INSIGHTS ON SYNTHESIS AND POTENTIAL APPLICATIONS OF GOSSYPOL, A PROMISING MULTIPURPOSE CONTRACEPTIVE AND ANTI-INFECTIONS AGENT: A REVIEW

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Abstract: Gossypol; a natural polyphenolic compound firstly extracted from cottonseeds oil has interestingly been reported to be an effective non-hormonal contraceptive agent, antimicrobial, and antiviral against human pathogenic bacteria, viruses, protozoa parasites and yeast-like microorganisms causing human infections including even some sexually transmitted infections (STIs). Multipurpose contraceptives are advanced drugs designed in one product indicated to prevent unwanted pregnancy and transmission of one or more STIs including HIV. More especially; the antifertility properties of gossypol combined with its multi-antimicrobial and antiviral properties may play a huge impact on reproductive health for further design and development of an effective multipurpose therapy providing a potential anti-infections protection and contraceptive barrier method. Gossypol and its derivatives showed complex chemical and biological properties with high reactivity through different mechanisms of action by which their various chemical forms exhibit polyvalent biological activities such as anticancer, antioxidant, antifertility, antimicrobial, antiviral, antiprotozoal, and antimalarial. This review discusses gossypol and its derivatives formation, synthesis, and current potential applications as an antifertility and anti-infections molecule.

Keywords: gossypol, cottonseed, contraceptive, antifertility, microorganisms, antimicrobial.

I. INTRODUCTION

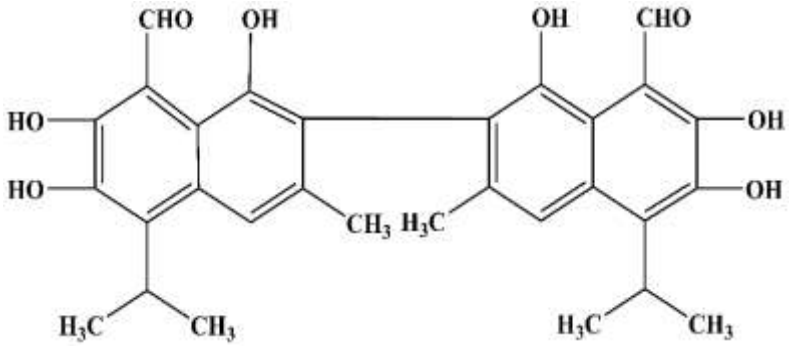
Gossypol is a natural polyphenolic extract from cottonseeds and other parts of cotton plants (*gossypium sp.*) belonging to *malvaceae* family (Fig.1).Gossypol is one of the complex organic terpenoids having aldehyde and hydroxyl groups (Fig.2) ready to be engaged in its high reactive properties. Through many studies concerning the polyvalent reactivity of gossypol; it has interestingly been discovered that gossypol has versatile biological activities including: its ability to act on most human chronic diseases as an effective anticancer [76, 147, 185], antioxidant [68], antimicrobial against main human pathogenic microorganisms such as bacteria, fungi, protozoa and yeasts [122], antiviral activities against herpes simplex virus [133], human immunodeficiency virus [89, 156], some arboviruses [114] and influenza type viruses [38], insecticidal [163] and antifertility activities [81, 116, 132]. Unfortunately, even though gossypol exerts these potential biological activities; its wide systemic toxicity is still a big limit from its clinical uses.

For example, the systemic toxicity of gossypol and its derivatives was reported to be mainly dose-dependent and also the duration of exposure and route of administration may play an important role on severity of its toxic effects manifestations. The general accepted standards of gossypol consumption have been set to be no more than 450-600mg/kg per day [16] or 0.8mg/kg/day for 6weeks [163] as the maximum safety doses human species can tolerate. Through series of clinical trials, no side effects were enhanced by small doses, even before humans have used gossypol-containing drugs (Chinese medicine for treatment of chronic bronchitis and cough) and foods but few and negligible related adverse consequences were reported [19, 20]. Gossypol was reported to have a high binding affinity with albumin and iron of the red blood cells hemoglobin; thereby forming a strong complex that could lead to generalized anemia, respiratory and cardiac difficulties and probably death depending on ingested amount and time of exposure [40, 41, 46]. Moreover, gossypol stimulates prostaglandins biosynthesis and inhibits Na-K-ATPase leading to renal potassium loss which can cause hypokalemia in some subjects [112, 131], and its high doses were also reported to cause irreversible aspermatogenesis trending to sterility after long term ingestion possibly due to its inhibition of spermatogenesis cycle [90, 136, 164].

In fact, To prevent gossypol systemic toxicity, some gossypol-based gel formulations were investigated to minimize gossypol-associated toxic effects by preventing its contact with the blood; and as results, neither side effects on monkeys and humans have be reported once gossypol-containing topical formulations are used at its appropriate low doses [21, 81, 184]; after the discovery of potential biological activities of gossypol and its derivatives, and studying the mechanisms of action of severity of its adverse effects; many studies have been conducted to counteract or minimizing its toxicity but, maintaining its pharmacological activities either by co-administration with selenium and potassium salts supplements or by suppressing its aldehyde groups [40, 86, 114].

This review highlights the recent progress of *in vitro* and *in vivo* collective researches clarifying mechanisms of action of gossypol, its complex chemistry and reactivity on which lies polyvalent biological activities of gossypol and its derivatives for therapeutic applications; discussing findings from multitudinous studies providing special persuasive evidences for its potential contraceptive effects and inhibition of most human infections-causing microorganisms mainly bacteria, viruses, fungi and yeast possibly involved even in STDs and other non-curable or chronic human diseases.

Figure 1: Descriptive parts of cotton plant



Chemical formula: $C_{30}H_{30}O_8$

Figure 2: Chemical structure of gossypol.

II. CHEMISTRY AND FORMATION OF GOSSYPOL

Gossypol; a yellow crude pigment was firstly discovered and successfully isolated in 1886 from cottonseed oil as a mixture of gums precipitates during refining of crude cottonseed oil [4, 97, 121]. Its chemical formula as $C_{30}H_{30}O_8$ was lately established and successfully synthesized and named 1,1', 6,6', 7,7'-hexahydroxy, 3,3'-dimethyl, 5,5'-diisopropyl, 2,2'-binaphthyl, 8,8'-dialdehyde (Fig.2) by Edwards (1958)[39]. The 8 polar groups of gossypol make it soluble in most organic solvents such as ethanol, methanol, butanol, glycol, ethylene, ethers, acetates, carbon tetrachloride, phenols, naphthalene, pyridine, dimethyl sulfoxide and vegetable oil at certain temperature but it remains less soluble in cyclohexane, glycerin, gasoline, petroleum ether, and benzene. Unfortunately, the presence of heavy dialkylnaphtalene groups in structure of gossypol makes it insoluble in water[103]. Through dissolution of gossypol in some solvents such as chloroform, diethyl ether, it has showed three different crystal forms with different melting points of 184, 199 and 214^{o}c respectively [4]. Lately, after the some deep studies on such solubility behaviors, it was concluded that only the sample with 214^{o}c was a non-soluble form of gossypol and the other two showing 184 and 199^{o}c were complexes with the solvents of diethyl ether and chloroform respectively, the demonstration of these polymorphic forms of gossypol revealed its presence into two enantiomers as (+) - gossypol and (-) - gossypol (Fig.3) [4, 70].

