-phase	5–15 μM	[20]
			61.11 μM	[21]
MDA-MB-231	Breast cancer	G2/M phase	51.98 μM	[21]
MCF10A	Normal Breast epithelial		137.9 μM	[21]
A498	Renal cancer		30 μM	[29]

Table 3: Cell signaling pathways affected by andrographolide treatment

Inhibition of cell signaling in LPS activated macrophages					
	ERK1/2	P38 (MAPKs)	NF-κB	JNK	Reference
Andrographolide (1-25 μg/ml)	-	No-effect	No-effect	No-effect	[55]
Neoandrographolide (15-90 μM)	No-effect	-	No-effect	No-effect	[72]

Inhibition of cell signaling pathways in cancer cell lines							
	Concentration	ERK1/2	P38 (MAPKs)	NF-κB	JNK	Cell line	Reference
Andrographolide	5 μM	-				Ts-V-Src	[42]
	0-10 μM			-		H3255	[44]
	15 μM				+	HepG2	[39]
	30 μM	-				RAW264.7	[43]
	10-50 μM	-		-	+	Jurkat E-6	[27]
	0-100 μM	+	+		+	Hep3B	[40]

+=Activates, -=Inhibits, JNK=Jun N-terminal kinase, MAPKs=Mitogen-activated protein kinases, ERK1/2=Extracellular signal-regulated kinase 1/2

that increased levels of LC3II was due to the suppression of autophagy in the late stages by andrographolide. Autophagosome–lysosome fusion was inhibited without affecting the lysosomal functions.[46] Autophagy was suggested to play a prosurvival role in cisplatin-induced cytotoxicity and pretreatment with andrographolide increased the sensitivity of HCT116 colon cancer cell line against cisplatin in a p53-independent manner.[46] These studies clearly indicate that andrographolide imparts its effect by altering mitochondrial functions. Since there is not much data available to demonstrate the effect of andrographolide on autophagy, more studies on different cancer cell lines are required to confirm its autophagy inhibitory property.

It was reported that andrographolide inhibits the proliferation of prostate cancer cells by affecting the cytokine pathways. Andrographolide suppressed the prostate cancer growth by downregulating both mRNA and protein levels of interleukin-6 (IL-6).[47] It induced the expression of D4 receptors by transcriptional up-regulation and sensitized TRAIL (tumor necrosis factor alpha-related apoptosis inducing ligand) resistant cancers for TRAIL-mediated apoptosis.[39] Recent studies showed that andrographolide directly binds to the guanine nucleotide exchange site of oncogenic Ras protein and inhibits its function[48] and also antagonizes V-Src oncoprotein function by promoting V-Src degradation.[42]

Andrographolide and its derivatives are reported to induce differentiation in mouse myeloblast and M1 cells.[9] Induced cell differentiation and free radical formation are closely associated process[49] and there are reports that andrographolide had induced reactive oxygen species in hepatoma and lymphoma cell lines.[31,32] Therefore, these evidence clearly state that cell differentiation induced in M1 cells by andrographolide may

be due to the generation of reactive oxygen species. It is possible that induction of cell differentiation is also associated with perturbation of mitochondrial functions. Andrographolide and its derivatives that induced cell differentiation in mouse myeloid leukemia cells are listed in Table 4.

In vivo cytotoxic effects of andrographolide

Various studies proved that andrographolide can impart cytotoxicity against tumors in *in vivo* condition. Treatment of nude mice bearing human lung adenocarcinoma cell line (CL1-5) with 4 mg/kg of andrographolide significantly reduced the tumor volume and tumor weight.[37] Andrographolide loaded in solid lipid nanoparticles showed better anticancer effect on Balb/c mice.[50] Andrographolide displayed significant antitumor activity against B16F0 melanoma syngenic and HT-29 xenograft models.[20] At a concentration of 100 mg/kg of body weight, andrographolide significantly arrested the proliferation of MCF7 cells placed in fibers inside the peritoneum or subcutaneous layer of Swiss albino mice.[51] Although some of the xenograft model studies listed here used a higher concentration of andrographolide, these reports clearly state that andrographolide can reach the tumor sites and impart its cytotoxic activity.

