DOI: 10.21276/sajp

Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2017; 6(6): 236-244 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Review Article

Gossypol: a potential promising anticancer agent

MUBANO Olivier Clément *
School of Pharmaceutical Science, Jiangnan University, Jiangsu Province, P.R. China

***Corresponding author**
MUBANO Olivier Clément
Email: muboc@yahoo.fr

Abstract: Despite the enormous amount of research and rapid developments seen during the past decades, cancer continues to be a killer and a major public health problem worldwide. Gossypol, a polyphenolic compound derived from cottonseeds, has been the target of many researches due to its multifaceted biological activities such as antifertility, antivirus, antioxidant, antitryphagosomal, antimicrobial, antimalarial and most importantly anticancer activities. In present review we summarized the results of studies obtained in a comprehensive search of Science Direct, PubMed, Scirus, Springer LINK E-books and Web of Science. Basing on its mechanism of action and the synergy with other anticancer agents we found gossypol to be a very good promising chemotherapeutic agent as it has shown its potentiality *in vitro*, *in vivo* using animal models and in clinical trials against several different types of cancer.
Keywords: Gossypol, Cancer; Synergy, Apoptosis, Nanocarrier.

INTRODUCTION

There has been a surge interest in gossypol since it has shown its therapeutic potential both *in vivo* and *in vitro*[1]. Gossypol is a polyphenolic compound extracted from the cotton plant (genus *Gossypium*) and *Thespesia populnea*, both members of the family Malvaceae[2]. It is mainly embedded in the cottonseed pigment glands, it occupy 20–40% of the gland weight and 0.4–1.7% of the whole kernel; but it can be found into the other parts of plant such as leaves, bark of roots, seed hulls, and flowers. Inside the plant gossypol is produced via dimerization of two molecules of hemi gossypol, from where is classified as dimeric-sesquiterpenoid. Sesquiterpenoids are terpenes with three isoprene units that protect a plant from pathogens and insects[3]. Historically gossypol is known since 1886 as a crude pigment from cottonseed oil foot [4], its formula $C_{30}H_{30}O_8$ was established forty one years later in 1927[5], while in 1958 its complete structure was verified as 1,10, 6,60, 7,70-hexahydroxy-3,30-dimethyl-5,50-diisopropyl-2,20-binaphthyl-8,80-dialdehyde [6] [Fig.1]. Due to its six hydroxyl and two aldehydic groups, it is soluble in numerous organic solvents such as dimethyl Sulfoxide, acetone, methanol, ethanol, isopropanol, butanol, ethylene glycol, dioxane, diethyl ether, chloroform, phenol, pyridine, melted naphthalene, and heated vegetable oil; but it is insoluble in water because of the presence of two heavy dialkyl naphthalene groups[7].

Gossypol exists in (+) and (-) enantiomers due to restricted rotation of its internaphthyl bond[8] [Fig.2] ,thus its structure consists of two naphthalene rings joined by a single internaphthyl bond between the 2- and 20-carbon atoms. Therefore**,** it is chemically reactive because of the reactivity of carbonyl and phenolic hydroxyl groups as well as its bulky binaphthalene structure, but also by the presence of two aldehyde groups. This reactivity contributed to its several biological activities such as antimicrobial activities, essentially *Staphylococcus aureus, Sarcina lutea, Escherichia coli, Candida utilis* and *Saccharomyces cerevisiae* (9); antiretroviral activities such as anti HIV-1[10, 11], it presents inhibitory effect on some arboviruses such as Sindbis virus, West Nile fever virus, Japanese encephalitis virus, and the virus of tick-borne encephalitis [12], it has stopped influenza, parinfluenza-3 [13], and also *herpes simplex virus* [14].In addition, it presents antifertility, antiparasitic protozoan activities and finally anticancer activity(6). Along this review we are going to provide an overview about cancer treatment and deeply describe the potential anticancer activity of gossypol by showing how much it is highly promising chemotherapeutic agent.

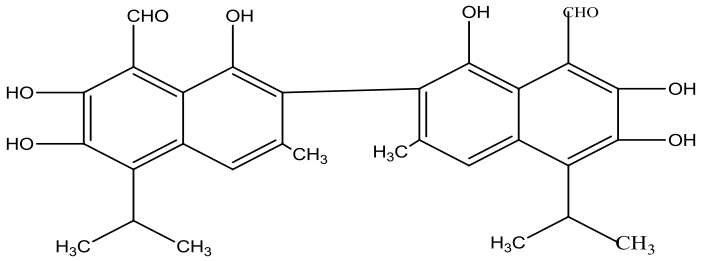
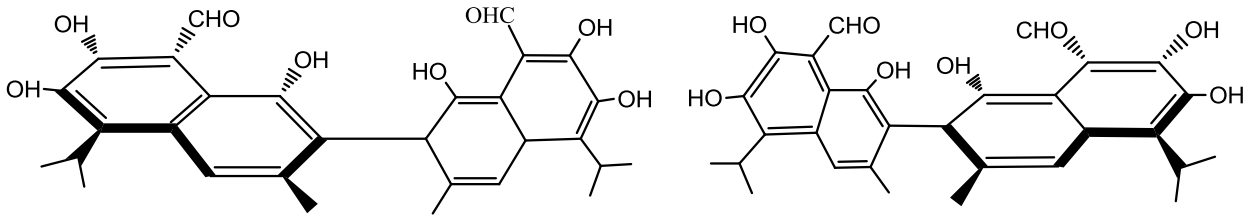


