A REVIEW OF THE ANTICANCER POTENTIAL OF THE ANTIMALARIAL HERBAL CRYPTOLEPIS SANGUINOLENTA AND ITS MAJOR ALKALOID CRYPTOLEPINE

C. ANSAH and K. B. MENSAH

Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Corresponding Author: *Prof. Charles Ansah* ***Email:*** *cansah.pharm@knust.edu.gh*

Conflict of Interest: *None declared*

SUMMARY

Cryptolepis sanguinolenta (Lindl.) Schltr (Periplocaceae), has a longstanding traditional use in the treatment of malaria in the West African region. Recent evidence suggests that the aqueous extract from the roots and the major alkaloid from the plant, cryptolepine, have prospects as cancer chemotherapeutic agents on account of their potent cytotoxicity to mammalian cells. Several mechanisms have been proposed to explain the cytotoxic activities of the agents. However, emerging evidence from their anti-inflammatory actions suggest that the mechanism of the cytotoxicity may be closely related to its anti-inflammatory activity. This review looks at the mechanisms of cryptolepis-induced cytotoxicity, its link with inflammation and its potential as anticancer agent. The elucidation of these interwoven mechanisms may be useful in the development of cryptolepine or other analogues as new anticancer agents.

INTRODUCTION

Cryptolepis sanguinolenta (Lindl.) Schltr (Periplocaceae) is a popular Central and West African anti-malarial plant that has attracted scientific research for the past four decades. Its numerous biological effects[1,2,3] have been attributed to its main alkaloid, cryptolepine (Figure 1). Recently, cryptolepine has shown great potential as a candidate anti-cancer agent.[4,5,6,7]

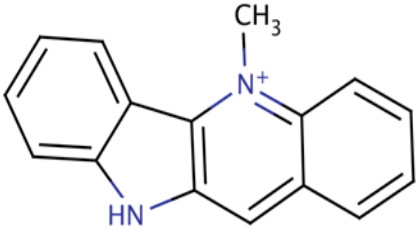

Figure 1 Cryptolepine, the main alkaloid from the aqueous root extract of *Cryptolepis sanguinolenta*.

The worldwide increase in cancer incidence, reported treatment failures and the side effects associated with most of the conventional anticancer medications call for further research and exploration for newer agents for cancer management. Several studies with cryptolepis and its major alkaloid show very potent cytotoxic activity[4,5,7] and multiple mechanisms of action have been suggested. Recent emerging evidence also suggests that the mechanism of the cytotoxicity may be closely related to its anti-inflammatory actions.

This paper examines the interwoven mechanisms involved in the cytotoxicity and anti-inflammatory activity of cryptolepis and its alkaloid, cryptolepine, and suggests possible roles that cryptolepis and cryptolepine could play in the management of certain forms of cancer.

Cytotoxicity of Cryptolepis and Cryptolepine

Prior to the discovery of cryptolepis's cytotoxicity against several mammalian cancer cell lines *in vitro*[4] its main alkaloid, cryptolepine, was empirically believed to have the potential to interact with DNA due to its apparent structural similarity to 9-aminoacridine, which intercalates DNA.[8] Subsequently, it was shown to bind to DNA and inhibit DNA synthesis.[9] At the turn of the 21st century, cryptolepine and its structural analogues were known to have DNA and topoisomerase II as their principal targets.[10]

Cryptolepine was first shown as cytotoxic due to its reported interference with the nuclear ubiquitous enzyme topoisomerase II.[10] Topoisomerase II inhibition is a major mechanism of several anticancer agents including etoposide to induce apoptosis.[11] However, cells treated with cryptolepine *in vitro* have very low survival rates.[4] This report together with the fact that cryptolepine unlike other topoisomerase II inhibitors had low mutagenicity[12,13] suggested that topoisomerase II inhibition may not wholly account for the cytotoxic action of cryptolepine.

Indeed in cell cultures, cryptolepine showed G1 and sub-G1 peaks characteristic of apoptotic cell population.[4,5]

It has already been proposed that p53 may be involved in the cellular response to DNA damage, producing arrest in the G1 phase of the cell cycle to allow efficient repair of DNA before entry to S phase, or cell death if the damage is too overwhelming to be repaired.[14,15] The work by Zhu and Gooderham, (2006)[5] in human lung adenocarcinoma A549 cells lines showed that cryptolepine provokes p53 accumulation at all concentrations. At the molecular level, cryptolepine has a unique planar structure allowing it to slide into DNA and intercalating it particularly at CG rich regions and non-alternating CC sites.[9] Furthermore, accumulation of DNA strand breaks is a well-known stimulus for elevating p53 protein levels and for activating p53-mediated signaling pathways.[16,17]

This alteration in DNA structure and function lead to activation of p53 and subsequently its transcriptional targets such as p21.[18] Cryptolepis treatment inhibits Bcl-2, a protooncogene blocking apoptotic cell death, and causes the release of cytochrome C into the cytosol.[4,5] The overall executioner of apoptosis is caspase[19] and cryptolepine efficiently promotes the activation of caspase-3 but has a modest effect on caspase 8, suggesting a mode of apoptotic cell death executed at least in part through the mitochondrial pathway.[4,5]

Pathophysiology of Cancer and the link with Inflammation

It has been shown that inflammation is closely linked to tumour promotion and nearly all tumors have inflammatory cells present in them irrespective of the underlying cause of the tumor.[20] Inflammation can alter the expression of oncogenes and tumor suppressor genes, induce genomic instability, increase angiogenesis, alter the genomic epigenetic state and increase cell proliferation to promote neoplastic transformation.[21] Carcinogenesis affects the expression of various inflammatory genes and leads to recruitment of inflammatory cells. Evidence from several studies demonstrates that certain anti-inflammatory drugs (NSAIDS) have been associated with reduced risk of several different types of cancer suggesting a link between inflammation and cancer.[22]

Molecular mediators common to inflammation and cancer

Several molecular pathways connect chronic inflammation with oncogenic transformation. Common inflammatory mediators including cytokines, chemokines, reactive oxygen and nitrogen species, COX-2 and NF-kB can lead to cellular conditions favorable for tumor promotion.