Mechanism of anti-inflammatory property of andrographolide

Anti-inflammatory effect of andrographolide is primarily due to the covalent inhibition of NF-κB transcription factor. Andrographolide covalently bind to the cysteine 62 residue of NF-κB p50 protein and prevent its transactivation.[52] Expression of inflammation associated genes such as ERK activator protein and Interferon regulatory factor were inhibited on andrographolide treatment.[53] Induced expressions of various cytokines and proinflammatory molecules were inhibited during andrographolide treatment. Andrographolide reduced the levels of tumor necrosis factor-alpha and nitric oxide production in lipopolysaccharide (LPS)-stimulated macrophage.[54,55] Tumor necrosis factor-alpha (TNF-alpha), IL-6, macrophage inflammatory protein-2 (MIP-2), and nitric oxide synthase (iNOS) are direct targets of the NF-κB transcription factor. Therefore, inhibition of TNF-alpha, IL-6, MIP-2, and iNOS by andrographolide could be due to direct inhibition of NF-κB.[52,56,57] Another interesting observation is, at 10 μM concentration andrographolide enhanced the IL-2 production in human PHA-stimulated peripheral blood lymphocytes, but at 20 μM concentration, IL-2 production was inhibited.[26] These reports indicate that immunomodulatory functions of andrographolide are strictly dosage dependent. It might be also possible that andrographolide effects the IL-2 induction and repressor signaling pathway at different concentrations.

Various *in vivo* studies also confirmed the anti-inflammatory property of andrographolide. Andrographolide inhibited the mRNA levels of tumor necrosis factor-alpha (TNF-α), IL-12a, and IL-12b in murine LPS-stimulated macrophage.[55] Several studies have demonstrated the consistent inhibition of iNOS by andrographolide. iNOS, which is a target gene of NF-κB transcription factor was inhibited by *in vivo* treatment of andrographolide. Ovalbumin-induced expression of inducible nitric oxide synthase in lung tissues of BALB/c mice was significantly reduced on andrographolide treatment.[58] These studies clearly establish that *in vivo* treatment of andrographolide can inhibit the activity of NF-κB inside the activated immune cells. Andrographolide increased the expression of GATA3 (TH2 specific transcription factor) and reduced the expression of T-bet and retinoid related orphan receptor-γt (RORγt) in nonobese diabetic mice (NOD mice) and delayed the symptoms of diabetes. NOD mice develop diabetes due to immune cell infilteration into the pancreas, and therefore serve as a model system for the autoimmune disease type 1 diabetes. Reduction in levels of IFN-γ, IL-2 and IL-17 and increased levels of IL-10 and transforming growth factor in NOD mice following andrographolide treatment is thought to modulate the T-helper cell differentiation and cytokine production.[59] This report strongly suggests the use of andrographolide against the treatment of autoimmune diseases.

There is ample evidence for the *in vivo* anti-inflammatory effect of andrographolide. Intraperitoneal treatment of 30 mg/kg of andrographolide inhibited the TNF-α and granulocyte-macrophage colony stimulating factor levels in the bronchoalveolar fluids of ovalbumin immunized mice and the lymphocyte and eosinophil accumulation was completely abolished.[60] Andrographolide prevented the inflammatory bone loss in animal model of osteolysis. A study showed that andrographolide treatment affects the adaptive immune responses. Intraperitoneal treatment of 1 mg/kg of andrographolide reduced the anti-HBs antibody production and the number of IL-4 producing splenocytes in female BALB/c mice.[50]

Table 4: Cell differentiation inducing diterpenoids from *Andrographis paniculata*