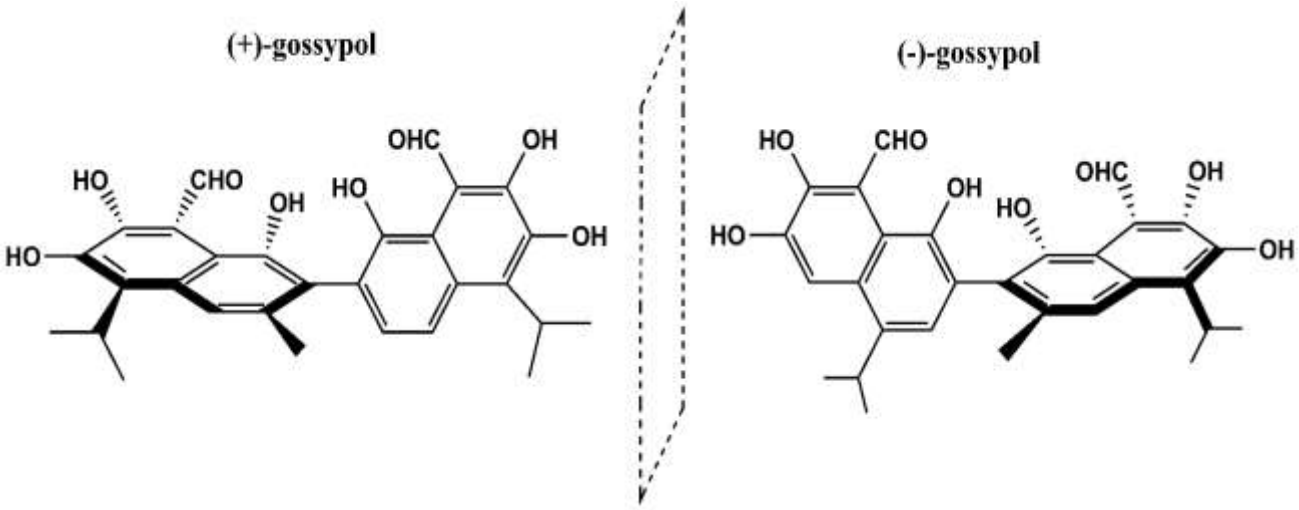


Figure 3: Structure of gossypol enantiomers

The two atropoisomers of gossypol are resulting from a restricted rotation of its internaphthyl bond. Gossypol has four naphthalene rings derived from sesquiterpenes as characteristic of the cardinanes formed in the biogenetic cascade from the bisabolane intermediate putative series of shift bonding and cyclization. During metabolism of cotton plant metabolites, gossypol as an aldehydic terpenoid compound is metabolically originating from acetate by an isoprenoid pathway as a result from radical coupling reactions of sesquiterpenes dimer (Fig.4) [22], the real gossypol biosynthesis begins by formation of farnesyl diphosphate known as sesquiterpene precursor resulting from combination of isopentenyl pyrophosphate with geranyl pyrophosphate, it is followed by formation of (+)-d-cadinene (2) as a result of cadinyl cation (1) catalyzed by (+)-d-cadinene synthase (E_1). The catalytic reaction facilitated by (+)-d-cadinene 8-hyroxylase (E_2) allows generation of 8-hydroxy-d-cadinene (3), obtained from homigossypol which is fundamental sesquiterpene aromatic unit formed from (+)-d-cadinene, 8-hydroxy-d-cadinene goes through various oxidative processes to form the deoxy-hemigossypol (4), which is oxidized by 1 electron into product 5, 6, and 7; the final product is obtained after a coupled phenol-based oxidation to the ortho- position of the phenol groups to produce gossypol (8) and the peroxidase enzyme (X_2) involved in such catalytic coupling were showed to be hydrogen peroxide dependent type to yield this final product with various proportions with other plant metabolites [36, 101]. Many researchers discovered the specific excess of (-) -gossypol in *gossypium barbardens* cotton plants species versus an excess of (+)-gossypol found in all other different *gossypium* species [24, 69].

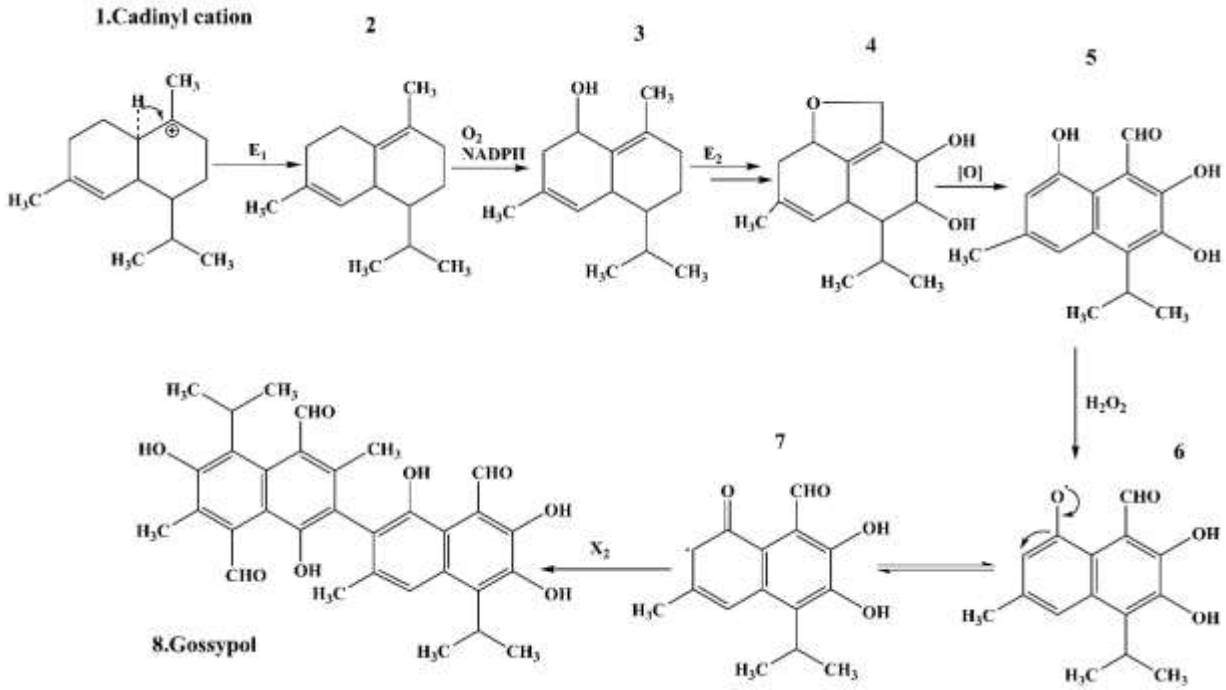


Figure 4: Metabolic formation of gossypol

The advance in use of nuclear magnetic resonance (NMR), UV spectrometry and mass spectrometry methods for gossypol analysis confirmed different structural changes of gossypol in various solvents and it has been proposed and demonstrated that gossypol exists in three tautomeric (aldehyde, ketone, and the hemiacetal) forms (Fig.5) through which it may interchange into different reactive sites with different activity levels [4]. In polar solvents with alkaline medium such as dimethyl sulfoxide (DMSO) gossypol exists as hemiacetal form in dynamic equilibrium with other 2 forms, in basic solvents medium as ketol form while in acidic conditions and usual inert solvents such as acetone, chloroform or benzene gossypol exists mainly in the aldehyde form [1, 152]. The versatile biological activities of gossypol may be potentially and simultaneously attributed to its different tautomeric forms existing once dissolved in DMSO for biological studies purpose. Gossypol is a highly reactive organic compound due to its carbonyl and polar groups (6 hydroxyls and 2 aldehydes) as well as to its bulky binaphtalene structure [163]. All phenolic groups of gossypol are readily reactive to form organic ethers and esters; whereas its aldehyde groups are highly susceptible to reaction of Schiff bases formation with amine compounds and they can react with organic acids to form heat labile products or gossypol may either also react with other products of the plant to form bound gossypol or remain as free gossypol which is attributed to be its most toxic form [83, 84].

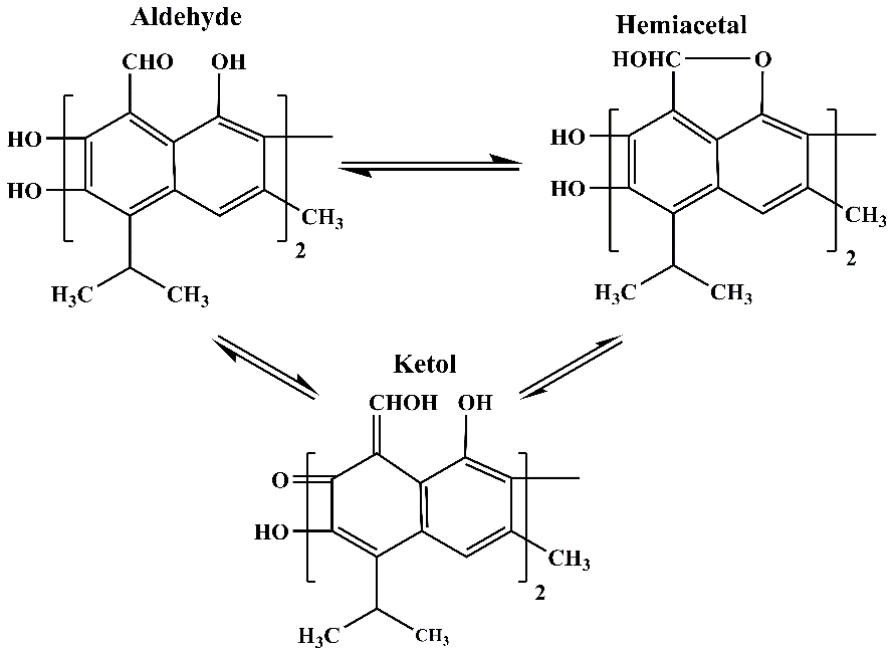


Figure 5: Tautomeric forms of gossypol.