Cyclooxygenase-2 (COX-2)

Prostaglandins (PGs), the main products of COX 2 activity affect cell proliferation, mitosis, cell adhesion, apoptosis and immune surveillance. Over-expression and up-regulation of COX-2 have been found in cancers of the breast, colon, lung, pancreas, head and neck cancers in humans.[23,24,25] different types of tumours and transformed cells.[26,27,28] COX-2 prolongs the survival of malignant or transformed cells, elevates Bcl-2 protein expression (anti-apoptotic factor) and diminishes levels of transforming growth factor beta-2 (TGFβ-2) receptor and E-cadherin.[25]

Inducible nitric oxide synthase

Inducible nitric oxide synthase (iNOS) is one enzyme that works synergistically with COX-2. Up-regulation of COX-2 and iNOS has been associated with pathophysiology of certain types of human cancers. iNOS catalyzes the oxidative deamination of L-arginine to produce NO, a potent pro-inflammatory mediator. NO has multiple roles in mutagenesis and carcinogenesis.[29,30,31,32,33] This is evident by the observation that inhibition of NO release cause profound suppression of PG production.[34] Over-production of reactive oxygen and nitrogen species (RONS), aberrant inflammatory cytokine and chemokine expression, increased COX-2 and nuclear factor kappa B (NF-kB) expression are some of the molecular factors that contribute to inflammation-induced carcinogenesis.

Nuclear Factor kappa Beta (NF-kB)

NF-kB is a transcription factor and a key mediator of inflammation-induced carcinogenesis. NF-kB has a variety of pro-tumorigenic activities. Under normal cellular conditions, NF-kB binds to and is negatively regulated by inhibitor of kappa B (ikb) in the cytoplasm. The NF-kB can be detected in its inactive form in the cytoplasm of almost all cell types.[35,36] Following an inflammatory episode, inhibitor of kappa B is phosphorylated and undergoes proteosomal degradation. This allows an activated NF-kB to translocate to the nucleus where it activates the transcription of target genes. NF-kB appears to be the endogenous link that induces expression of COX-2, TNFa and iNOS.[37,38,39,40]

Although experimental data clearly suggest that NF-kB is a major regulator of the inflammatory reaction by controlling the expression of pro-inflammatory molecules in response to cytokines, oxidative stress and infectious agents[41,42], NF-kB also regulates genes

including c-Jun-N-terminal kinase, inhibitors of apoptosis proteins 1 and 2, TNF receptor-associated factors 1 and 2, protein 53 (p53), B-cell CLL/ lymphoma 2, chemokine (C-X-C motif) receptor 4, vascular endothelial growth factor and angiopoetin, leading to pro-tumorigenic angiogenesis, metastasis and increased cell resistance to necrosis and apoptosis.[21,43]

Cryptolepis/Cryptolepine as potent anti-inflammatory agents

Cryptolepis is used traditionally to manage fevers and other febrile conditions. Bamgbose and Noamesi[44] reported the potent anti-inflammatory activity of cryptolepine, the major alkaloid of cryptolepis. The study reported on the ability of cryptolepine to directly antagonize the actions PGE_2 without affecting prostaglandin synthesis in the perfused lung of the rainbow lizard. At the time of the study, very little was known about the isoenzymes of COX but the study well established that the mechanism of the anti-inflammatory activity of cryptolepine may be slightly different from that of conventional NSAIDS.

Subsequently, Olajide *et al.,* had argued that the anti-inflammatory activity of cryptolepis and cryptolepine was due to inhibition of COX-2 and hence its apparent low ulcerogenic activity in rats.[45,46,47,48] Together, the above findings are in agreement that cryptolepis/cryptolepine inhibits prostaglandin E_2 activity. In addition, other key promoters of inflammation such iNOS and NF-kB were all affected by cryptolepine.

Is the cytotoxic action of cryptolepis/cryptolepine linked with its anti-inflammatory activity?

Substances with potent anti-inflammatory activity inhibit carcinogenesis and mutagenesis.[49,50] This has been shown for several anti-inflammatory agents. Inhibition of COX-2 activity is thought to be one of the mechanisms by which NSAIDs exert their antineoplastic effects.[51,52] More than 85% of human cancers have increased levels of COX-2 when compared with adjacent normal tissue.[53,54] Cryptolepine exhibits potent cytotoxicity and potent anti-inflammatory activity.[45,46,47,48] Protein 53 (p53) and NF-kB are key mediators of both processes.

Protein 53 is the cellular gate keeper of cytotoxicity. The p53 protein is up-regulated in response to a diverse array of cellular stresses, including DNA damage, hypoxia, oxidative stress, ribonucleotide depletion, and oncogene activation.[55,56] This tumour suppressor protein inhibits cell growth through activation of cell-cycle arrest and apoptosis and more than half of all human tumours have either mutation within the p53 gene or defects in the ability to induce p53.

NF-kB is a major regulator of the inflammatory reaction by controlling the expression of pro-inflammatory molecules in response to cytokines, oxidatives stress and infectious agents.[41,57] Pro-inflammatory mediators promote tumourigenesis and carcinogenesis. NF-kB acts as anti-apoptotic by inducing the expression of the inhibitors of apoptosis[58,59,60] and some members of the anti-apoptotic Bcl-2 family[61,62] which also antagonize the function of the pro-apoptotic members including A1/BFL1 and Bcl-XL of the family, and subsequently suppress apoptosis induced by both extrinsic and intrinsic pathways through direct inhibition of effector caspases (caspases-3, -6, -7, and 9).

In transformed cells, molecular alterations result in impaired regulation of NF-κB activation leading to loss of its inducibility and activation constitutively. This leads to deregulated expression of genes under NF-κB control. Among them are genes involved in regulation of apoptosis, cell cycle control, adhesion or migration. Since alterations in all these processes participate in development and progression of cancer, there is a clear link between NF-κB and carcinogenesis.