Cell differentiation inducing diterpenoids from *Andrographis paniculata*	Reference
Andrographolide, 14-epi-Andrographolide, Isoandrographolide, 14-Deoxyandrographolide, 14-Deoxy-12-methoxyandrographolide, 12-epi-14-Deoxy-12-methoxyandrographolide, 14-Deoxy-12-hydroxyandrographolide, 14-Deoxy-11-hydroxyandrographolide, 14-Deoxy-11,12-dihydroandrographolide	[9]

CYTOTOXIC AND ANTI-INFLAMMATORY MECHANISM OF IMPORTANT DERIVATIVES OF ANDROGRAPHOLIDE

14-deoxy-11, 12-didehydroandrographolide

14-deoxy-11, 12-didehydroandrographolide has no cytotoxic effect on lung carcinoma cell line (A549), human lung epithelial cell line (BEAS-2B) and rat basophilic leukemia (RBL-2H3) cells. However, our studies showed that 14-deoxy-11,12-didehydroandrographolide induced GSH dependent cytotoxicity on human promonocytic cell line THP-1.[61] This compound reduced the expression of apoptosis marker caspase-3, fibrosis marker TGF-β, and PAI-1 in mesangial cell line (MES-13).[62] 14-deoxy-11,12-didehydroandrographolide also showed anti-inflammatory effect against ovalbumin-induced inflammation. 14-deoxy-11,12-didehydroandrographolide treatment reduced the eosinophill counts and levels of IL-4, IL-5, and IL-13 in lavage fluid and reduced ovalbumin specific–IgE production in mouse model of allergic asthma. Platelets are known to secrete various inflammatory mediators and also directly interact with leukocytes therefore, reducing platelet activation might alleviate the inflammation associated with pathological conditions such as sepsis. Treatment of 14-deoxy-11,12-didehydroandrographolide reduced the platelet aggregation by inhibiting the phosphorylation of extracellular signal-regulated kinase1/2 (ERK1/2).[63] Therefore, 14-deoxy-11,12-didehydroandrographolide might reduce the severe inflammatory signals induced by platelets during severe sepsis. 14-deoxy-11,12-didehydroandrographolide also inhibits the translocation of transcription factor NF-κB-p65 into the nucleus and

inhibits the transcription of various mediators of inflammation.[64] Andrographolide is known to antagonize Ras proteins; therefore, its structurally close derivative 14-deoxy-11,12-didehydroandrographolide might also shows Ras antagonizing property; hence, inhibition of ERK1/2 pathway in platelets might be due to inhibition of Ras function. Comparative study showed that cytotoxicity of 14-deoxy-11,12-didehydroandrographolide was less than andrographolide with IC_{50} values above 40 μM.[26] 14-deoxy-11,12-didehydroandrographolide is a potent inhibitor of alphaglucosidase enzyme which break the alpha 1,4 linkages in maltose and starch to form glucose[65,66] However, there are no reports claiming that inhibition of alphaglucosidase activity is responsible for the cytotoxicity or anti-inflammatory properties of this derivative.

Isoandrographolide

Isoandrographolide exhibited cytotoxic property. It induced cell differentiation in myeloid leukemia cell line (M1 cells) and showed significant inhibition of proliferation on human promyelocytic cell line, HL-60.[9,67] Isoandrographolide inhibited the LPS-induced IL-6, nitric oxide, and prostaglandin 2 release in J774A.1 macrophages.[68] It also inhibited the interleukin production from LPS-stimulated macrophages and thromboxane 2 release from A23187 activated HL-60 promyelocytic cells.[69] The evidence from multiple studies clearly shows that target genes of NF-κB such as IL-6 and iNOS are consistently inhibited by isoandrographolide. This indicates that isoandrographolide might be directly inhibiting the NF-κB only like andrographolide.