The chemical status of gossypol in cottonseed products may be determined in terms of free gossypol and its derivatives which can be extracted by 70% aqueous acetone and bound gossypol which is an insoluble portion of gossypol in acetone resulting from reactions of gossypol and its analogues with other cotton plant products during processing while the both forms are defined as total gossypol after including all its analogues and derivatives readily reactive with 3-amino-1-propanol in dimethylformamide solution. In the cottonseed products, the biggest part of gossypol exists as Schiff bases resulting from condensation reactions between aldehydic groups and amino acid groups of cottonseed proteins (Fig.6) [25, 99] and it was lately concluded that gossypol has a strong ability of binding many different amino acids at once and producing insoluble protein complexes. Some advanced spectral analysis methods such as nuclear magnetic resonance and circular dichroism were conducted for further understandings of interactions between gossypol and proteins and other amino acids-containing compounds, and reported the existence of the reversible ionic and hydrophobic interactions [33, 104, 155].

Furthermore, gossypol was also reported to have ability to be chelated by iron of the cottonseed products to form insoluble metal complexes which may form gossypol polymers or oxidized into other products, its phenolic groups react with carboxylic and phenol compounds of the plant to form natural esters or ethers [10, 58, 113]; more interesting studies discovered that gossypol binds competitively not only to bilirubin-binding site of human serum albumin but also to iron of hemoglobin of human red blood cells and forms stable complexes, its bilirubin-binding site is linked to high affinity with oxygen in the blood and other many hydrophobic residues able to fix one or more positively charged amino acids [141]. Furthermore; with deeper studies on complexes of gossypol with human amino acids, peptides or proteins such as human serum albumin, protamine, lactate dehydrogenase, lysozyme, and poly-*l*-lysine found out that the rate of reaction of gossypol

and amino acids-containing compounds increases with an increase in PH ranging between 5.7 and 7.5, and the binding affinity of (+)- gossypol and (+/-)- gossypol with human serum albumin was the same[167, 168].

III. REACTIVITY OF GOSSYPOL AND FORMATION OF ITS DERIVATIVES

Gossypol is particularly susceptible to form Shiff base products at alkaline PH ranges which remain less stable until they are reduced into secondary or tertiary amines (Fig.6). In aqueous solutions, Shiff bases hold a labile bond ready to undergo a reversible hydrolysis and probably stabilized by chemical reductions [63]. The reduction of gossypol into a Schiff base is facilitated by an addition of sodium borohydride ($NaBH_4$) or cyanoborohydride ($NaBH_3CN$); the both are used for a reductive amination, the borohydrides focus on simultaneous reduction of aldehyde groups to hydroxyls and convert Schiff base into secondary amine while cyanoborohydrides are most effective for Shiff bases reduction rather than even in mild reductive amination reactions as they eventually showed to be a highly selective reducing agents on functional organic groups. In fact, cyanoborohydride is the best reducing agent to produce the best yield of schiff base products through addition reaction of $NaBH_3CN$ compared to $NaBH_4$ based on the fact that it entirely results in reduction of schiff base intermediate into a stable secondary amine [8, 63, 88].The combination of gossypol and neutral amino acids with aliphatic groups were studied and showed versatile biological activities of research interests with lower toxicity [9, 178].

Figure 6: Gossypol Schiff base formation

The oxidation of gossypol is also favorable in alkaline conditions. The pure gossypol and some of its derivatives are unstable with high susceptibility of undergoing oxidation reactions into various products under different conditions either with catalysts or usual solvents [28, 83]. During oxidation studies with gossypol by pure oxygen in alkaline medium and late analysis of its chemical products through X-ray diffraction method; detected the crystal structure changes of gossypol naphthalene nucleus into indane nucleus structure confirming the chemical formation of a new derivative of gossypol (Fig.7) finally named gossindane [74] while an early stage reaction of gossypol and oxygen by using Dakin-type reaction conducted to the formation of gossypol derivatives with interesting activities such as gossypol-*o*-binaphthoquinone[157] and gossypolone (*p*-binaphthoquinone) obtained through reaction of gossypol with ferric chloride hexahydrate in acetone or acetic

acid medium (Fig.8); characteristic of the orange color appeared correspondingly with gossypolone formation [143, 149]. Gossypol undergoes ozonolysis in acetic acid solution and results in formation of oxalic acid as major product and gossypolic acid (Fig.9) at low yield, and it is highly susceptible to chemical oxidations by potassium permanganate ($KMnO_4$) in sodium hydroxide (NaOH) medium resulting in its degradation into carbonic dioxide, acetic acid, formic acid, and *n*-butyric acid or *iso*-butyric acid (Fig.7) [29].

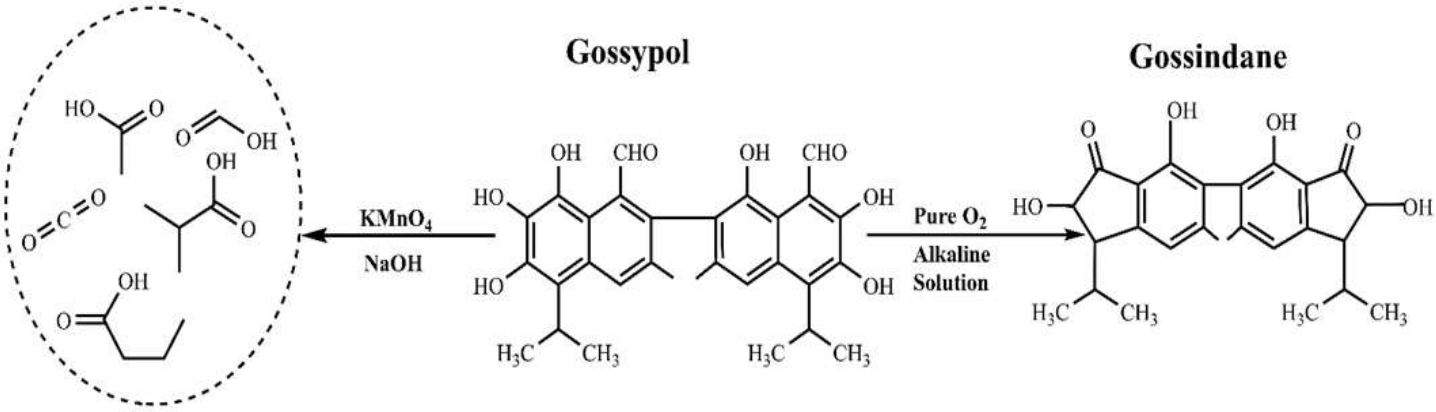


Figure 7: Oxidative degradation of gossypol and formation of gossindane

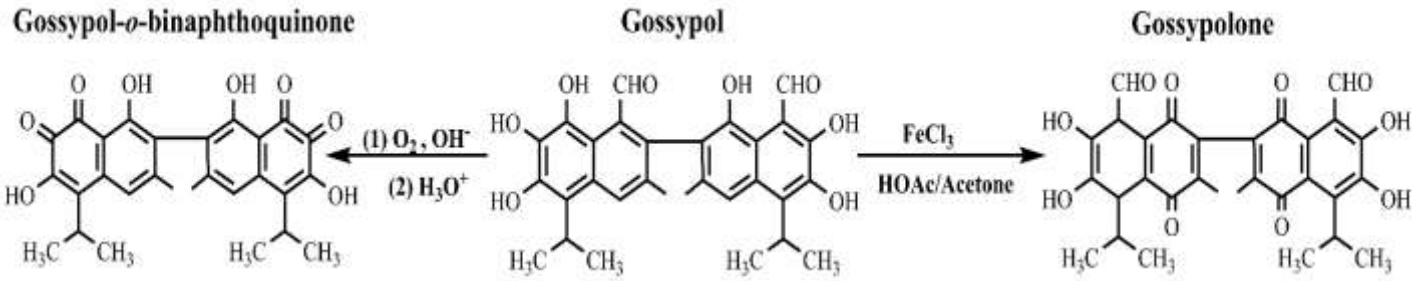


Figure 8: Oxidation of gossypol: formation of gossypolone and gossypol-*o*-binaphthoquinone.