Fig 1: Chemical structure of gossypol



(+)-GOSSYPOL (-)-GOSSYPOL

Fig 2: Structure of gossypol enantiomers

Overview on cancer

Cancer is the name given to a collection of related diseases. There are more than one hundred different types of cancers and their name are derived from the organ or type of cell in which it starts, for example cancer that starts from breast is called breast cancer, the one which starts from prostate is called prostate cancer. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place; [*Cancer Fact sheet N°297". World Health Organization. February 2014*] when cancer develops, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive instead of dying, consequently new cells are formed when they are not needed. These extra cells can divide without stopping and may form growths called tumors, even though there are some kinds of cancer which don't form tumors such as blood cancer. Cancerous tumor can spread and invade the tissue around; but also cancer cells can travel beyond nearby tissue to distant place via blood or lymph system to grow new tumors. (*Fact sheet: cancer; available on http://www.who.int/mediacentre/factsheets/fs297/en/*).

Cancer continues to be a killer and major public health problem worldwide, despite the enormous amount of research and rapid developments seen during the past decade. According to recent statistics, in 2017, 1,688,780 new cancer cases and 600,920 cancer deaths are projected to occur in the United States (*Cancer statistics, 2017; available on www.ncbi.nlm.nih.gov/labs/articles/28055103/*). According to GLOBOCAN data, 14.1 million new cases and 8.2 million deaths from cancer were estimated in 2012 [15]. By 2020, the world population is expected to have increased to 7.5 billion; of this number, approximately 15 million new cancer cases will be diagnosed, and 12 million cancer patients will die [16]. These terrifying statistics remind us the statement of Dr. John Bailer's May 1985 from US national cancer program who recognized war against cancer as a "qualified failure" 15 years after President Nixon campaign against cancer. Even until now cancer remains a huge problem and we are still losing war against cancer even though most of the time it can be preventable by changing life style [17]; in fact, lifestyle risk factors such as tobacco use, diet and obesity, lack of exercise, alcohol consumption, and excessive exposure to sunlight; environmental and occupational exposures to carcinogens and mutagens (including chemicals and radiation); infectious agents (Helicobacter pylori, hepatitis B virus, hepatitis C virus, human papillomavirus, Epstein-Barr Virus); chronic inflammation; hormone metabolism; family history; ethnicity and socioeconomic status are frequently the cause of increasing cancer in addition to aging [Fig.3]. The American Association for Cancer Research (AACR) believes that the conquest of cancer, through further scientific progress in cancer etiology, prevention, diagnosis, treatment, and quality healthcare delivery, must become an international priority[18].

Despite huge advances in anticancer drug research, the current approach to face cancer treatment remain minimal[19]. Drugs which target only one

molecular abnormality or cancer pathways has displayed a good clinical responses that have modestly affected survival in some cancers. However, targeting a single hallmark or pathway with a single drug is not enough to treat cancer. In contrast, drug combinations against several molecular alterations or cancer hallmarks, in same way as it has been done with HIV treatment, might be a promising therapeutic strategy to treat cancer in upcoming years. Therefore new anticancer agents are warmly welcome to participate in this combinatorial treatment. It is in this framework that researchers continue to struggle in order to find additional anticancerous molecules, one of them is gossypol.