NF-kB has pro-apoptotic activity as well. Other reports indicate that induction of NF-kB is also associated with growth arrest and cellular differentiation.[63,64,65] In particular, NF-kB stimulates the tumor suppressor gene p53[63], which is key to apoptotic cell death. The dual role of NF-kB in cell survival and cell death may be due to several reasons and mechanisms as well as the structure and function of NF-kB. The mammalian NF-kB transcription factor family is not a single gene but a family of closely related genes producing five proteins of two subfamilies: Rel subfamily, (Rel B, p65 (Rel A), and c-Rel)[42,66] which contain C-terminal transactivation and p50 and p52 proteins, which lack transactivating C-terminal region domains.[42,66] The different NF-kB family members can form hetero- or homodimers to produce 15 possible NF-kB transcription factor complexes Hetero- or homo-dimers containing Rel proteins are activators of transcription whilst homodimers of p50 or p52 act as repressors of gene transcription.[42,66] This explains why NF-kB acts as activator or repressor of transcription in normal cells.

Inhibition of NF-kB activity and up-regulation of p53 is responsible for anti-inflammatory and cytotoxicity of cryptolepis/cryptolepine

Current trends suggest that the interplay between NF-kB and p53 determine cell survival and cell death.

Under normal conditions in cells, Hdm2 inhibits p53-induced apoptosis. The Hdm2 promoter contains an NF-kB binding site. This process prevents p53 from triggering apoptosis. Aberrant expression and activation of NF-kB suppress the activation of p53 thereby inhibiting the induction of p53 target genes and DNA damage-induced apoptosis leading to increase cell survival and resistance to apoptosis.[67] Once the activity of NF-kB has been reduced, Hdm2 inhibition of p53 is attenuated and the cell proceeds through a p53-mediated apoptotic pathway. This leads to the inhibition of the expression of inflammatory genes and consequent reduction in tumor incidence.

Cryptolepine as inhibitor of NF-kB activity and an inducer of p53

Cryptolepine inhibits the activity of NF-kB.[46] The interaction of cryptolepine with NF-kB may be different from other conventional anti-inflammatory agents, which act by inhibiting IKK. Cryptolepine acts downstream by interfering with the DNA binding capacity of NF-kB and inhibiting NF-kB-mediated gene expression.[45] This impairs the initiation of transcription of not only pro-inflammatory proteins, but also regulators of the NF-kB pathway such as IkB. Cryptolepine does not interfere with phosphorylation and degradation of IkB or the nuclear entry of NF-kB. Evidence supporting this pathway is that, substances with thiol groups such as DTT are able to reduce the effects of cryptolepine on NF-kB as they compete with cryptolepine for CC sites of DNA reducing intercalation.[46]

The cytotoxicity of cryptolepine is likely to be mediated by activation of p53 as genes that activate cryptolepine induced-cell death are regulated by expression of p53.[4,5] The key to effective cancer therapy is activation or re-introduction of p53 induced apoptosis in tumour cells.

Cryptolepis/cryptolepine as candidate anticancer agents

The potential of cryptolepine as an anticancer agent has never been disputed. Cryptolepine also posseses several advantages over mainstream anticancer agents. Preclinical studies with cryptolepine, the main alkaloid of cryptolepis, show that it has activity against several solid human tumours with breast tumours being the most sensitive.[7] Cryptolepine, however showed differences in activity in patient tumour samples in both solid tumours and haematological malignancies.[7] Interestingly, cisplatin a standard cytotoxic drug is known to exhibit that property. Perhaps the reason why cryptolepine shows prominent activity in breast tumours may be related to its ability to affect cyclins D1, D2, D3 and cyclin E, which control cell cycle progression, as NF-kB-induced cyclin D1 expression appears to be a key element in mammary gland development and breast carcinogenesis.[68]

Cryptolepis/cryptolepine and prostatic disorders

We recently observed in our laboratory that cryptolepis is potently antiandrogenic in rodents and aves. Its potency by our estimation is thirty times less than cyproterone acetate.[69]Androgens are required for the normal growth and development of the prostate and have been implicated in the pathogenesis of Benign Prostatic Hyperplasia (BPH).[70] With its potent cytotoxicity, anti-inflammatory, anti-androgenic activity and its ability to antagonize NF-kB, we reasoned that cryptolepis/cryptolepine may be more effective in the management of androgen sensitive prostate cancers in men either as monotherapy or in combination with other anticancer agents.

Cryptolepis and cryptolepine may also be beneficial in other forms of prostate disorders particularly those that are non-androgen dependent. There is considerable experimental and epidemiological data suggesting the involvement of obesity, insulin resistance, hyperinsulinaemia, hyperlipidemia in the pathogenesis of Benign Prostatic Hyperplasia (BPH).[71--74] Many risk factors for BPH such as insulin, insulin like growth factors (IGFs), and dyslipidaemia might act through androgen-independent mechanisms.[75-78]

Insulin-resistance generally precedes Type 2 diabetes by 10 to 20 years[79] and is associated with a group of disorders such as obesity, dyslipidemia, hyperglycaemia, hyperinsulinemia and hypertension. Recently it has been reported that the higher serum LDL is associated with greater risk of BPH [73] and might be one of the important risk factors in the pathogenesis of BPH.[80] Report by Luo *et al.,* 1998 [81] showed that cryptolepis/cryptolepine caused significant decrease in plasma glucose when given orally to a mouse model of diabetes. Traditionally, cryptolepis is used for such purposes. This reduction in glucose consequently leads to significant decline in plasma insulin concentration in hyperinsulinaemia.

Cryptolepis/cryptolepine can interact with insulin and COX-2 in managing BPH. IGFs, especially IGF-II, are directly involved in tumorigenesis.[82] IGF-II exerts its mitogenic activity through the insulin-like growth factor Type 1 IGF receptor (IGF-IR) [83] and activation of IGF-IR up-regulates phosphatidylinositol 3-kinase/AKT-kinase (PI3k/AKT) signaling and increases proliferation and survival. Among mechanisms regulating IGF-IR expression, the PI3k/Akt pathway plays a crucial role in its expression.[84]

COX-2 activates the Akt pathway through PGE_2 in colon cancer cells [53], indicating that COX-2/PGE_2 is involved in IGF-IR expression and subsequent inhibition leads to decrease in tumorigenesis.

Cryptolepis and resistance to chemotherapy.