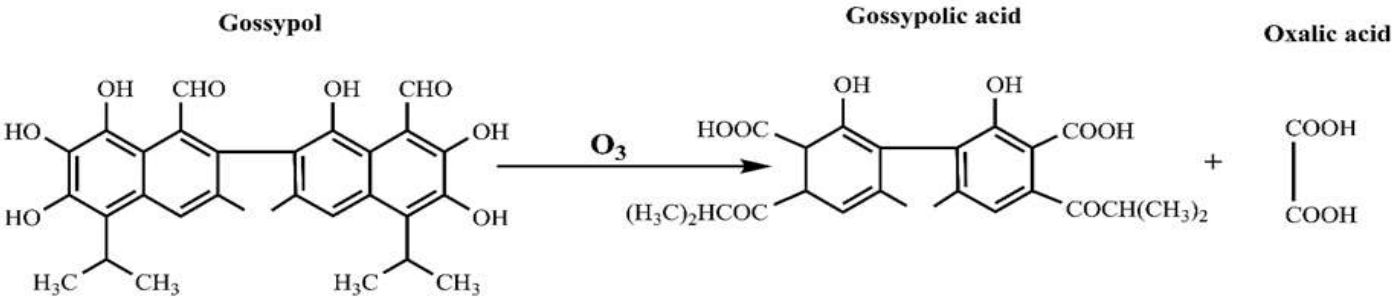


Figure 9: Ozonolysis of gossypol

The multifaceted reactivity of gossypol influence its potential wide application in many different biological activities. Through novel advanced nanotechnology by trying to get rid of gossypol toxicity, some studies were conducted to combine gossypol and fullerene through synthetic pathway to make nanoparticle-based therapeutic gossypol-fullerene hybrids (Fig.10) and their therapeutic interests still need to be deeply investigated and developed; mainly N-methyl,2-phenylfulleropyrrolidine (1), 1,2,2-trimethylfulleropyrrolidine(3), and N-methylfulleropyrrolidine (2) as different fulleropyrrolidines having different reactivity levels, and able to be chemically and physically characterized by different methods such

as IR, MS and X-rays methods [180]. Moreover other technics such as emulsification-volatilization, encapsulation advanced drugs delivery systems are nowadays employed to prepare low doses gossypol-loaded nanoparticles [62, 76, 96].

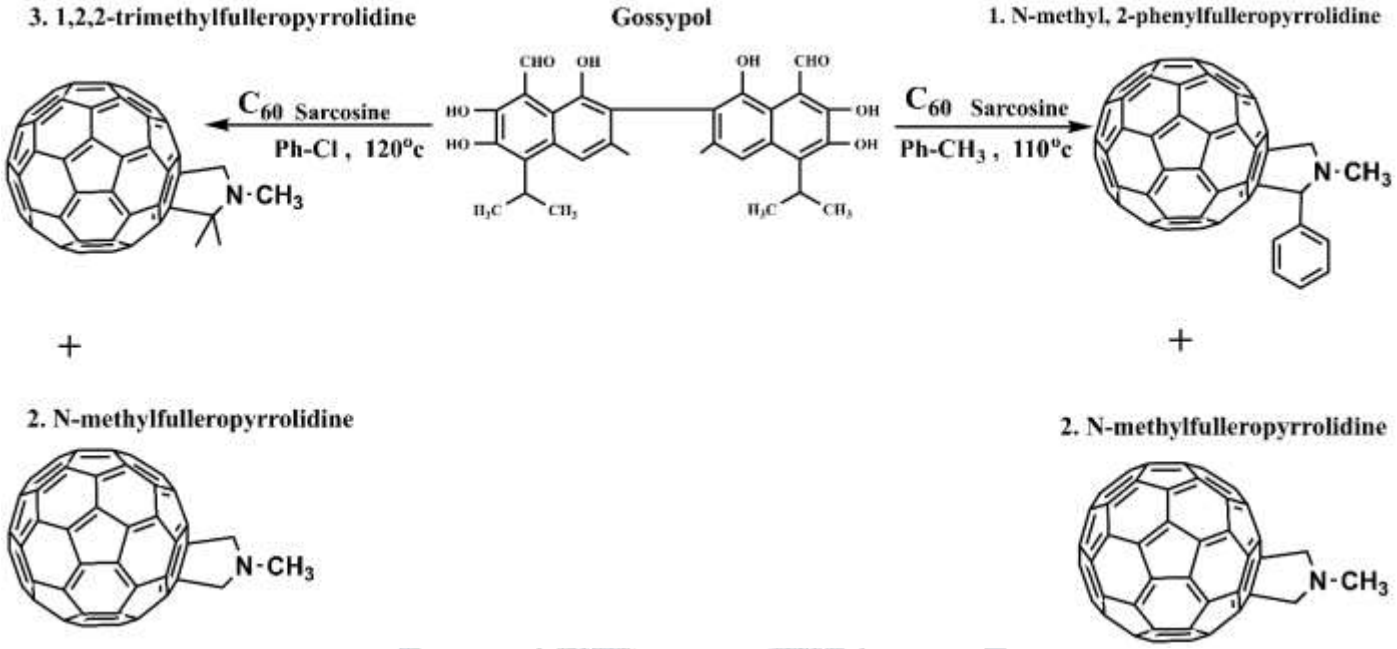


Figure 10: Synthesis of gossypol-fullerene hybrids

The stability of gossypol ethers after series of methylation reactions is firmly declared by the formation of a final more stable gossypol hexamethyl ether by using a combination of some great organic methylation agents such as methyl iodide, dimethyl sulfate and diazomethane in ether medium (Fig.11) [2]. The reaction between gossypol and dimethyl sulfate in methanolic potassium hydroxide may also result in formation of gossypol tetramethyl ether and gossypol hexamethyl ether; the first one ends up by undergoing a complete methylation into gossypol hexamethyl ether as a final and stable form [57, 67] and its formation is characterized by the appearance of an orange color in the medium. Further demethylation of gossypol hexamethyl ether in acetic acid and concentrated sulfuric acid (H_2SO_4) medium results in gossypol dimethyl ether (Fig.11) [3]. Through different reactivity and stability levels of these gossypol ethers it was found that they also display different biological activities with various toxicity rates [171].