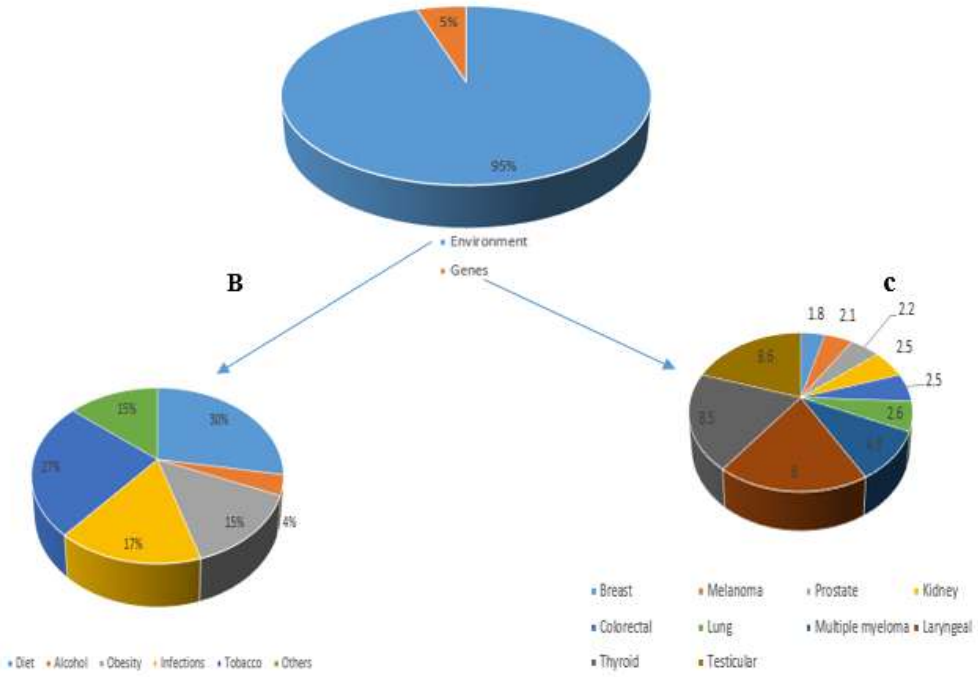


Fig 3: The role of genes and environment in the development of cancer

A The percentage contribution of genetic and environmental factors to cancer. The contribution of genetic factors and environmental factors towards cancer risk is approximately 5% and 95% respectively. **B** Percentage contribution of each environmental factor. The percentages represented here indicate the attributable-fraction of cancer deaths due to the specified environmental risk factor. **C** Family risk ratios for selected cancers. The numbers represent familial risk ratios, defined as the risk to a given type of relative of an affected individual divided by the population prevalence. The data shown here is taken from a study conducted in Utah to determine the frequency of cancer in the first-degree relatives (parents + siblings + offspring). The familial risk ratios were assessed as the ratio of the observed number of cancer cases among the first degree relatives divided by the expected number derived from the control relatives, based on the years of birth (cohort) of the case relatives. In essence, this provides an age-adjusted risk ratio to first-degree relatives of cases compared with the general population.

Anticancer activity of gossypol

Different studies have been conducted in purpose to exploit the anticancer properties of gossypol:

In vitro and mechanisms of action

Gossypol is capable of inhibiting the growth of a variety of cell lines including breast, colon, prostate, and leukemia cells [6].The table 1 highlights the IC_{50} of gossypol on different forms of human cancer lines *in vitro*. In fact, apoptosis is a highly regulated process that involves the activation of a cascade of molecular events leading to cell death, and it is characterized by cell shrinkage, membrane blebbing, chromatin condensation, and formation of a DNA ladder with multiple fragments caused by internucleosomal DNA cleavage[20]. So gossypol intervenes in apoptosis and in anti-proliferation through different ways; the inhibitory effects of gossypol on proliferation of human prostate cancer cells (PC-3) has been proven to be associated with enhancing the secretion of transforming growth factor β1 protein ($TGF\beta_1$), a negative growth regulator that regulates the expression and the functions of the cell cycle regulatory proteins , cyclin D1 and Rb, proteins involved in cell cycle progression from G1-phase to S-phase in prostate cancer cells [21]; this finding suggests that $TGF\beta_1$ is a potential physiological regulator of normal prostate cells, cancer cells and human breast cancer cells. Thus, gossypol might regulate cell cycles by modulating the expression of cell-cycle regulatory proteins Rb and cyclin D1 and the phosphorylation of Rb protein. On the other study on PC-3 cells, it was shown that gossypol can induce their apoptosis through mitochondrial signal transduction pathway by inhibiting the hetero dimerization of Bcl-XL/Bcl-2 with pro-apoptosis molecules Bak, Bax, and Bim, then followed

by a caspase-dependent and –independent procedure which involves the release of apoptosis inducing factor (AIF), a toxic protein which triggers the apoptosis cascade ,from mitochondria to cytosol [22]. Besides, gossypol-induced apoptosis in ovarian cancer cells results in cell death through oxidative stress, by enhancement of Reactive Oxygen Species (ROS) production and by induction of decreasing of cellular levels of glutathione (GSH), aspartic acid and a part of the electron transport respiratory chain (FAD) which is central to energy production) [23]. In human breast cancer cells gossypol inhibits expression of both Mouse double minute 2 (MDM2) and vascular endothelial growth factor (VEGF), important molecules involved in tumor progression (24). A mechanistic study further demonstrated that, through disrupting the interaction between MDM2 protein and VEGF mRNA, gossypol induced MDM2 self-ubiquitination and decreased VEGF translation simultaneously, which resulted in both apoptosis and anti-angiogenesis effects. Furthermore, gossypol blocks DNA synthesis in HeLa cells by inhibiting key nuclear enzymes responsible for DNA replication and repair, such as DNA polymerase α [25] and topoisomerase II [26]. The inhibition of DNA synthesis can also be achieved with 10 µM gossypol by blocking the G_1/S checkpoint in human mammary cancer cell line cells **(**MCF-7) after 24 h of incubation; this can be explained by the fact that gossypol decreases the expression level of the Rb , pRb and cyclin D_1 proteins in MCF-7 cells which are critical for G_1 to S progression [27]. At 50 µM for 6 h gossypol induces apoptosis in human promyelocytic leukemia cells (HL-60),and the truncation of Bid protein, the loss of mitochondrial membrane potential, cytochrome c release from mitochondria into cytosol, and activation of caspases-3,-8, and -9 [28].In fact, released cytochrome *c* can activate caspase-9 which in turn cleaves and activates executioner caspase-3. After caspase-3 activation, some specific substrates for caspase-3 such as poly (ADP-ribose) polymerase (PARP) are cleaved, and eventually lead to apoptosis[29].