Resistance during chemotherapy is one of the main problems encountered in cancer treatment. This has led to the use of more than one anticancer agent for cancer management. This practice increases the side effects as well as the cost of chemotherapy. Laryea *et al.*, (2009)[7] reported that cryptolepine was largely insensitive to established anti-cancer drug resistance mechanisms in preclinical studies. It has been hypothesized that tumors with constitutive NF-kB activation usually show increased resistance to chemotherapy and NF-kB may be responsible for blocking the efficacy of chemotherapy and radiation in some types of tumor cells.[85]

One of these target proteins is the FLICE-like inhibitory protein (FLIP) which shares a high degree of homology with caspase- 8 but lacks protease activity. FLIP competes with caspase-8 for the binding to the Death-Inducing Signaling Complex and FLIP expression may explain the resistance to death receptor apoptosis in some types of tumors.[86-88]It has been demonstrated that inhibition of NF-kB improves the apoptotic response to radiation therapy and chemotherapy.[89] It has been suggested that NF-kB may induce the expression of the multidrug resistance P-glycoprotein. It is probable that the insensitivity of cryptolepine to established anti-cancer, antiplasmodial and antimicrobial mechanisms and its activity against chloroquin resistant falciparium[7,90,91] is due to its effects on NF-kB and its interaction with multidrug resistance P-glycoprotein. This raises the possibility that cryptolepine may enhance sensitivity of cancer cells to chemotherapy and radiotherapy.

Low mutagenicity of cryptolepis/cryptolepine.

Contrary to the mutagenic and genotoxic effects associated with many anticancer agents and cytotoxics, chronic cryptolepis usage may offer chemoprotection against the development of some cancers such as breast, prostate and colorectal cancers. In *in vivo* and *in vitro* models, cryptolepis/cryptolepine exhibits low mutagenic potential.[12,13] This appears to be closely linked to chemoprotective effects on carcinogenesis and mutagenesis exhibited by anti-inflammatory agents.[49,50] Several mechanisms employed by mutagens are also targets for cryptolepis.

It appears that the genesis of mutagenesis is alterations in essential DNA processes, which do not overwhelm the cell to cause death, but rather stimulate the initiation of repair of DNA aberrations in surviving populations [92-96]. NF-kB upregulates genes such as COX-2, iNOS [97-101] and other non NF-kB targets such as topoisomerase II and the multiple pathways either singly or synergistically promote mutagenesis. Interestingly, cryptolepis/cryptolepine inhibits most of the pathways needed for neoplastic and metastatic transformation.

Potential limitations of cryptolepis/cryptolepine as novel anticancer agents

Despite its promising activity in preclinical and *in vitro* studies, cryptolepine has not left the confinement of the laboratory and has never entered any clinical study for assessment of its potential in cancer treatment. This may be related to the notion that anticancer agents that target DNA are relatively non-selective as both normal and tumour cells could be affected. There have been many structural modifications on the parent molecule in many laboratories to reduce its DNA intercalation and potential toxicity. It is thought that in some forms of cancers, local application and tumour targeted delivery of cryptolepine may be beneficial and may minimize systemic toxicity.

In recent times, there has been considerable interest in the use of cryptolepis rather than cryptolepine. In our laboratory, a thin layer chromatogram of cryptolepis shows only cryptolepine as the major constituent suggesting that other components may be minor in relation to cryptolepine.[69] Cryptolepis is used traditionally for the management of several diseases and has recently undergone a clinical study in Ghana as an antimalarial.[102] It is relatively safe with its LD_{50} estimated to be above 5000 mg/kg in rats.[103] It is possible that other minor components may modulate the effects of cryptolepine in the aqueous extract and that property may be lost when cryptolepine is used as a pure compound.

Potential effect of cryptolepis/cryptolepine on the renal system

Cryptolepis/cryptolepine is anti-inflammatory and cytotoxic with the inhibition of COX-2 and NF-kB as principal mechanisms. We hypothesized that the kidneys could be a principal target for cryptolepine cytotoxicity as renal failure is a common complication of NSAIDs abuse[104, 105] and cancer chemotherapy.

In our laboratory, we noticed that cryptolepis at a dose beyond 100 mg/kg; *p.o* progressively induced an apoptotic-like cell death in the kidneys of mice. [69] The process begins with condensation of groups of cells to form a multinucleated cell in tissues of the renal cortex and parts of the medullary regions. These cells pick up stain and appear dark, an indication of a condensation

of their nuclear material. Many groups of cells undergo this process in the renal cortex but the surrounding tissues remain undisturbed. The bulk of the damage occurred in the renal cortex but major necrotic lesions were observed in the medulla.

To the best of our knowledge, this is the first demonstration of apoptosis induced by aqueous *Cryptolepis sanguinolenta in vivo*.

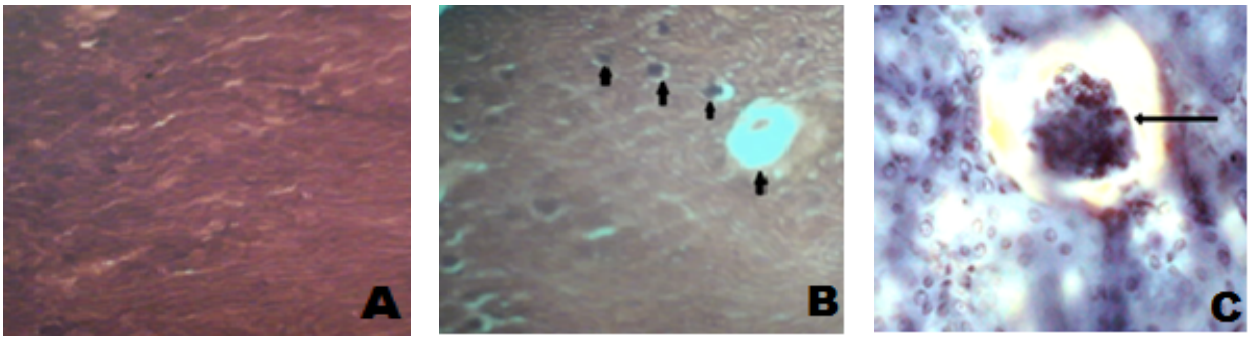

Figure 2 Induction of apoptotic-like cells in the renal cortex of mice treated with cryptolepis. (A) represents photomicrograph of the renal cortex of untreated mouse. (B) represents the renal cortex of mice after 14 days treatment with cryptolepis (100 mg/kg; *p. o*). Arrows show systematic renal damage by cryptolepis: condensation of groups of cells and renal lesions. (C) is a renal interstitium of mice showing a multinucleated cell after 14-day treatment with cryptolepis (100 mg/kg *p.o)*