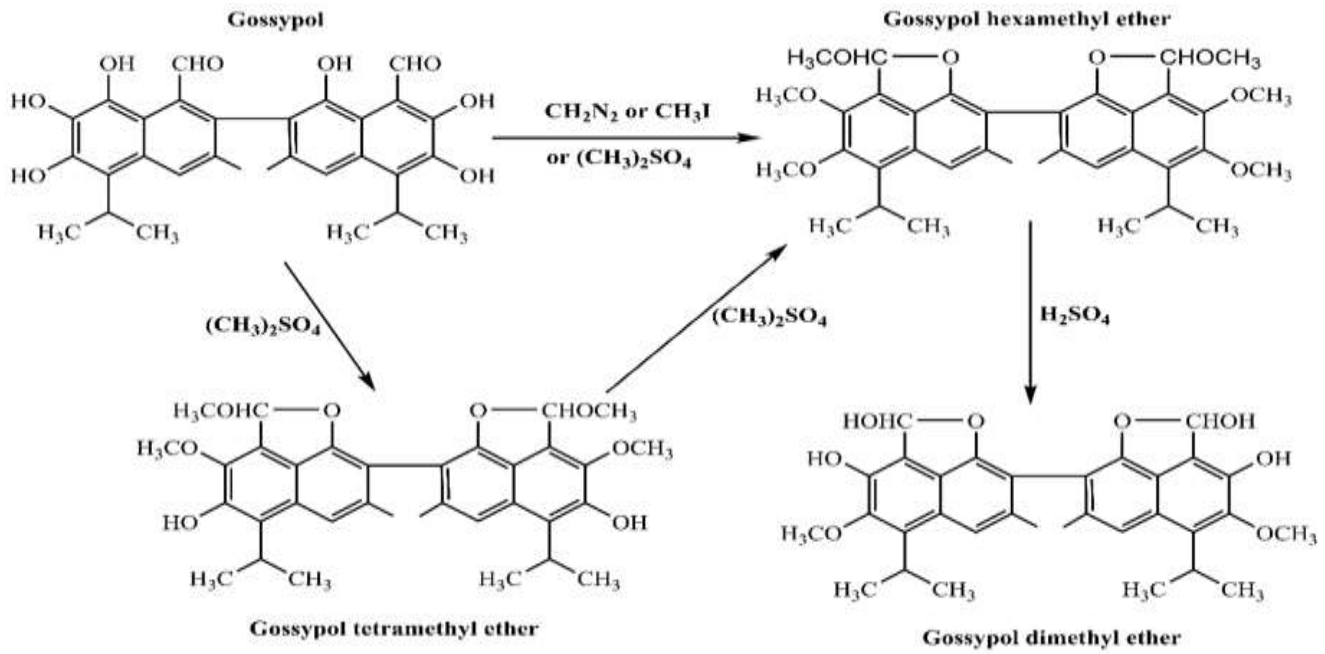


Figure 11: Methylation of gossypol

Gossypol may also be reduced with some selective reducing agents of organic functional groups like sodium cyanoborohydride ($NaBH_3CN$), Lithium aluminium hydride ($LiAlH_4$) or H_2 catalyzed by platinum, the reactions through which the aldehyde groups are reduced into methanol [35] or methyl groups [118, 148, 150]. Gossypol reacts with aqueous sodium hydroxide concentrated around 40% at temperature above 85°c under nitrogen atmosphere to give apogossypol (Fig.12) which is a gossypol-dealdehyde derivative. Apogossypol can be more stabilized by transforming it into apogossypol hexamethyl ether by using dimethyl sulfate as the most stable gossypol ether form but which can also be used to prepare didesisopropyl apogossypol hexamethyl ether by using concentrated sulfuric acid (H_2SO_4) [110].

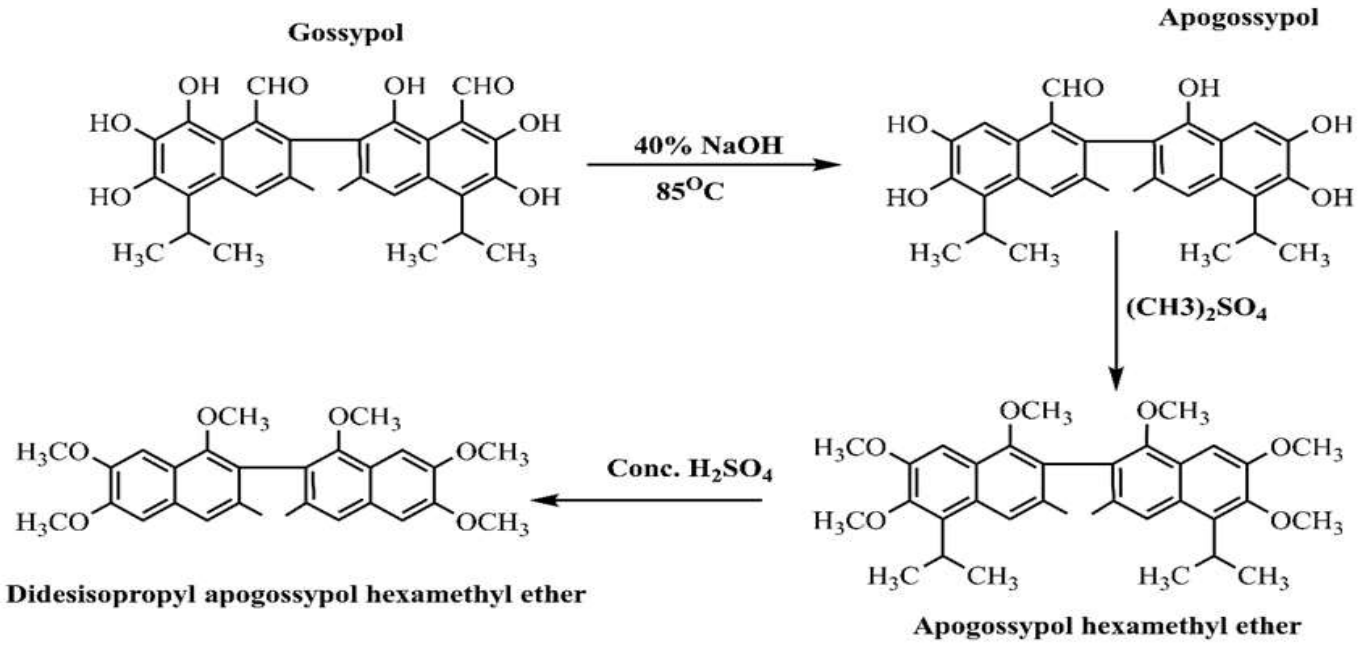


Figure 12: Formation of apogossypol and its derivatives

IV. POTENTIALITY OF ANTIFERTILITY ACTIVITY OF GOSSYPOL

The contraceptive effects of gossypol were extensively experienced even before 1930s by a big number of reproductive families using cottonseeds oil without realizing any fertility concern related to its constituents, and up to 1957 when Chinese researchers remarked a notable change in fecundity rate trending to infertility of couples and female menstrual disturbances at the same time, and after a period of time of replacement of cooking with crude soybean oil by cottonseed oil [95]. In 1972; the scientists of Chinese National Coordinating Group on Male Antifertility Agents conducted first clinical trial on 4000 fertile Chinese men using gossypol pills of 15-40mg/kg of body weight per day for 0.5 to 4 years and clinical analysis of the semen showed 99.89% antifertility efficacy, short number of men reported hypokalemia, quasi-irreversible spermatogenesis recovery after long term treatment and no other obvious toxic effects observed up to 2 years of the treatment, and it was reported that gossypol acetic acid, pure gossypol and gossypol formic acid were the three kinds of tablets which have been prepared for clinical studies [116].

Lately; gossypol which was only considered as toxic waste from cottonseed products started to be interestingly investigated by different worldwide researchers after being reported the antispermatogenic effects of crude cottonseed oil in male rats and monkeys for a certain feeding period [79, 162, 165]. The development of gossypol extraction methods and initiation of *in vitro* and *in vivo* intensive studies concerning contraceptive effects of gossypol were urgently conducted on various animal models such as rats [59, 91, 92] and mice [30], hamsters and rabbits [26, 161], rams [124] and bulls [12], monkeys [145] and humans [5] for better understandings of the effects of gossypol and its derivatives both on male and female reproductive system. On the other hand; some topical formulations of gossypol were made for clinical investigations as either vaginal spermicidal contraceptive on female monkeys [21] and humans [81] or antimicrobials use, and experimental findings revealed its effectiveness without causing neither any tissues irritations nor systemic toxicity at appropriate doses [158].

In animal studies, the high doses of gossypol acetic acid of 20-30mg/kg of body weight per day for 7 weeks given orally showed a great ultrastructural epididymal and testicular damage leading to a significant decrease of sperms production in rats [66] but 40mg/kg/day for only 18days decreased semen volume and sperm concentration in cocks[111] and ostriches [18] while 5-10mg/kg/day for 14weeks produced sterility in male rats and hamsters but only decreased sperms motility and concentration in rabbits [26, 92] and in monkeys when taken up to 6 months by oral feeding[145] without causing any detectable interferences with plasma testosterone levels, the same as in another study conducted with gossypol acetic acid at dose of 15-40mg/kg per day for 2-4 weeks resulted in antifertility outcomes to male rats without causing any harmful effect [116]. Through studies of contraceptive activity of enantiomeric forms of gossypol, the oral feeding of 30mg/kg per day of (-)-gossypol showed antifertility effects after only 7 days in male rats while the same dose of (+)-gossypol showed neither antifertility effects nor any other toxic effects on male rats after 14 days but small testicular ultrastructural damages were observed after 4 weeks. It was concluded that (-)-gossypol has a better

contraceptive activity than (+)-gossypol [115], and gossypolone as an oxidized gossypol metabolite showed less spermicidal activity than the isomers of the parent compound on rats, hamsters, rabbits, monkeys and human sperms [85].