However ,it has been found that (-)-gossypol is more active than racemic one in inhibiting activity of breast cancerous epithelial cells (cEC) and cancerous stromal cells (cSC) by reduction of cell-cycle regulator, cyclin D1,and induction of the cell proliferation inhibitor TGF-β [30]. In lymphoma cell line WSU-DLCL2 (-)-gossypol inhibits their growth by inducing complete cytochrome c release from mitochondria, increasing caspases-3 and -9 activity, and causing apoptotic death without affecting protein levels of Bcl-2, Bcl-X(L), Bax, and Bak(31); (-)-gossypol also acts as a BH3 mimetic binding to the BH3-binding domain in various proapoptotic proteins of the Bcl-2 family, displacing prodeath partners to induce apoptosis [32].

Between two enantiomers of gossypol (Fig.2), (–)-gossypol has proven to be more potent inhibitor of cancer cell growth due to the higher affinity for Bcl-2 and Bcl-XL, the anti-apoptotic proteins commonly overexpressed in cancer cells and considered as attractive therapeutic targets; hence it has mostly been attributed these anticancer effects[33].

Table 1: Gossypol activity against different type of cancer cell lines in vitro

Human cancer cell lines	**Gossypol activity IC_{50} (µM)**			**References**
	Racemic gossypol	**(-)-gossypol**	**(+)-gossypol**	
Breast				
MCF-7	24			(34)
T47-D	5	3	20	(35)
SKOV-3	5.7			(36)
Carvix				
KB-3	3.8			(37)
KB-A1	2.9			(37)
KB-V1	4			(37)
SiHa	47	30	>50	(38)
SiHa	14			(34)
Ovarian				
SKOV-3	30			(23)
OVCA-432	2.4	1.1	10.2	(39)
OVCAR-3	1.5	0.6	5.8	(39)
Prostate				
PC-3	9.6			(40)
PC-3	10			(41)
Leukemia				
HL-60	8.3			(42)
Table. 2 continued				

Human cancer cell lines	Gossypol activity IC_{50} (µM)			References
K562	15			(43)
HL-60	15			(43)
Lung				
H69	30	20	50	(38)
Pancreatic				
MiaPaC		3	20	(35)
Melanoma				
SK-MEL-19	25	20	>30	(44)
WM9	6.2	3.1	14.3	(39)
Medullary thyroid				
TT	18.9			(36)
Glioma				
HS 683	25			(45)
U373	64			(45)
U87	69			(45)
U138	97			(45)

In vivo and synergic studies

As described above, anticancer activities of gossypol has extensively exploited *in vitro*, further animal modal studies have been also conducted. For *in vivo* inhibition of proliferation of prostate cancer cell line, PC-3, Male BALB/c nude mice were inoculated with cell suspension of PC-3 cells in order to be treated by (-)-gossypol after developing tumors. The tumor volume were significantly decreasing after treatment; the concentration of 10 mg kg-1 (–)-gossypol achieved a T/C % of 39.9%, indicating that it possesses significant antitumour activity [46], according to National Cancer Institution (NCI) criteria, T/C % < 42% is considered significant antitumour activity; T/C % < 10% is considered to indicate highly significant antitumour activity [47]. Additionally, the cytotoxicity of gossypol against BRW in vivo, using the nude mouse xenograft Model was tested [45]; Given at a dose of 30 mg/kg per day five days a week for four weeks orally via gavage, was found to decrease the mean tumor weight of treated xenografts by more than 50% as compared to untreated xenografts. *Keith et al.* have shown that (-)-Gossypol inhibits growth and promotes the apoptosis of Human Head and Neck Squamous Cell Carcinoma (HNSCC) *in vivo* [48], there were no significant differences in tumor inhibition between the two dosing levels used; following drug withdrawal, there was arrest of tumor growth for 3 weeks. Therefore, the antitumor growth effect of (-)-gossypol against HNSCC tumors, although significant, was incomplete when it was used as a single agent; its combination with other standard chemotherapeutic agents may lead to a more dramatic reduction in tumors with reduced drug concentrations.

However, gossypol was used combined synergically with other anticancer agent where this combination has proven to be more active than used alone. In this purpose, (-)-Gossypol-loaded Pluronic P85 was found to be a more potent radio sensitizer *in vitro* [49]. Pluronic P85, the ABA triblock copolymers of propylene oxide (PO) and ethylene oxide (EO), has increased the anti-proliferative activity of (-)-gossypol against adeno carcinomic human alveolar basal epithelial cells (A549 cells) (82 ± 42 versus 190 ± 60 nM). In fact, the combination of P85 and (-)-gossypol effectively reduced clonogenic survival of A549 cells: (11 ± 5%) compared to (-)-gossypol and P85 alone (62 ± 27% and 93 ± 13%, respectively), and enhanced radiation cancer cell killing. *In vivo*, P85 (200 mg/kg/day) and (-)-gossypol (15 mg/kg/day) has been safely injected intravenously over 5 days and enhanced radiation-related tumor control in an A549 xenograft model. P85 alone showed little effect on tumor growth, but when combined with (-)-gossypol it exerted a considerable tumor growth delay [49]. In the same framework, Valproic Acid enhances the anti-tumor effect of (-)-gossypol to Burkitt Lymphoma Namalwa Cells [50], 2 mmol/L valproic acid and 5 µmol/L (-)-gossypol has better suppressed dramatically the proliferation of cells when used in combination than when used separately[50].