The reason for the present observation is that prostaglandins are essential for renal function. The kidney synthesizes prostaglandins from different locations, including afferent and efferent arteriole, glomeruli, and tubular system. It is highly possible that this effect of cryptolepis may be related to its inhibition of the activity of prostaglandins. COX-2 null mice develop severe nephropathy.[59,106] In humans, selective COX-2 inhibition causes sodium and water retention and transient decrease in glomerular filtration rate.[104,107]

CONCLUSION

Overall, this review has shown that the cytotoxicity and anti-inflammatory activity of cryptolepis/cryptolepine are closely linked and are likely to be mediated by interference with NF-kB activity leading to down-regulation of inflammatory and anti-apoptotic genes such COX-2, iNOS, TNFa, Bcl-2 and up-regulation of pro-apoptotic genes such p53, p21, Bax, caspase and cytochrome C. Interference of NF-kB activity downstream by cryptolepis/cryptolepine may be exploited in cancer treatment or to enhance sensitivity of cancer cells to chemotherapy and radiotherapy. The properties of the agents clearly suggest that they have prospects as anticancer agents particularly in breast and prostate cancers.

REFERENCES

1. Boye, G.L., and Ampofo,O. Clinical uses of Cryptolepis sanguinolenta (Asclepiadaceae). In, Boakye Yiadom, K., & Bamgbose S. O. A. (ed*). Proceedings of the first International symposium on cryptolepine*, 1983; Abstr 4. University of Science and Technology Kumasi Ghana.
2. Bamgbose, S.O., and Noamesi, B.K.Preliminary report of some pharmacological actions of cryptolepine, *7th International Congress Pharmacology (Tokyo, Japan)*. 1978 Abstr 1289.
3. Boakye –Yiadom, K.Antimicrobial properties of some west African medicinal plants II. Antimicrobial activity of aqueous extracts of Cryptolepis Sanguinolenta (Lindl.) Schlechter. QuartJ *Crude Drug Res* 1979;17: 78-80
4. Ansah, C., and Gooderham, N.J. The Popular herbal, antimalarial, Extract of Cryptolepis is potentially cytotoxic. *Toxicol Sci*.2002;70:245-251.
5. Zhu, H., and Gooderham, N.J. Mechanisms of Induction of Cell Cycle Arrest and Cell Death by Cryptolepine in Human Lung Adenocarcinoma A549 Cells. *Toxicol Sci*. 2006;91: 132-139.
6. Ansah, C., and Gooderham, N.J.Cryptolepine provokes changes in the expression of cell cycle proteins in growing cells. *Am J Pharmacol Toxicol*. 2009; 4: 177-185.
7. Laryea, D., Isaksson, A., Wright, C.W., Larsson, R., and Nygren, P. Characterization of the cytotoxic activity of the indoloquinoline alkaloid cryptolepine in human tumour cell lines and primary cultures of tumour cells from patients. *Invest New Drugs* 2009;27: 402–411.
8. Wright, C.W., Phillipson, J.D., Awe, S.O., Kirby, G.C., Warhurst, D.C., Quertin-Leclerq,

J., Angenot, L. Antimalarial activity of cryptolepine and some other anhydronium bases. *Phytother Res* 1996;10:361-363

9. Lisgarten, J.N., Coll, M., Portugal, J., Wright, C.W., and Aymani, J. The anti-malarial and cytotoxic drug Cryptolepine intercalates into DNA at cytosine-cytosine sites. *Nat Struct Biol* 2001;9:57-60.
10. Bonjean, K., De Pauw-Gillet, M.C., Defresne, M.C., Colson, P., Houssier, C., Dassonneville, L., Baily, C., Greimers, R., Wright, C., Quetin-Leclercq, J., et al. The DNA intercalating alkaloid cryptolepine interferes with topoisomerase II and inhibits primarily DNA synthesis in B16 melanoma cells. *Biochemistry* 1998; 37:5136-5146.
11. Kluza, J., Lansiaux, A., Wattez, N., Mahieu, C., Osheroff, N., Bailly, C. Apoptotic Response of HL-60 Human Leukemia Cells to the Antitumor Drug TAS-103. *Cancer Res.*2000; 6: 4077
12. Ansah, C., Khan, A., and Gooderham, N.J. *In vitro* genotoxicity of the West African anti-malarial herbal Cryptolepis sanguinolenta and its major alkaloid cryptolepine. *Toxicology*; 2005*:*208,141-147.
13. Ansah, C., Mensah, K. B. Woode, E. and Duweijua M. Reproductive and developmental toxicity of Cryptolepis sanguinolenta in mice. *Res J Pharmacol* 2010;4(1):9-14.
14. Li H.L., Zhang, H.W.,and Ren, X.D. Synergism between heparin and adriamycin on cell proliferation and apoptosis in human nasopharyngeal carcinoma CNE2 cells. *Acta Pharmacol Sin* 2002;23:167-172
15. Li H.L., Ye, K.H.,and Ren, X.D. Heparin induced apoptosis in human nasopharyngeal carcinoma CNE2 cells. *Cell Res* 2002;11:311-315
16. Jayaraman, J. and Prives, C. Activation of p53 sequence specific DNA binding by short single strands of DNA requires the p53 C-terminus. *Cell*. 1995;81:1021–1029
17. Huang, L.C., Clarkin, K..C., and Wahl, G.M. Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. *Proc. Natl. Acad. Sci. U.S.A*. 1996;93:4827–4832.
18. Cox, S.L. Multiple pathways control cell growth and transformation: overlapping and independent activities of p53 and p21Cip1/WAF1/Sdi1. *J Pathol* 1997;183:134–140
19. Cohen, G.M. Caspases: the executioners of apoptosis. *Biochem J* 1997;326: 1-6
20. Medzhitov, R.Origin and physiological roles of inflammation. *Nature.*2008;454:428–435.
21. Schetter, A.J., Heegaard, H.H.N., and Harris C.C. Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. *Carcinogenesis* 2010;31:37–49
22. Cuzick, J., Otto, F., Baron J.A., Brown, P.H., Burn, J., Greenwald, P.,Jankowski, J., La Vecchia, C. Meyskens, F., Jörg Senn, H. Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. *Lancet Oncol*. 2009;10:501–507.
23. Goodwin, J.S., and Ceuppens, J. Regulation of immune response by prostaglandins. J *Clin Immunol* 1983;3:295–314.
24. Qiao, L., Kozoni, V., Tsioulias, G.J., Koutsos, M.I., Hanif, R., Shiff, S.J., and Rigas, B. Selected eicosanoids increase the proliferation rate of human colon carcinoma cell lines and mouse colonocytes in vivo. *Biochim Biophys Acta* 1995;1258: 215–223.
25. Tsujii, M., and DuBois, R.N. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 1995;83: 493–501.
26. Xie, W. and Herschman, H.R. v-src induces prostaglandin synthase-2 gene expression by activation of the c-Jun N-terminal kinase and c-Jun transcription factor. *J Biol Chem* 1995; 270:27622–27628.
27. DuBois, R.N., Radhika, A., Reddy, B.S., and Entingh, A.J. Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. *Gastroenterology* 1996;110:1259–1262.
28. Muller-Decker, K., Kopp-Schneider, A., Marks, F., Seibert, K., and Furstenberger, G. Localization of prostaglandin H synthase isozymes in murine epidermal tumors: suppression of skin tumor promotion by inhibition of prostaglandin H synthase-2. *Mol Carcinogen* 1998;23: 36–44.
29. Nguyen, T., Brunson, D., Crespi, C.L., Penman, B.W., Wishnok, J.S., and Tannenbaum, S.R. DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc Natl Acad Sci USA* 1992;89:3030–3034.
30. Ohshima, H., and Bartsch, H.Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat Res*.1994;305, 253–264.