Neoandrographolide

Neoandrographolide, a glucose bound derivative of 14-deoxy-11,12-didehydroandrographolide showed very less cytotoxicity and cell differentiation in M1 cells.[9] Neoandrographolide exhibited very good free radical scavenging activity and it was hypothesized that neoandrographolide may scavenge the free radical by donating the allylic hydrogen of unsaturated lactone ring.[70] This hypothesis is consistent with the data that neoandrographolide suppressed the respiratory burst induced by phorbol-12-myristate-13-acetate and inhibited the nitric oxide and TNF-α production induced by LPS activated macrophages.[71] Anti-inflammatory effect of *A. paniculata* extracts is mainly due to neoandrographolide.[71] Inordinate production of inflammatory agents such as prostaglandin $E^{[2]}$ and nitric oxide from LPS-stimulated macrophages were significantly reduced on treatment with neoandrographolide. Treatment of neondrographolide on LPS-stimulated macrophages inhibited p38 mitogen-activated kinases (MAPK), but the activation of other proteins such as JNK, ERK1/2, or NF-κB was not inhibited.[72] This study showed that anti-inflammatory effect of neoandrographolide is not only by inhibition of NF-κB but also due to inhibition of p38 mitogen-activated protein kinase, possibly by reducing the respiratory burst by scavenging the free radicals.

14-deoxyandrographolide

14-deoxyandrographolide was cytotoxic against human breast carcinoma cell line (T47D) and human promonocytic leukemia (HL-60) cell line but found noncytotoxic in human T-cell leukemia cells (MT2-cells).[11,22,73] It was reported to inhibit platelet activating factor-induced flux of calcium in the presence of extra cellular calcium and tyrosine phosphorylation of ERK1.[74] 14-deoxyandrographolide-induced iNOS as a result of activation of adenylate cyclase enzyme.[75] 14-deoxyandrographolide-induced nitric oxide production in hepatocytes by inducing iNOS and showed enhanced or accentuated microsomal Ca-GTPase activity.[75] Similarly, it was shown to enhance the nitric oxide production in endothelial cells and also induces calcium-mediated relaxation of rat uterine smooth muscles.[77,78] Comparative study of cytotoxicity of andrographolide, 14-deoxy-11,12-didehydroandrographolide, and 14-dehydroandrographolide identified 14-dehydroandrographolide to be weakly cytotoxic with IC_{50} value above 100 μM.[26] Further studies on various cell lines are required to disclose the mechanism of 14-dehydroandrographolide.

Andrograpanin

Andrograpanin was reported to have no cytotoxicity in human T-cell leukemia (MT2 cells).[11] Andrograpanin induced anti-inflammatory activity by downregulating the p38 MAPKs signaling pathways in LPS-stimulated macrophages[79] and thereby reducing the levels of proinflammatory molecules such as TNFα, IL-6, and IL-12p70. Although NF-κB target genes levels are inhibited the ability of andrograpanin to directly bind the NF-κB transcription factor is not demonstrated. Since andrograpanin and neoandrographolide shares similar structure, andrograpanin might also show a free radical scavenging activity by donating the allylic hydrogen from furan ring. Andrograpanin inhibited the chemokine (SDF1α) directed movement of Jurkat and THP-1 cells and this effect is attributed to the inhibition on internalization of CXCR4 receptor. This study shows the interference of andrograpanin in the endocytosis process.[12]

Bisandrographolide

Bisandrographolide are covalent dimers of andrographolides. Bisandrographolide ether is potentially cytotoxic against MT2 cells (human T-cell leukemia).[11] Bisandrographolide specifically activates TRPV4 channels, which are nonselective Ca^{2+} permeable transmembrane channels normally activated during physicochemical stimuli.[6] TRPV4 channel activation can induce apoptosis through the activation of MAPK and downregulation of phosphatidyl inositol 3 kinase signaling pathways.[80] Therefore, cytotoxicity-induced by bisandrographolide might be due to the activation of MAPK pathways.