Furthermore; based on the facts that gossypol not only promotes pregnancy loss and negatively interferes with embryo implantation in female animals such as rats and rabbits either through inhibition of histamine release or blocking luteinizing hormone effects [87, 93, 94, 182], but also its spermicidal and antimotility effects *in vitro* and *in vivo* through injection in epididymis as well as application in the vagina [21, 79, 170], also showed a strong ability to reduce the penetration of spermatozoa through cervical mucus and zona pellucida of the ovum [6].

Many researchers studied the mechanisms of antifertility activity of gossypol and reported various effects interfering with spermatogenesis process such as through deterioration of seminiferous tubes and epididymis causing less sperms production which are even completely weak and abnormal to reach the female fertilization site [119], reducing seminal plasmid lipids concentration leading to decreased sperms count [144], and gossypol also showed the ability to block T-type Ca^{2+} spermatogenic cells [13] the same as not only inhibiting acrosomal enzymes mainly hyaluronidase, acid phosphatase, β-glucuronidase [183] but also acrosomal plasminogen activator / plasmin system main factors involving in ovum fertilization process. Gossypol showed an antimotility effects on human spermatozoa through competitive inhibition of nicotinamide adenine dinucleotide hydrogenase (NADH), binding to lactate dehydrogenase(LDH) [181] and adenosine triphosphate (ATPase); essential enzymes participating in energy production system required for sperm cells growth, maturation and motility [173].

The antifertility activity of gossypol through cellular energy inhibition starts by inhibition of mitochondrial LDH-x as target of action present in testicular seminiferous cells [181] and concomitantly affects the ribonucleotide reductases [107] and dehydrogenase enzymes complex such as malate dehydrogenase, glyceraldehyde-3-phoshate dehydrogenase and cytoplasmic phospholipase A_2 [72]; this enzymatic complex is the fundamental key of acrosomal reaction in sperm maturation to acquire fertilizing ability [37]. The selective non-competitive inhibition of gossypol with human 5-α-reductase enzymes may be effectively developed for treatment of some disorders associated to androgen-dependence such as prostate cancers, benign prostatic hyperplasia, hirsutism, alopecia areata and some others [65]. Gossypol as natural non-steroidal contraceptive agent showed a significant inhibition of spermatozoa production and motility of various male animals including humans without causing any detectable interactions with hormones but it mostly interferes with enzymes system involving in maturation and production of spermatogenic cells and spermatozoa [31].

In fact; after reviewing different animal studies describing the reproductive effects of gossypol on reproductive organs and behaviors of different male (Table 1) and female (Table 2) animals, it can be

concluded that the antifertility and toxic effects of gossypol are somehow intrinsically dose-dependent especially to species, gender and treatment duration variations. Firstly; hamsters, rats and monkeys seem to be the most tolerant to gossypol [26], dogs and rabbits are the least but mostly female hamsters showed to be less tolerant than males [174]; rabbits are only very sensitive to toxic effects of gossypol but no antifertility effects based on very noticeable toxicological manifestations seen after few days of dosing as the same case as for the dogs and only rabbits still fertile even in few days before their death [142] the same as the fatal doses of gossypol in dogs induced slight spermatogenesis disturbances and animal dies after few months of treatment [11, 73] while monkeys tolerate the toxicity of gossypol but are mildly sensitive to its antispermatogenic effects; rats are sensitive to normal ranges of antifertility doses (7.5mg//kg/day for 12 weeks) without any harmful effects up to its 10 times (30mg/kg/day for 16 weeks) where minor toxic effects may appear in small number of treated animals and still infertile, normal mating and no effects on visceral histology [14, 176].

Table 1: Some experimental studies on antifertility effects of gossypol on male animals' reproductive system.

Animal tested	Dose in mg/kg/d	Treatment Duration	Reproductive effects	Toxic manifestations	References
Rats	7.5	12 weeks	-majority Infertile	None	186
Rats	10-20	6 weeks	-Spermatocytes degeneration -Decreased sperm counts & motility - Lowered testosterone, FSH & LH	-Slight hepatocytes damages at high doses	43, 60
Rats	10	14 weeks	-Retarded body& sex organs growth -Tubular degeneration & infertility	-Digestive troubles -liver necrosis	47, 165
Rats	25	26 weeks	-tubular & Sertoli cells degeneration -Reduced sperm count and motility -Sterility & lowered testosterone	-Liver cells damages -Body weight loss	64
Mice	40	8 weeks	-No degeneration	None	60
Hamsters	10	12 weeks	-Spermatocytes degeneration	None	60
Bulls	8	56 days	-Increased abnormal sperms *Effects were reversible after 210 days of gossypol feeding withdrawal	None	61
Bulls	8200	12 weeks	-Reduced sperm production, motility -Reduced normal sperm counts	None	27
Rabbits	16	14-41 days	-Fertile till few days of their death	-death	125
Rabbits	80	8-17 days	-Normal spermatozoa	-weight loss	142
	20-40	23-84days	-Normal sperms motility	-Paralysis & death	
Dogs	1.5-3	50-140days	-Slight spermatogenesis inhibition	-heart &lung failure	11

	30	18-28days		-All animal died	
Monkeys	4	4months	-Decreased normal sperm counts	-liver swelling	80
	8	14months	-Reduced sperms motility	-Weight loss	
Humans	15-50	≥6months	-≥99% infertile -Irreversible aspermatogenesis	-hypokalemia after 1year of treatment	116
Humans	7.5-15	52-58wks	-Infertile -reversible spermatogenesis	None	31, 175

Doses of gossypol are expressed in mg per kg of body weight per day; FSH: Follicles-stimulating Hormone; LH: Luteinizing Hormone.

Table 2: Some experimental studies on antifertility effects of gossypol on female animals' reproductive system.

Animal tested	Dose in mg/kg/d	Treatment Duration	Reproductive effects	Toxic manifestations	References
Rats	5-10	13days	-Longer diestrus cycles	-Body weight loss	56
Rats	20-25	60 days	-Lowered estradiol levels -Prolonged mating time -Inhibited ovum implantation - Decreased pregnancy rates	-Decreased body weight gain -liver damages	87, 93
Mice	40-80	19days	-pregnancy inhibition at 90%	None	60
Hens	200-400	18days	-Reduced egg production -Egg yolk discoloration	None	82, 98
Cows	50	64 days	-No any interferences	-Reduced weight gain	48
Cows	5000	210days	-Reduced number of ovarian follicles	-Weight gain loss	134

Doses of gossypol are expressed in mg per kg of body weight per day.

V. ANTIMICROBIAL POTENTIALS OF GOSSYPOL AND ITS DERIVATIVES

Gossypol has shower a general antimicrobial activity as an antifungal, antiprotozoal and antibacterial agent, sometimes bactericidal or bacteriostatic on different strains with LD_{50} ranging from 10 to 100ppm of pure gossypol [15], and gossypol has been reported to have an inhibitory effect on microorganisms including most of aerobic spore-forming *lactobacilli* bacteria and some oxidative yeasts (Table 3) [122].

Gossypol was reported to be more potent antibacterial agent against most strains of resistant gram positive bacteria like *staphylococcus spp, bacillus spp*. and *streptococcus spp*. than gram negative organisms such as *Escherichia coli*, *Salmonella spp*, *Proteus spp*., *Klebsiella pneumoniae*, *Shigella spp*., and *Pseudomonas aeruginosa*; it was concluded that antibacterial inhibitory effect of gossypol is effectively dependent to the amount of peptidoglycans present in the bacteria cell wall which may act as target site influencing the transport of gossypol [159]. Some gossypol derivatives also showed a strong inhibitory effects on plants and animal pathogenic bacteria such as *Edwardsiella ictaluri* [179] and fungi such as *Fusarium oxysporum* [108, 130], and *Aspergillus flavus* [109] which produces aflatoxins possibly causing some types of hepatitis, hepatocellular carcinoma and pulmonary aspergillosis in immunosuppressant humans [77].

Table 3: Inhibitory effects of gossypol on some microorganisms

Microorganisms	Minimum inhibitory concentration (µg/ml)
Staphylococcus aureus	10
Sarcina lutea	25
Bacillus polymyxa	50
Bacillus megaterium	50
Bacillus licheniformis	25
Bacillus cereus	50
Bacillus thermocidurans	50
Leuconostoc mesenteroides	10
Lactobacillus delbruckii	20
Escherichia coli	>200
Proteus mirabilis	>200
Pseudomonas aeruginosa	>200
Saccharomyces cerevisiae	>200
Saccharomyces carlsbergegensis	>200
Zygosaccharomyces mellis	>200
Hansenula anomala	200[a]
Hanseniaspora sp.	200[a]
Candida utilis	>200
Debaryomyces nicotianae	100
Pichia membranefaciens	25[b]
Cryptococcus neoformans	25
Rhodotorula mucilaginosa	>200

[a] *Caused a slight antimicrobial inhibition*
[b] *Caused a complete suppression of film growth.* Adapted Margalith(1967)[122].