31. Maeda, H., and Akaike, T. Nitric oxide and oxygen radicals in infection, inflammation, and cancer. *Biochemistry* 1998;63:854–865.
32. Wink, D., Vodovotz, Y., Laval, J., Laval, F., Dewhirst, M.W., and Mitchell, J.B. The multifaceted roles of nitric oxide in cancer. *Carcinogenesis* 1998;19:711–721.
33. Zhuang, J.C., Lin, C., and Wogan, G.N. Mutagenesis associated with nitric oxide production in macrophages. *Proc Natl Acad Sci USA* 1998;95: 8286–8291
34. Salvemini, D., Seibert, K., and Marino, M.H. New concepts in inflammation and therapy. *Drug News Perspect* 1996;9: 204–219.
35. Barnes, P.J.,and Karin, M. Nuclear factor-kB, a pivotal transcription factor in chronic inflammatory disease. *New Eng J Med* 1997;336: 1066-1071.
36. Huang, S., Li, J.Y., Wu, J., Meng, L., Shou, C.C. Mycoplasma infections and different human carcinomas. *World J Gastroenterol* 2001;7:266-269
37. Posadas, I., Terencio, M.C., Guillen, I., Ferrandiz, M.L., Coloma, J., Paya, M., and Alcaraz, M.J. Co-regulation between cyclooxygenase-2 and inducible nitric oxide synthase expression in the time-course of murine inflammation. Naunyn-Schmiedeberg's *Arch Pharmacol* 2000;361: 98–106.
38. Salvemini, D., Manning, P.T., Zeifel, B.S., Seibert, K., Curie, M.G., Needleman, P., and Masferrer, J.L. Dual inhibition of nitric oxide and prostaglandin production contributes to the anti-inflammatory properties of nitric oxide synthase inhibitors. *J Clin Invest.1995*;96: 301–308.
39. Tetsuka, T., Baier, L.D., and Morrison, A.R. Antioxidants inhibit interleukin-1-induced cyclooxygenase and nitric oxide synthase expression in rat mesangial cells. *J Biol Chem* 1996;271: 11689 – 11693.
40. Takahashi, M., Mutoh, M., Kawamori, T., Sugimura, T., and Wakabayashi, K. Altered expression of catenin, inducible nitric oxide synthase and cyclooxygenase-2 in azoxymethane-induced rat colon carcinogenesis. *Carcinogenesis* 2000 ;21: 1319–1327.
41. Kipp, E. and Ghosh, S. Inhibition of NF-kB by sodium salicylate and aspirin. *Science* 1994; 265: 956-959
42. Ghosh, S,and Karin, M. Missing pieces in the NF-kappaB puzzle. *Cell*.2002;109:S81-S96.
43. Helbig, G., Christopherson, K.W., Bhat-Nakshatri, P., Kumar, S., Kishimoto, H., Miller, K.D. Broxmeyer, H. E., and Nakshatri, H. NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. *J. Biol. Chem* 2003;278:21631–21638.
44. Bamgbose, S.O., and Noamesi, B.K.Studies On Cryptolepine II: Inhibition of Carrageenan-Induced Oedema by Cryptolepine. *Planta Med* 1981;41:392–396.
45. Olajide, O.A., Pinheiro de Oliveira, A., Unekwe, J., Wright, C., and Fiebich, B. Cryptolepis sanguinolenta (Lindl.) Schltr. root extract inhibits prostaglandin production in IL-1b stimulated SK-N-SH neuronal cells. *Planta Med* 2010;76:601.
46. Olajide, O.A., Ajayi, A.M., and Wright, C.W. Anti-inflammatory Properties of Cryptolepine. *Phytother Res*.2009; 23:1421-1425.
47. Olajide, O.A., Heiss, E.H., Schachner, D., Wright, C.W., Vollmar, A.M., and Dirsch, V.M. Synthetic cryptolepine inhibits DNA binding of NF-kappaB. *Bioorg Med Chem* 2007; 15:43-49.
48. Olajide, O.A., Wright, C.W., and Fiebich, B.L. Effects of Cryptolepis sanguinoleta root extract in lipopolysaccharide – stimulated human primary monocytes. *Planta Med* 2007a; 73: 077.
49. Thun, M.J., Namboodiri, M.M., and Heath, C.W. Aspirin use and reduced risk of fatal colon cancer. *N Engl J Med*.1991; 325:1593–1596.
50. Kawamori, T., Rao, C.V., Seibert, K., and Reddy, B.S. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. *Cancer Res*.1998; 58:409 – 412.
51. Eberhart, C.E., Coffey, R.J., Radhika, A., Giardiello, F.M., Ferrenbach, S., and Dubois, R.N. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994;107: 1183–1188
52. Kargman, S. L., O'Neill, G.P., Vickers, P.J., Evans, J.F., Mancini, J.A., and Jothy, S. A. Expression of prostaglandin G/H synthase-1 and -2 protein. *Cancer Res*.1995;55: 2556–2559.
53. Sheng, H., Shao, J., Washington, M.K., and Dubois, R.N.Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. *J. Biol. Chem*.2001;276: 18075–18081,
54. Williams, C.S., Mann, M., and DuBois, R.N. The role of cyclooxygenases in inflammation,