Cytotoxicity of semi-synthetic and synthetic derivatives of andrographolide

3,19-isopropylideneandrographolide showed selective cytotoxicity toward leukemia and colon cancer cell lines. Another derivative 14-acetylandrographolide showed selectivity toward ovarian, leukemia, and renal cancer cells.[81] An *in vitro* study showed that benzylidine derivatives of andrographolide-induced cell cycle arrest at G1 phase and were highly cytotoxic against colon and breast cancer cells.[82] 12-aminoandrographolide analogs showed promising anticancer activity against murine leukemia cell line (P-388), human epidermoid cancer of mouth (KB), human colon cancer (COL-2), human breast cancer (MCF-7), human lung cancer cell line (LU-1), and rat glioma cell line (ASK).[83] Noval C-14 ester analogs of andrographolides-induced apoptosis in the kidney (HEK293) and breast cancer (MCF-7) cell lines.[84] Halogenated di-spiropyrrolizidino oxindole derivatives of andrographolide were reported to be more cytotoxic than andrographolide and most cytotoxic derivative showed reactive oxygen depended, mitochondrial pathway-mediated cell death in colon cancer (HCT116), pancreatic cancer (MiaPaCa-2), and hepatocellular carcinoma (HepG2) cell lines.[24] Halogenated di-spiropyrrolizidino oxindole derivative-induced cell death by blocking cell cycle at G1 phase, treated cells showed upregulated proapoptotic proteins Bax and Bad, P53 levels, caspase 3-9, cleaved PARP levels and at the same time downregulated Bcl-2, cystolic NF-κB-P65, PI3-K, and p-AKT.[24] Dispiro andrographolide derivatives induced caspase-mediated apoptosis in MCF-7 breast cancer cell lines.[85]