Lately, Vadehra *et al*. (1985) investigated the effects of gossypol on the growth of a variety of bacteria and on spore formation and germination in *Bacillus cereus*, found that all of the Gram-positive organisms tested were completely inhibited at a concentration ranging between 10 to 100µg/ml and none of the Gram-negative strains was inhibited at concentration less than 200µg/ml. It was concluded that the potency of antibacterial properties of gossypol were linked to Gram character by the fact that the presence of high amount of peptidoglycans and lack the outer membrane in cell wall may facilitate the easier transport of gossypol in Gram-positive rather than in Gram-negative bacteria [159]. The same research group found that some yeasts such as *Saccharomyces cereviseae, S. uvarum, S. diasticu* were sensitive to gossypol and their growth at 50ppm were completely inhibited.

The antibacterial and antifungal activities of gossypol and its derivatives were tested in *vitro* and their good activity was observed at minimum inhibitory concentration (MIC) values of standard ranging between 12.5–100 µg/ml, moderate activity between 100 and 200 µg/ml and low antibacterial activity from 200 to >400 µg/ml on tested strains of *staphylococcus aureus* [129]. Another research reported that fungi *Paecilomyces fumosoroseus* (associated with cutaneous and disseminated infections in dogs and cats) were the highest resistant to gossypol up to 500 µg/ml but, totally inhibited at concentration ≥1000 µg/ml [127]. Through the mechanisms of selective inhibition of vital enzymes in different parasites; gossypol and its derivatives were reported to exert a strong antiparasitic protozoal activity against *Plasmodium falciparum* [135, 140], *Toxoplasma gondii*, *Entamoeba histolytica, Trypanosoma cruzi* and *T.brucei* strains; the causing agents of malaria, toxoplasmosis, amoebiasis and trypanosomiasis also called sleeping sickness disease , respectively [17, 34, 53].

The toxicity of gossypol was reported to be linked to its aldehydic groups and it was found its ability to inhibit some enzymes activity such as ATPase, Lactate dehydrogenase, lipid peroxidase and protease of some insects larvae leading to its insecticidal activity against some pests such as *helicoverpa zea* and others [42]*;* the racemic gossypol was found to be more insecticidal than its two enantiomers [153, 154].

VI. GOSSYPOL AGAINST SOME SEXUALLY TRANSMITTED INFECTIONS

Sexually transmitted infections (STIs) are commonly spreading either by sexual intercourses or non-sexual contacts, but open contact with contaminated blood/ body fluids, breastfeeding, or during childbirth and sometimes leading to life-threatening consequences like pelvic inflammatory diseases, ectopic pregnancies, endometritis infertility aspects, possibly even up to morbidity or mortality [50, 71]. According to reports of World Health Organization and Center for Disease Control and Prevention, there has been reported over 30 different pathogen microorganisms including bacteria, viruses, fungi, protozoa parasites involved in sexually transmitted infections [120]. The bacterial STIs include syphilis, chlamydia and gonorrhea; and parasitic

protozoa like trichomoniasis while viral infections include HSV causing genital herpes, human immunodeficiency virus (HIV) causing AIDS, human papillomavirus (HPV) and hepatitis B virus (HBV).

The world health organization (WHO) data in 1999, reported approximately more than 340 million of global distribution of new cases of curable STIs (Fig.13) mainly chlamydia, gonorrhea, trichomoniasis and syphilis every year; these above mentioned STIs counted 75-85% in developing countries by impacting high incidence of sexually transmissions of HIV, HSV, HBV and HPV and leading to 17% of global deaths as the second greatest worldwide death-causing problem challenging all categories of population at high incidence [169]. The availability of effective multipurpose drug therapies targeting the prevention of unwanted pregnancies and STIs became among the WHO priorities.

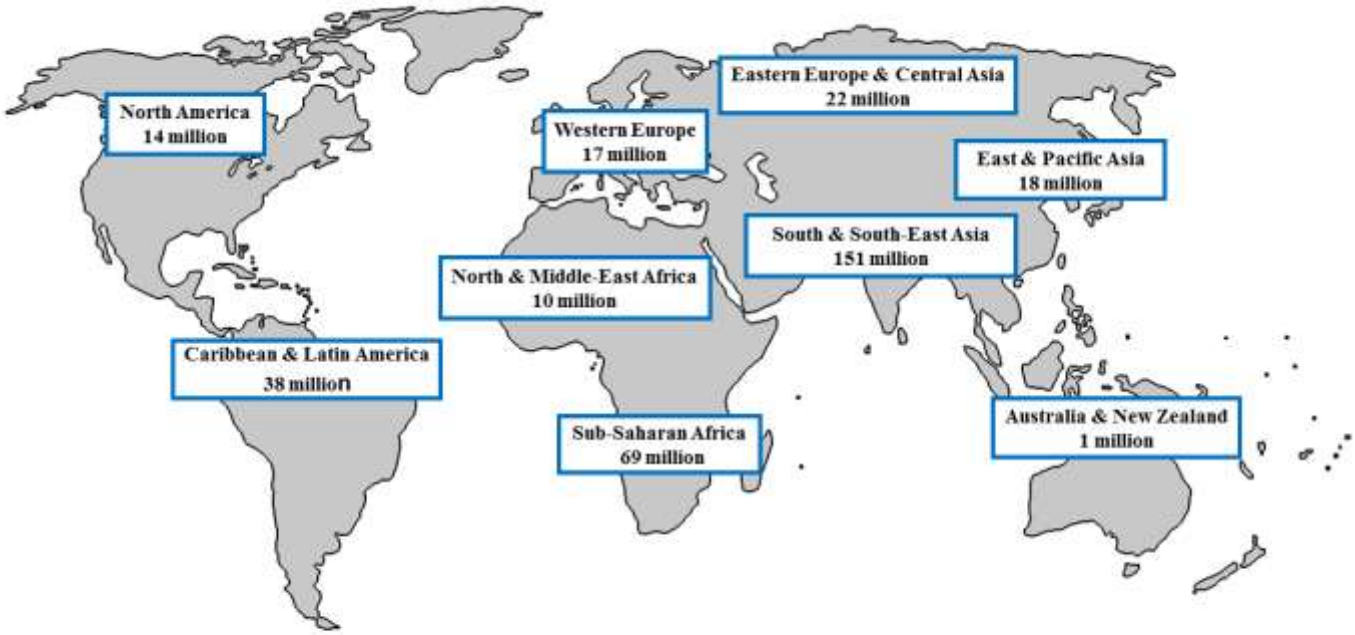


Figure 13: Estimated new cases of curable STIs among adults in 1999.

Recently; in 2015 global statistics showed a huge increase of STIs where it was estimated more than 1 million of new cases of STIs acquired every day making more than 1.1 billion people contaminated with STIs, HIV/AIDS excluded [51]. In 2012, there has significantly been estimated 357 million new infections with alarming increasing rate of curable STIs of chlamydia with 131 million, gonorrhea with 78 million, about 5.6 million, 143 million with trichomoniasis and around 290 million women living with human papilloma virus infections while more than 530 million new cases of HSV infections were reported [7]. The lowest prevalence and incidence with chlamydia was relatively very low in African regions but with the highest one in syphilis infections while the regions of Americas and the Western Pacific were the highest in chlamydia, and the prevalence and incidence of these four infection were the lowest in European and South-East Asian regions

possibly thanks to increased condoms use, lower number of partners, better and socio-economic conditions maintaining the clinical services of STIs mainly in terms of prevention and treatments strategies [120] . Unfortunately; these 4 non-viral infections increased about 17 billion dollars to the health care costs of the country each year, resulted in approximately 108,000 deaths in 2015 in USA alone [7, 102]. By the global prevalence of these most epidemic sexually transmitted infections estimated more than two third of the global population has herpes infections but mostly in dormant stages; worldwidely 0.8% of women versus 0.6% of men have gonorrhea (Table 4).