Overexpression of antiapoptotic genes, such as the Bcl-2 family play a critical role in conferring resistance to conventional anticancer therapies[51], and gossypol has revealed the ability to overcome this drug resistance to other conventional chemotherapeutic drugs [52].Therefore gossypol can be used in combination with chemotherapeutic drugs to improve the efficiency of inducing apoptosis in cancer therapy. *Cengiz E et al.* observed that the combined treatment of gossypol and docetaxel could synergistically induced apoptosis in PC-3 prostate cancer cell line[53], and *Foong et al.* revealed that therapy combination of gossypol with gemcitabine showed synergism against gemcitabine resistance in cancer cells with high BCL-2 expression [54]; these observations suggested that gossypol

reversal of gemcitabine resistance involves the downregulation of anti-apoptotic proteins (Bcl-2 and/or Bcl-xl) and upregulation of pro-apoptotic proteins (Noxa and Mcl-1S). Moreover, gossypol, paclitaxel and cyclopamine combined in Poly (ethylene glycol)-block-poly (ε-caprolactone) micelles has been delivered intraperitoneal injection into *in vivo* in xenograft models of ovarian cancer, resulting in tumor growth inhibition and prolonged survival of mice over paclitaxel alone.

Clinical trial

Even though gossypol has undergone several different anticancer tests *in vitro* and *in vivo* by resulting in satisfying outcomes, it is unfortunately not the case for clinical trials; few cases have been reported to date, may be because its toxicity resulting in lack of volunteers or because some trials which have been conducted have not delivered the expected results. In fact, when gossypol was given to patients who had histologically or cytologically confirmed to have small cell lung cancer (SCLC) and previously treated with chemotherapy regimen, at the time of planned interim evaluation, none of the 14 evaluable patients enrolled in the first stage had shown any response to therapy, and the study was closed permanently for further accrual [55]; another study was carried out to 20 patients within none among them displayed an evidence of tumor regression [56]. A possible explanation is that the agents do not reach sufficient concentration in the tumor cells to suppress the Bcl-2 pathway in vivo and the long half-life of Bcl-2 protein, the possibility of off target cytotoxic mechanisms and a failure to inhibit apoptotic induction specifically. But some studies have shown an anticancer activity even though it appeared to be minimal; *Van Poznak et al.;* have demonstrated that gossypol is safe, but with limited activity in doxorubicin and taxane refractory metastatic breast cancer [57], among 20 patients who participated in study only one has shown a small regression of disease. Although this study demonstrated only one minor response and no partial or complete responses, three patients experienced a decrease of > 50% in tumor markers serum breast cancer antigen (BR2729) and/or carcinoembryonic antigen (CEA). Furthermore, during a test on humans with metastatic adrenal cancer whose tumors were refractory to other chemotherapeutic agents gossypol has shown its power [58]; 21 patient who were receiving an oral gossypol at doses of 30-70 mg/day three out of them had partial tumor responses (> or = 50% decrease in tumor volume) that lasted from several months to over 1 year. Also another group of 27 patient with recurred glial tumors after radiation therapy were orally given 10mg of gossypol twice a day until the patient had evidence of progressive disease, two patients exhibited a partial response, one of which lasted 78 weeks while 4 of them remained stable for approximately 12 days [59].

To our knowledge neither combinatorial nor synergic study of gossypol with other chemotherapeutic agents have been conducted on level of clinical trial while, as mentioned above, it has increased both safety and efficacy *in vitro* and *in vivo*, so one can hypothesize that the same scenario can happen after clinical study. It is also noted that the research was focused only on racemic gossypol while (-)-enantiomer has shown to be of considerable therapeutic significance as it is more cytotoxic *in vitro* [6]. Therefore it will be a great significance to pursuit further clinical trials by focusing on combination of (-)-gossypol with other anticancer agents but also on using nanocarriers in purpose to increase its bioavailability and decreasing toxicity [40]. However, gossypol has not been used as an antitumor agent due to a number of limitations, including poor water solubility, single-route drug administration and low bioavailability. In addition, at high concentrations, (-)-gossypol may be highly toxic to the liver and intestinal tract [46]. So, nano technique is being applied to gossypol in order to get rid from such side effects, and the outcome is good and promising as too few related researches already done have revealed the antitumor effect of gossypol nanoparticles to be stronger than that of free gossypol [40, 60].

CONCLUSION

Gossypol has been extensively exploited for its several biological activities especially for its apoptotic and anti-proliferative effects against different cancer cell lines. The numerous studies outlined above show that gossypol has the potential for prevention and therapy of various cancer even though much more further studies are still in need to be used in clinic, mostly clinical trials which involve gossypol combined with other chemotherapeutic agents; but also it would be of great significance to use (-)-enantiomer of gossypol and its encapsulated form in nanocarriers like nano liposomes or Nano micelles to prove its antitumor potential already revealed *in vitro*. In fact, gossypol has its own potentiality to fight against cancer, thereby it deserves much more attention and interest to researchers as it may participate in eradicating or at least in decreasing the incessant deaths caused by cancer.