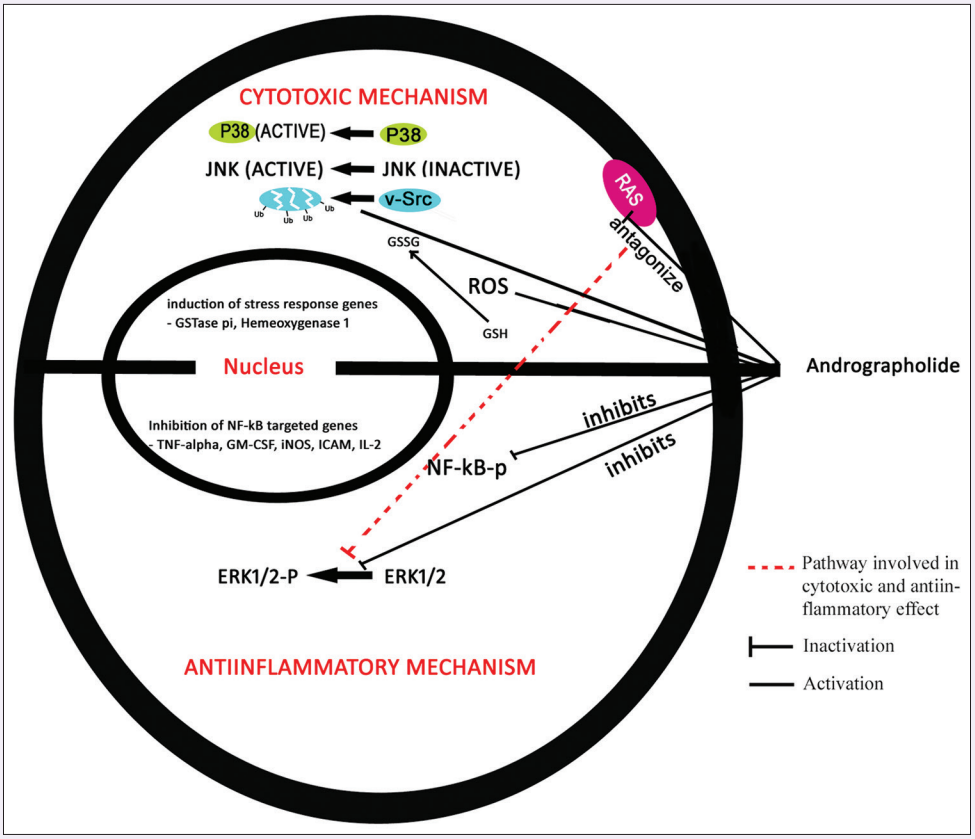

Figure 2: Cytotoxic and antiinflammatory pathways of andrographolide

IMPACT ON DRUG DETOXYFYING ENZYMES AND IMPROVEMENT OF BIOAVAILABILITY OF ANDROGRAPHOLIDE

Effect of andrographolie on drug detoxifying enzymes

Andrographolide is reported to induce the expression of various drug detoxifying enzymes in the liver. In rat hepatocytes, andrographolide-induced high expression of CREB (cyclic AMP response element binding proteins) proteins and subsequent increase in mRNA expression of the antioxidant enzyme glutathione S-transferase π.[86] Increased expression of glutathione S-transferase may adversely affect the combination treatment if the drug is susceptible to detoxification by glutathione S-transferase. Andrographolide increased the expression of cytochrome p450 family genes CYP1A1 and CYP1A2 in mouse hepatocytes.[4] A study in Caco2 cell model (intestinal epithelial barrier model) shows that treatment of andrographolide can downregulate the mRNA expression and protein levels of cytochrome P450 3A4 (CY3PA4) in intestinal cells.[87] These studies show that andrographolide treatment can reduce the expression of intestinal cytochrome P450 3A4 enzymes but increases the expression of CYP1A1 in liver cells. CY3PA4 is responsible for metabolism of 60% of the commercially available drug; therefore, combination treatment of CY3PA4 susceptible drugs with andrographolide might synergistically increase the bioactivity of those drugs in intestine.

Pharmacokinetic studies of andrographolide

Pharmacokinetic studies revealed that andrographolide has poor bioavailability, short plasma half-life, and inappropriate tissue localization.[88] It was showed that andrographolide remained in human plasma only for 4 h after administration of 50 mg of capsule.[89] Metabolic research showed that andrographolide undergoes sulfation in rats and in humans, it undergoes both sulfation and glucuronidation to form various metabolites.[90-93] Administration of andrographolide by both oral and intravenous route in BalB/c mice showed poor bioavailability and moderate terminal half-life.[21] In addition, it was reported that poor oral availability of andrographolide is due to the increased metabolization in duodenum and jejunum to form an impermeable sulfonated metabolite and due to extensive removal from the cells by P-glycoprotein.[94] Studies in rats showed that pharmacokinetic parameters for phase one metabolite of andrographolide were significantly less compared to andrographolide.[85] *In silico* studies showed that andrographolide molecule could be potentially targeted by glutathione S-transferase enzyme.[96] The outcome of this interaction is not studied so far. It was showed that efficacy of andrographolide administered orally can be improved using drug carrier hydroxy-propy-betacyclodextrin.[97] Similarly, poly (lactic-co-glycolic) acid-nanoparticulation improved the bioavailability of andrographolide and increased the lifespan of Ehrlich ascites cancer bearing mice by 79.08% compared to the increased life span of 23.5% for andrographolide alone.[98] Loading of andrographolide into solid-lipid nanoparticles increased the bioavailability of andrographolide by 241% in intestine.[99] Therefore, incorporating andrographolide into lipid-based nanoparticle or Poly (lactic-co-glycolic) acid can reduce its chemical modification inside the intestinal segments and enhance the bioavailability during oral administration.