cancer, and development. *Oncogene*.1999;18: 7908–7916.
55. Ko, L.J., and Prives, C. p53: Puzzle and paradigm. *Genes Dev*.1996;10:1054–1072.
56. Giaccia, A.J. and Kastan, M.B. The complexity of p53 modulation: Emerging patterns from divergent signals. *Genes Dev*.1998;12:2973–2983.
57. Giardina, C., Boulares, H., Inan, M.S. NSAIDs and butyrate sensitize a human colorectal cancer cell line to TNF and Fas ligation: the role of reactive oxygen species. *Biochim Biophys Acta* 1999;1448: 425-438
58. Deveraux,Q.L., Roy, N., Stennicke, H.R., Van Arsdale, T., Zhou, Q., Srinivasula, S.M., Alnemri, E.S., Salvesen, G.S., Reed, J.C. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J* 1998;17: 2215–2223
59. Wang, C.Y., Mayo, M.W., Korneluk, R.G., Goeddel, D.V., Baldwin, A.S. Jr. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* 1998;281:1680–1683
60. Takahashi, R., Deveraux, Q., Tamm, I., Welsh, K., Assa-Munt, N., Salvesen, G.S., Reed, J.C. A single BIR domain of XIAP sufficient for inhibiting caspases. *J Biol Chem* 1998;273:7787–7790
61. Lee, J.U., Hosotani, R., Wada, M., Doi, R., Kosiba, T., Fujimoto, K., Miyamoto, Y., Tsuji, S., Nakajima, S., Nishimura, Y., Imamura, M. Role of Bcl-2 family proteins (Bax, Bcl-2 and Bcl-X) on cellular susceptibility to radiation in pancreatic cancer cells. *Eur J Cancer* 1999; 35:1374–1380
62. Chen, Q.M., and Tu, V.C. Apoptosis and heart failure: mechanisms and therapeutic implications. *Am J Cardiovasc Drugs* 2002;2:43–57
63. Wu,Y.L., Sun, B., Zhang, X.J., Wang, S.N., He, H.Y., Qiao, M.M., Zhong, J.,Xu, J.Y. Growth inhibition and apoptosis induction of Sulindac on human gastric cancer cells. *World J Gastroenterol*.2001;7:796-800
64. Bash, J., Zong, W.X., and Gelinas,C. Molc-Rel arrests the proliferation of HeLa cells and affects critical regulators of the G1/S-phase transition. *Mol. Cell. Biol*. 1997;17: 6526–6536.
65. Perkins, N.D., Felzien, L.K., Betts, J.C., Leung, K., Beach, D.H., and Nabel, G.J. Regulation of NF-κB by Cyclin-Dependent Kinases Associated with the p300 Coactivator. *Science* 1997;275:523–527
66. Karin, M.,and Lin, A. NF-kappaB at the crossroads of life and death. *Nat. Immunol.* 2002; 3:221-227.
67. Karin, M., Luo, J., and Kamata, H. IKK/NF-κB signaling: balancing life and death – a new approach to cancer therapy. *J Clin Invest*.2005;115(10):2625–2632
68. Yu, Q., Geng, Y., and Sicinski, P. Specific protection against breast cancers by cyclin D1 ablation. *Nature*.2001;411:1017–1021
69. Mensah K.B. Reproductive and developmental toxicity of the aqueous root extract of the antimalarial herbal Cryptolepis sanguinolenta (LINDL.) SCHLTR (Periplocaceae) in experimental animals. (PhD Thesis 2011)
70. Marker, P.C., Donjacour, A.A., Dahiya, R., Cunha, G.R. Hormonal, cellular, and molecular control of prostatic development. *Dev. Biol*. 2003; 253, 165–174
71. Nandeesha, H. Benign prostatic hyperplasia: dietary and metabolic risk factors. *Int. Urol. Nephrol.* 2008;40:649–656.
72. Nandeesha, H., Koner, B.C., Dorairajan, L.N., Sen, S.K. Hyperinsulinemia and dyslipidemia in non-diabetic benign prostatic hyperplasia. *Clin. Chim. Acta* 2006;370:89–93.
73. Parsons, J.K., Bergstrom, J., and Barrett-Connor, E. Lipids, lipoproteins and the risk of benign prostatic hyperplasia in community-dwelling men. *BJU Int*. 2008;101: 313–318.
74. Parsons, J.K., Sarma, A.V., McVary, K., Wei, J.T. Obesity and benign prostatic hyperplasia: clinical connections, emerging etiological paradigms and future directions. *J.Urol*.2009; 182:S27–S31.
75. Vikram, A., Jena G,and Ramarao, P. Insulin-resistance and benign prostatic hyperplasia: The connection. *Eur J Pharmacol* 2010;641:75–81.
76. Peehl, D.M., Cohen, P., Rosenfeld, R.G.The insulin-like growth factor system in the prostate. *World J. Urol*. 1995;13:306–311.
77. Lucia, M.S., Lambert, J.R.Growth factors in benign prostatic hyperplasia: basic science implications. *Curr. Urol. Rep.* 2008;9:272–278.
78. Ikeda, K., Wada, Y., Foster Jr., H.E., Wang, Z., Weiss, R.M., Latifpour, J. Experimental diabetes-induced regression of the rat prostate is associated with an increased expression of transforming growth factor-beta. *J. Urol*. 2000;164:180–185.