ACKNOWLEDGMENT

We really acknowledge Jiangnan University for its support in providing us the access on its online books and papers.

REFERENCES

1. Keshmiri-Neghab H, Goliaei B. Therapeutic potential of gossypol: an overview. Pharmaceutical biology. 2014 Jan 1;52(1):124-8.
2. Jaroszewski JW, Strøm-Hansen T, Hansen SH, Thastrup O, Kofod H. On the botanical distribution of chiral forms of gossypol. Planta medica. 1992 Oct;58(05):454-8.
3. Stipanovic RD, Stoessl A, Stothers JB, Altman DW, Bell AA, Heinstein P. The stereochemistry of the biosynthetic precursor of gossypol. Journal of the

Chemical Society, Chemical Communications. 1986(2):100-2.
4. Longmore J. Cotton-seed oil: its colouring matter and mucilage, and description of a new method of recovering the loss occurring in the refining process. J. Soc. Chem. Ind. 1886;5:200.
5. Clark EP. Studies on gossypol i. The preparation, purification, and some of the properties of gossypol, the toxic principle of cottonseed. Journal of Biological Chemistry. 1927 Dec 1;75(3):725-39.
6. Wang X, Howell CP, Chen F, Yin J, Jiang Y. Gossypol-a polyphenolic compound from cotton plant. Advances in food and nutrition research. 2009 Dec 31;58:215-63.
7. Campbell K, Morris R, Adams R. The structure of gossypol. I. Journal of the American Chemical Society. 1937;59(9):1723-8.
8. Freedman TB, Cao X, Oliveira RV, Cass QB, Nafie LA. Determination of the absolute configuration and solution conformation of gossypol by vibrational circular dichroism. Chirality. 2003 Jan 1;15(2):196-200.
9. Margalith P. Inhibitory effect of gossypol on microorganisms. Applied microbiology. 1967 Jul;15(4):952.
10. Lin TS, Schinazi R, Griffith BP, August EM, Eriksson BF, Zheng DK, Huang LA, Prusoff WH. Selective inhibition of human immunodeficiency virus type 1 replication by the (-) but not the (+) enantiomer of gossypol. Antimicrobial agents and chemotherapy. 1989 Dec 1;33(12):2149-51.
11. Tai-Shun L, Schinazi RF, Zhu J, Birks E, Carbone R, Yikang S, Kemei W, Liang H, Prusoff WH. Anti-HIV-1 activity and cellular pharmacology of various analogs of gossypol. Biochemical pharmacology. 1993 Jul 20;46(2):251-5.
12. Baram NI, Biktimirov L, Ziyaev KL, Paizieva RZ, Ismailov AI. Antiviral and interferon-inducing activities of gossypol and its dernatives. Chemistry of Natural Compounds. 1995 May 1;31(3):299-303.
13. Dorsett PH, Kerstine EE, Powers LJ. Antiviral activity of gossypol and apogossypol. Journal of pharmaceutical sciences. 1975 Jun 1;64(6):1073-5.
14. Radloff RJ, Deck LM, Royer RE, Vander Jagt DL. Antiviral activities of gossypol and its derivatives against herpes simplex virus type II. Pharmacological research communications. 1986 Nov 1;18(11):1063-73.
15. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer. 2015 Mar 1;136(5):E359-86.
16. Bray F, Møller B. Predicting the future burden of cancer. Nature Reviews Cancer. 2006 Jan 1;6(1):63-74
17. Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical research. 2008 Sep 1;25(9):2097-116.
18. Biemar F, Foti M. Global progress against cancer—challenges and opportunities. Cancer biology & medicine. 2013 Dec;10(4):183.
19. Zugazagoitia J, Guedes C, Ponce S, Ferrer I, Molina-Pinelo S, Paz-Ares L. Current challenges in cancer treatment. Clinical therapeutics. 2016 Jul 31;38(7):1551-66.
20. Steller H. Mechanisms and genes of cellular suicide. Science. 1995 Mar 10;267(5203):1445.
21. Jiang j, sugimoto y, liu s, chang hl, park ky, kulp sk, lin yc. The inhibitory effects of gossypol on human prostate cancer cells-PC3 are associated with transforming growth factor beta1 (TGFβ1) signal transduction pathway. Anticancer research. 2004 Jan 1;24(1):91-100.
22. Zhang M, Liu H, Tian Z, Griffith BN, Ji M, Li QQ. Gossypol induces apoptosis in human PC-3 prostate cancer cells by modulating caspase-dependent and caspase-independent cell death pathways. Life sciences. 2007 Jan 30;80(8):767-74.
23. Wang J, Jin L, Li X, Deng H, Chen Y, Lian Q, Ge R, Deng H. Gossypol induces apoptosis in ovarian cancer cells through oxidative stress. Molecular BioSystems. 2013;9(6):1489-97.
24. Xiong J, Li J, Yang Q, Wang J, Su T, Zhou S. Gossypol has anti-cancer effects by dual-targeting MDM2 and VEGF in human breast cancer. Breast Cancer Research. 2017 Mar 9;19(1):27.
25. Rosenberg LJ, Adlakha RC, Desai DM, Rao PN. Inhibition of DNA polymerase α by gossypol. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression. 1986 May 5;866(4):258-67.
26. Wang YC, Rao PN. Effect of gossypol on DNA synthesis and cell cycle progression of mammalian cells in vitro. Cancer research. 1984 Jan 1;44(1):35-8.
27. Ligueros M, Jeoung D, Tang B, Hochhauser D, Reidenberg MM, Sonenberg M. Gossypol inhibition of mitosis, cyclin D1 and Rb protein in human mammary cancer cells and cyclin-D1 transfected human fibrosarcoma cells. British journal of cancer. 1997;76(1):21.
28. Hou DX, Uto T, Tong X, Takeshita T, Tanigawa S, Imamura I, Ose T, Fujii M. Involvement of reactive oxygen species-independent mitochondrial pathway in gossypol-induced apoptosis. Archives of biochemistry and biophysics. 2004 Aug 15;428(2):179-87.
29. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell. 1997 Nov 14;91(4):479-89.
30. Liu SU, Kulp SK, Sugimoto Y, Jiang JI, Chang HL, Dowd MK, Wan P, Lin YC. The (-)-enantiomer of gossypol possesses higher anticancer