Table 4: Global prevalence of main viral and microbial STIs by WHO 2015

STIs	Infected population	References
Herpes	4,000,000,000	166
Human papillomavirus	800,000,0000	45
Gonorrhea	500,000,000	117
Chlamydia	450,000,000	117
Hepatitis B	356,000,000	49
Syphilis	45,400,000	160
HIV/AIDS	36,700,000	44

The safe sex or the use of appropriate and effective barrier methods such as condoms or vaginal microbibicidal spermicides is the main known concomitant ways to prevent unplanned pregnancies and STIs transmissions at once, and female controlled methods were reported to be more trustful for easy controls of provision of such dual function therapy. The repeated uses of some vaginal formulations having either bactericidal or/and virucidal properties such as nonoxynol-9 were reported to cause disruption of vaginal epithelium leading to the increasing of susceptibility of the users to some incurable diseases such as HIV, Hepatitis and others [106, 146]. Based on the strange facts that most of infections with trichomoniasis and chlamydia in women are asymptomatic and stay untreated, the new and repeated infections cases between sexually active partners are commonly difficult to control and sometimes may contribute to development of antimicrobial resistances of the STIs-causing pathogens to usual antibiotic therapeutic doses [78, 128]. The longer average duration of infection of STIs contributed to the higher frequency of new cases with about 69% in men versus 48% in women [128]. The use of potentially effective multi-purpose contraceptive agents having properties of acting on these STIs-causing pathogens like gossypol and/or its derivatives may play a great impact on stopping such dramatic infection cases.

In fact; through research tragedies by a group of researchers at the University of Helsinki, Finland reported the ability of gossypol of stopping the multiplication of *herpes simplex virus* type II (HSV-2) causing genital

herpes/warts, continuously their intensive studies using laboratory cultures of human tissue cells, they demonstrated that gossypol inhibits the growth of cultured *gonococci* strains such as *Neisseria Gonorrhea*, the gonorrhea causing bacteria [126]. At the same time, a group of Argentinean researchers have investigated gossypol's effects on *Trypanosoma cruzi*, the protozoa parasite responsible for Chagas disease and reported that gossypol inhibited the parasite's growth, multiplication, motility and some metabolic enzymes during an early stage of the parasite's life-cycle but not the latest stage infectious to humans and this disease was called" neglected tropical disease" which is mostly striking central and south America countries. By this global burden epidemic issue requires some widely strong and emerging multipurpose therapeutic drugs to considerably reduce the alarming rate of these transmitted infections which are a horrific worldwide problem.

Afterwards; based on antibiotic therapies side effects, mutagenicity and microbial resistance to certain antimicrobials, many studies noted that gossypol-containing combinations such as gossypol-metronidazole therapy showed to be as effective as each drug alone against *Trichomonas Vaginalis* and the most efficacious anti-amoebic, especially less toxic to the hosts [23, 32, 100]. Lately, it was published that gossypol and its derivatives are also toxics to protozoa parasites such as *Plasmodium falciparum*[139], *Entamoeba histolytica* [54] and *Giardia lamblia* [105], causing malaria, amoebiasis and giardiasis respectively, and final results demonstrated that the gossypol anti-amoebic activity is mainly based on its content of (–)-gossypol rather than (+)-isomer and racemic gossypol properties[55].Further researches on STIs reported an *in vitro* inhibitory effects of gossypol, formulated as a pessary against *Trichomonas Vaginalis* at IC_{50}=50µM as effective potency [52] and due to an increasing *T. vaginalis* resistance to metronidazole as its effective medication, alternative therapies must to be investigated and gossypol can be trusted to be safely effective at its spermicidal doses [123], assumed that most agents inhibiting growth of *Trichomonas vaginalis* can also inhibit *candida albicans* [151] but, further confirming studies are still suggested.

More interestingly; the strong inhibition of viral replication of human immunodeficiency virus type 1 by gossypol and some of its derivatives has been reported in some studies [138] and (-)-gossypol was found to be the best and effective inhibitor of HIV-1 compared to the (+)-gossypol (with IC_{50}=5.2µM) Vs. IC_{50}=50.7µM, respectively) or the racemic mixture [89]. Gossypol also showed antiviral activity in multiple enveloped viruses including herpes simplex virus type II (HSV-2)[172], influenza virus, and para-influenza virus [75]. Royer *et al.* (1995) studied inhibitory effects of main gossypol derivatives on HIV-1 and it was noticed that 1,1'-dideoxygossylic acid (DDGA) was the most effective inhibitor of HIV-1 replication and growth *in vitro* with EC_{50} less than 1µM while 1,1'-dideoxygossypol (DDG), 8-deoxyhemigossypol (DHG) and 1,1'-dideoxygossylic acid (DDGA) showed some antiviral activities less effective than that of gossypol except 8-deoxyhemigossylic acid (DHGA) which was ineffective against HIV-1. Meanwhile, all those gossypol derivatives showed very lower affinity with albumin than gossypol, this would possibly enhance the antivirus activity of the gossypol derivatives *in vivo*. In the same context of gossypol derivatives, periacylated gossylic

nitriles, gossylic nitrile 1,1-diacetate/divalerate and gossylic iminolactone/lactone have showed inhibition of replication of most enveloped viruses and low toxicity compared to gossypol either with less interferences with proteins or with reduced toxic mechanisms to the host cells and leading to low gossypol-related systemic toxicity [9, 133, 138].

The combination of gossypol and the neutral amino acid with aliphatic group derivatives such as *l*-valine, *l*-alanine, *l*-leucine and others were tested for their antiviral activity and their toxicity levels; the results showed that the combination of (-)-gossypol with neutral amino acids conjugates play a wide and strong anti-HIV-1 by interfering with viral replication through mechanism of inactivation of reverse transcriptase. By mechanism of action, it was demonstrated that *l*-alanine conjugated with (-)-gossypol inhibited HIV-1 entry into the host cells neither by blocking viral CD4 binding site nor interacting with CX-CR4 viral co-receptor but by inhibiting and blocking the formation and activation of the complex between host cell and viral receptors by binding and covering different core domains of viral proteins [9]. Gossypol showed a wide antiviral activities through its mechanism of interactions with viral coat by completely inactivating RNA-containing viruses such as parainfluenza and influenza viruses (H1N1, H2N2, H3N2 and H5N1) [75, 177], Newcastle disease; and DNA-containing viruses such as herpes virus strains and virus of Aujeszky's disease but no effect on non-enveloped polioviruses [38, 137]; its ability of suppression of some arboviruses such as sindbis virus, encephalitis type viruses and West Nile fever virus was also underlined [114]. The potentiality of developing an effective gossypol-based drugs will respond to the emerging worldwide health problems associated with sexually and non-sexually viral and non-viral transmitted infections with high transmission rate including human papillomavirus and hepatitis B virus even though some have vaccines; the researches on the activity of gossypol and its novel derivatives on such kind of dangerous viruses could be taken into considerations for deep investigations.

VII. CONCLUSION AND RECOMMENDATIONS

Gossypol is a versatile and highly reactive compound with multiple biological properties required for development of multi-function drugs for various human microbial and viral infections combined with its contraceptive effects but, its systemic toxicity is a big limit from its clinical use, the deep studies of appropriate doses and the clear investigation of the nature of active compounds are still needed for its therapeutic exploitation. Notwithstanding that many researches focused on antifertility effects of gossypol as single factor of reproductive health, but evidently sexual health goes with effects of STIs, many *in vitro* and *in vivo* intensive studies concerning inhibitory effects of gossypol and its derivatives on most human pathogenic microorganisms mainly causing STIs are still highly required with advanced assays of their mechanisms of action which may be useful for further design of successful development of gossypol into potentially advanced dosage forms for future clinical interests, more interestingly using micro-encapsulation with carriers like nano-liposomes or nano-micelles advanced technologies.

In fact; There still an urgent need of more studies establishing mechanisms of action of gossypol and its derivatives for its clinical potentials as antimicrobial agent capable of preventing most STIs and having drastic antiviral activity possibly with safer use as vaginal contraceptive dosage forms without interactions with hormonal functions, hope the information collected from this review will provide to the researchers the valuable data showing an emergency of a wide and advanced therapeutic exploration and synthesis of other different gossypol derivatives for further design and development of effective gossypol-based drugs for multiple clinical uses in the future.

VIII. CONFLICTS OF INTEREST

None of the authors declared any kind of conflicts of interest.

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