CONCLUSION

Literature study showed that among the diterpenoids isolated from *A. paniculata*, andrographolide showed cytotoxicity against broad range of cancers. Glucose derivatives of andrographolide are found to be less

Table 5: Diterpenoids from *Andrographis paniculata* that inhibits NF-κB

Diterpenoids inhibiting the NF-κB transactivation	Mode of inhibition	Reference
Andrographolide	Covalent binding to NF-kB	[52]
14-deoxy-11,12-didehydroandrographolide	Not available	[101]
14-deoxy-14,15-dehydroandrographolide	Not available	[101]
19-oacetyl-14-deoxy-11,12-didehydroandrographolide	Not available	[101]

cytotoxic.[9] On comparing the cytotoxic mechanisms of andrographolide and few its derivatives, it is clear that they induce cytotoxicity by generating reactive oxygen species by an unknown mechanism. In addition, andrographolide also blocked autophagosome maturation and increased the expression of cell cycle inhibitors such as p16 and p21. Studies showed that andrographolide is activating JNK and inhibiting ERK1/2 pathways in different cell lines [Table 3]. Inhibition of ERK1/2 pathway by andrographolide could be possibly due to Ras antagonizing property. Since andrographolide antagonize Ras proteins, treatment of andrographolide will inhibit the ability of cells to respond to the growth factors. Studies demonstrating the cytotoxicity and anti-inflammatory effect of andrographolide have showed that andrographolide can inhibit ERK1/2 pathway, therefore inhibition of ERK1/2 could be due to Ras antagonization. Figure 2 shows the cytotoxic and anti-inflammatory pathways reported in various cell lines. Consistent inhibition of ERK1/2 pathway observed in three different studies indicated that Ras antagonizing property of andrographolide might be a key event to induce cytotoxicity and anti-inflammatory functions.[42,43,55] NF-κB p50, Ras and alphaglucosidase enzyme are the known direct targets of andrographolide. There are no reports showing the correlation between alphaglucosidase inhibiting property of andrographolide with its anticancer or anti-inflammatory effect. However, it is showed that andrographolide can directly inhibit alphaglucosidase enzyme activity and reduce the plasma glucose levels in mice. Therefore, antidiabetic property of andrographolide has been associated with the inhibition of alphaglucosidase enzyme.[100]

Studies showed that cytotoxic mechanism of andrographolide was depended on concentration and cell type. This observation was substantiated by the evidence that andrographolide inhibited proliferation of HL-60 cells and MCF-7 at G0-G1 phase and Jurkat, MDA-MB-231 and HepG2at G2/M phase.[1,20-22,31] IC_{50} values of andrographolide against proliferation of various cancer cell lines from multiple studies were listed, IC_{50} value of andrographolide against the proliferation of leukemia, cervical and colon cancer cell lines were less than 5 micromolar. Therefore, it is proposed that colon, cervical and leukemic cell lines could be more sensitive to andrographolide. Protective effect of andrographolide includes induction of antioxidant enzymes such as glutathione S-transferase and hemeoxygenase 1. As andrographolide was reported to induce glutathione S-transferase-π in the liver, combinational treatment of andrographolide with certain drugs may not be successful due to drug detoxification effect. Andrographolide showed potent anti-inflammatory activity against asthma and autoimmune disease in mouse models. These reports strongly recommend the use of andrographolide as an anti-inflammatory agent. The glucose conjugated derivative of andrographolide (neoandrographolide) showed less cytotoxicity and high anti-inflammatory effects. Low cytotoxicity of neoandrographolide indicates that it may have less ability to generate reactive oxygen species. A report showed that neoandrographolide inhibits p38 MAPKs in LPS-stimulated macrophages but did not inhibit JNK, NF-κB, or ERK1/2.[72] From these reports, it is evident that neoandrographolide has no NF-κB inhibiting property, but it induces anti-inflammatory effect through inhibition of p38 MAPKs. Derivatives of andrographolide that inhibits NF-κB transactivation are listed in Table 5. Literature study shows that neoandrographolide has promising anti-inflammatory potential; hence, this compound can be used to develop nonsteroidal anti-inflammatory drugs.

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Conflicts of interest

There are no conflicts of interest.

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