potency than racemic gossypol in human breast cancer. Anticancer research. 2001 Dec;22(1A):33-8.
31. Mohammad RM, Wang S, Aboukameel A, Chen B, Wu X, Chen J, Al-Katib A. Preclinical studies of a nonpeptidic small-molecule inhibitor of Bcl-2 and Bcl-XL [(−)-gossypol] against diffuse large cell lymphoma. Molecular cancer therapeutics. 2005 Jan 1;4(1):13-21.
32. Balakrishnan K, Wierda WG, Keating MJ, Gandhi V. Gossypol, a BH3 mimetic, induces apoptosis in chronic lymphocytic leukemia cells. Blood. 2008 Sep 1;112(5):1971-80.
33. Shelley MD, Hartley L, Fish RG, Groundwater P, Morgan JJ, Mort D, Mason M, Evans A. Stereo-specific cytotoxic effects of gossypol enantiomers and gossypolone in tumour cell lines. Cancer letters. 1999 Jan 29;135(2):171-80.
34. Zhang H, Wang X, Chen F, Androulakis XM, Wargovich MJ. Anticancer activity of limonoid from Khaya senegalensis. Phytotherapy Research. 2007 Aug 1;21(8):731-4.
35. Benz CC, Keniry MA, Ford JM, Townsend AJ, Cox FW, Palayoor SA, Matlin SA, Hait WN, Cowan KH. Biochemical correlates of the antitumor and antimitochondrial properties of gossypol enantiomers. Molecular pharmacology. 1990 Jun 1;37(6):840-7.
36. Leblanc ML, Russo J, Kudelka AP, Smith JA. An in vitro study of inhibitory activity of gossypol, a cottonseed extract, in human carcinoma cell lines. Pharmacological research. 2002 Dec 31;46(6):551-5.
37. Jaroszewski JW, Kaplan O, Cohen JS. Action of gossypol and rhodamine 123 on wild type and multidrug-resistant MCF-7 human breast cancer cells: 31P nuclear magnetic resonance and toxicity studies. Cancer research. 1990 Nov 1;50(21):6936-43.
38. Shelley MD, Hartley L, Groundwater PW, Fish RG. Structure-activity studies on gossypol in tumor cell lines. Anti-cancer drugs. 2000 Mar 1;11(3):209-16.
39. Band V, Hoffer AP, Band H, Rhinehardt AE, Knapp RC, Matlin SA, Anderson DJ. Antiproliferative effect of gossypol and its optical isomers on human reproductive cancer cell lines. Gynecologic oncology. 1989 Mar 1;32(3):273-7.
40. Jin CL, Chen ML, Wang Y, Kang XC, Han GY, Xu SL. Preparation of novel (-)-gossypol nanoparticles and the effect on growth inhibition in human prostate cancer PC-3 cells in vitro. Experimental and therapeutic medicine. 2015 Mar 1;9(3):675-8.
41. Zhang M, Liu H, Tian Z, Huang J, Remo M, Li QQ. Differential growth inhibition and induction of apoptosis by gossypol between hct116 and hct116/bax−/–colorectal cancer cells. Clinical and experimental pharmacology and physiology. 2007 Mar 1;34(3):230-7.
42. Hou DX, Uto T, Tong X, Takeshita T, Tanigawa S, Imamura I, Ose T, Fujii M. Involvement of reactive oxygen species-independent mitochondrial pathway in gossypol-induced apoptosis. Archives of biochemistry and biophysics. 2004 Aug 15;428(2):179-87.
43. Moon DO, Kim MO, Lee JD, Kim GY. Gossypol suppresses NF-κB activity and NF-κB-related gene expression in human leukemia U937 cells. Cancer letters. 2008 Jun 18;264(2):192-200.
44. Blackstaffe L, Shelley MD, Fish RG. Cytotoxicity of gossypol enantiomers and its quinone metabolite gossypolone in melanoma cell lines. Melanoma research. 1997 Oct 1;7(5):364-72.
45. Coyle T, Levante S, Shetler M, Winfield J. In vitro andin vivo cytotoxicity of gossypol against central nervous system tumor cell lines. Journal of neuro-oncology. 1994 Feb 1;19(1):25-35.
46. Zhang XQ, Huang XF, Mu SJ, An QX, Xia AJ, Chen R, Wu DC. Inhibition of proliferation of prostate cancer cell line, PC-3, in vitro and in vivo using (-)-gossypol. Asian journal of andrology. 2010 May 1;12(3):390.
47. Alessandri G, Filippeschi S, Sinibaldi P, Mornet F, Passera P, Spreafico F, Cappa PM, Gullino PM. Influence of gangliosides on primary and metastatic neoplastic growth in human and murine cells. Cancer research. 1987 Aug 15;47(16):4243-7.
48. Wolter KG, Wang SJ, Henson BS, Wang S, Griffith KA, Kumar B, Chen J, Carey TE, Bradford CR, D'Silva NJ. (−)-gossypol inhibits growth and promotes apoptosis of human head and neck squamous cell carcinoma in vivo. Neoplasia. 2006 Mar 1;8(3):163-72.
49. Tomoda K, Chiang HC, Kozak KR, Kwon GS. Injectable (−)-gossypol-loaded Pluronic P85 micelles for cancer chemoradiotherapy. International journal of radiation biology. 2017 Apr 3;93(4):402-6.
50. Yi GO, NI ZH, ZHANG X, CHEN XH, ZOU ZM. Valproic Acid Enhances the Anti-tumor Effect of (-)-gossypol to Burkitt Lymphoma Namalwa Cells. Biomedical and Environmental Sciences. 2015 Oct 1;28(10):773-7.
51. Castilla C, Congregado B, Chinchón D, Torrubia FJ, Japón MA, Sáez C. Bcl-xL is overexpressed in hormone-resistant prostate cancer and promotes survival of LNCaP cells via interaction with proapoptotic Bak. Endocrinology. 2006 Oct;147(10):4960-7.
52. Oliver CL, Miranda MB, Shangary S, Land S, Wang S, Johnson DE. (−)-Gossypol acts directly on the mitochondria to overcome Bcl-2-and Bcl-XL-mediated apoptosis resistance. Molecular cancer therapeutics. 2005 Jan 1;4(1):23-31.
53. Cengiz E, Karaca B, Kucukzeybek Y, Gorumlu G, Gul MK, Erten C, Atmaca H, Uzunoglu S, Karabulut B, Sanli UA, Uslu R. Overcoming drug resistance in hormone-and drug-refractory prostate cancer cell line, PC-3 by docetaxel and gossypol

