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, which 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 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_{18}H_{26}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.
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](image)

**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 (TGFβ1), 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 TGFβ1 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 G1/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 D1 proteins in MCF-7 cells which are critical for G1 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**

<table>
<thead>
<tr>
<th>Human cell lines</th>
<th>cancer</th>
<th>Gossypol activity IC50 (µM)</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Breast</td>
<td></td>
<td>Racemic gossypol</td>
<td>(-)-gossypol</td>
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<td>MCF-7</td>
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<td>3</td>
<td>(34)</td>
</tr>
<tr>
<td>T47-D</td>
<td>5</td>
<td>20</td>
<td>(35)</td>
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<tr>
<td>SKOV-3</td>
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<tr>
<td>Carcix</td>
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<td></td>
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<td>KB-3</td>
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<td>KB-A1</td>
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<td>10</td>
<td>(37)</td>
</tr>
<tr>
<td>KB-V1</td>
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<td></td>
<td>(37)</td>
</tr>
<tr>
<td>SiHa</td>
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<td>&gt;50</td>
</tr>
<tr>
<td>SiHa</td>
<td>14</td>
<td></td>
<td></td>
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<tr>
<td>Ovarian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKOV-3</td>
<td>30</td>
<td></td>
<td>(23)</td>
</tr>
<tr>
<td>OVCA-432</td>
<td>2.4</td>
<td>1.1</td>
<td>10.2</td>
</tr>
<tr>
<td>OVCAR-3</td>
<td>1.5</td>
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<tr>
<td>PC-3</td>
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<td>Leukemia</td>
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<tr>
<td>HL-60</td>
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Table. 2 continued
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 adenocarcinomic 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

<table>
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<th>Human cell lines</th>
<th>Gossypol activity IC₅₀ (µM)</th>
<th>References</th>
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<tbody>
<tr>
<td>K562</td>
<td>15</td>
<td>(43)</td>
</tr>
<tr>
<td>HL-60</td>
<td>15</td>
<td>(43)</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H69</td>
<td>30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Pancreatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MiaPaC</td>
<td>20</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-MEL-19</td>
<td>25</td>
<td>(44)</td>
</tr>
<tr>
<td>WM9</td>
<td>6.2</td>
<td>14.3</td>
</tr>
<tr>
<td>Medullary thyroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>18.9</td>
<td>(36)</td>
</tr>
<tr>
<td>Glioma</td>
<td></td>
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<tr>
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<td>U87</td>
<td>69</td>
<td>(45)</td>
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<tr>
<td>U138</td>
<td>97</td>
<td>(45)</td>
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Available online at http://saspublisher.com/sajp/
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 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 synergetic 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.

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