79. Riccardi, G., Giacco, R., Rivellese, A.A. Dietary fat, insulin sensitivity and the metabolic syndrome. *Clin. Nutr*. 2004; 23:447–456.
80. Li, P.J., Zhang, X.H., Guo, L.J., Na, Y.Q. Correlation of benign prostatic hyperplasia with hyperlipemia. *Zhonghua Wai Ke Za Zhi* 2005;43:387–389
81. 81. Luo, J., Fort, D.M., Carlson, T.J., Noemesi, B.K., Nii-Amon-Kotei, D., King, S.R., Tsai, J., Quan, J., Hobensack, C., Lapresca, P*., et al.* Cryptolepis sanguinolenta:an ethnobotanical approach to drug discovery and the isolation of a potentially useful new antihyperglycaemic agent. *Diabet Med* 1998 15: 367-374.
82. Werner, H., and LeRoith, D. The role of the insulin-growth factor system in human cancer. *Adv. Cancer Res*.1996;68: 183–222
83. Humbel, R. E. Insulin-like growth factors I and II. *Eur. J. Biochem*. 1990;190: 445–462.
84. Tanno, S., Tanno, S., Mitsuuchi, Y., Altomare, D.A., Xiao, G.H., and Testa, J.R.Akt activation up-regulates insulin-like growth factor I receptor expression and promotes invasiveness of human pancreatic cancer cells. *Cancer Res.*2001;61: 589–59.
85. Matias-Guiu, Dolcet, X., Llobet, D., Pallares, J. NF-kB in development and progression of human cancer. *Virchows Arch*. 2005;446: 475–482
86. Panka, D.J., Mano, T., Suhara, T., Walsh, K., Mier, J.W. Phosphatidylinositol 3-kinase/Akt activity regulates c-FLIP expression in tumor cells. *J Biol Chem*. 2001; 276:6893–6896
87. Bullani, R.R., Huard, B., Viard-Leveugle, I., Byers, H.R., Irmler, M., Saurat, J.H, Tschopp, J., French, L.E.Selective expression of FLIP in malignant melanocytic skin lesions. *J Invest Dermatol* 2001;117:360–364
88. Olsson, A, Diaz, T., Aguilar-Santelises, M., Osterborg, A., Celsing, F., Jondal, M., Osorio, L.M. Sensitization to TRAIL induced apoptosis and modulation of FLICE-inhibitory protein in B chronic lymphocytic leukemia by actinomycin D. *Leukemia* 2001;15:1868–18
89. Shen, H.M. and Tergaonkar,V. NFkappaB signaling in carcinogenesis and as a potential molecular target for cancer therapy. *Apoptosis* 2009;14:348–363.
90. Cimanga, K., De Bruyne, T., Pieters, L., Vlietinck, A.J., Turger, C.A. In vitro and in vivo antiplasmodial activity of cryptolepine and related alkaloids from Cryptolepis sanguinolenta. *J Nat Prod* 1997; 60:688-691
91. Kirby, G.C., Paine, A., Warhurst, D.C., Noamesi, B.K., Phillipson, J.D. In vitro and in vivo antimalarial activity of cryptolepine, a plant-derived indoloquinoline. *Phytother Res* 1995; 9: 359-363
92. Baguley, B.C., and Ferguson, L.R. (1998. Mutagenic properties of topoisomerase-targeted drugs. *Biochim Biophys Acta* 1998; 1400: 213–222.
93. Fortune, J.M., and Osheroff, N. Topoisomerase II as a target for anticancer drugs: when enzymes stop being nice,. *Prog Nucleic Acid Res Mol Biol* 2000; 64: 221–253.
94. Kaufmann, S.H. Cell death induced by topoisomerase-targeted drugs: more questions than answers. *Biochim Biophys Acta* 1998; 1400: 195–211.
95. Wilstermann, A.M., and Osheroff, N. Stabilization of eukaryotic topoisomerase II–DNA cleavage complexes. *Curr Top MedChem* 2003; 3: 1349–1364.
96. Walker, J.V., and Nitiss, J.L. DNA topoisomerase II as a target for cancer chemotherapy. *Cancer Invest* 2002; 20: 570–589.
97. Nguyen, T., Brunson, D., Crespi, C L., Penman, B W., Wishnok, J. S,. Tannenbaum, S. R. DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc. Natl. Acad. Sci. U.S.A.*1992; 89: 3030–3034
98. Ohshima, H., & Bartsch, H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis, *Mutat. Res*.1994; 305 : 253–264
99. Wink, D., Vodovotz, Y., Laval, J., Laval, F., Dewhirst, M. W., Mitchell, J. B. The multifaceted roles of nitric oxide in cancer*, Carcinogenesis* 1998;19: 711–721
100. Zhuang, J. C., Lin, C., Wogan, G. N. Mutagenesis associated with nitric oxide production in macrophages, *Proc. Natl. Acad. Sci.* U.S.A.1998;95 : 8286–8291.
101. Maeda, H., & Akaike, T. Nitric oxide and oxygen radicals in infection, inflammation, and cancer*, Biochemistry* 1998; 63 :854–865
102. Bugyei, K.A., Boye, G.L., and.Addy, M.E. Clinical efficacy of a tea-bag formulation of *Cryptolepis sanguinolenta* root in the treatment of acute uncomplicated falciparum malaria. *Gh. Med. J*. 2010;1(44): 3-9
103. Ansah, C., Mfoafo, E.A., Woode, E., Opoku-Okrah, C., Owiredu, W.K.B.A, and Duwiejua, M. Toxicological evaluation of the antimalarialherb *Cryptolepis sanguinolenta* in

rodents. *J. Pharmacol. Toxicol.*2008;3: 335-343

104. Komhoff, M., Grone, H.J., Klein, T., Seyberth, H.W., and Nusing, R.M. Localization of cyclooxgenase-1 and 2 in adult and fetal human kidney: implication for renal function,. *Am J Physiol* 1997.
105. Perazella, M.A.COX-2 inhibitors and the kidney. *Hospital Practice* 2001;3,43–56.
106. Williams, C.S., Mann, M., and DuBois, R.N. The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene*.1999;18:7908–7916.
107. Rossat, J., Maillard, M., Nussberger, J., Nrunner, H.R., and Burnier, M. Renal effects of selective cyclooxgenase-2 inhibitor in normotensive salt-depleted subjects. *Clin Pharmacol Ther* 1999;66: 76–84✪