combination. Molecular biology reports. 2010 Mar 1;37(3):1269-77.
54. Wong FY, Liem N, Xie C, Yan FL, Wong WC, Wang L, Yong WP. Combination therapy with gossypol reveals synergism against gemcitabine resistance in cancer cells with high BCL-2 expression. PLoS One. 2012 Dec 4;7(12):e50786.
55. Baggstrom MQ, Qi Y, Koczywas M, Argiris A, Johnson EA, Millward MJ, Murphy SC, Erlichman C, Rudin CM, Govindan R, Mayo Phase 2 Consortium. A phase II study of AT-101 (Gossypol) in chemotherapy-sensitive recurrent extensive-stage small cell lung cancer. Journal of Thoracic Oncology. 2011 Oct 31;6(10):1757-60.
56. Van Poznak C, Seidman AD, Reidenberg MM, Moasser MM, Sklarin N, Van Zee K, Borgen P, Gollub M, Bacotti D, Yao TJ, Bloch R. Oral gossypol in the treatment of patients with refractory metastatic breast cancer: a phase I/II clinical trial. Breast cancer research and treatment. 2001 Apr 1;66(3):239-48.
57. Van Poznak C, Seidman AD, Reidenberg MM, Moasser MM, Sklarin N, Van Zee K, Borgen P, Gollub M, Bacotti D, Yao TJ, Bloch R. Oral gossypol in the treatment of patients with refractory metastatic breast cancer: a phase I/II clinical trial. Breast cancer research and treatment. 2001 Apr 1;66(3):239-48.
58. Flack MR, Pyle RG, Mullen NM, Lorenzo BE, Wu YW, Knazek RA, Nisula BC, Reidenberg MM. Oral gossypol in the treatment of metastatic adrenal cancer. The Journal of Clinical Endocrinology & Metabolism. 1993 Apr;76(4):1019-24.
59. Bushunow P, Reidenberg MM, Wasenko J, Winfield J, Lorenzo B, Lemke S, Himpler B, Corona R, Coyle T. Gossypol treatment of recurrent adult malignant gliomas. Journal of neuro-oncology. 1999 May 1;43(1):79-86.
60. Zhai G, Wu J, Zhao X, Yu B, Li H, Lu Y, Ye W, Lin YC, Lee RJ. A liposomal delivery vehicle for the anticancer agent gossypol. Anticancer research. 2008 Sep 1;28(5A):2801-5.