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Potential Mechanisms of Action of Gossypol, an Anti-spermatogenic Male Contraceptive Agent: A Review

Mutabazi Francois[1,2], Mubano O. Clement[1], Tang Luhong[1*], Wang Honghua[3*]

[1]*Laboratory of Natural Pharmaceuticals, School of Pharmaceutical Sciences, Jiangnan University, P.R China*

[2]*School of Medicine and Health Sciences, University of Rwanda, Kigali-Rwanda*

[3]*Center of Reproductive Medicine, Wuxi Maternity and Child Care Hospital, Wuxi, P.R. China*

[*]***Corresponding author:*** *Tang Luhong, Laboratory of Natural Pharmaceuticals, School of Pharmaceutical Sciences, Jiangnan University, P.R. China, E-mail: tangluhong@msn.com*

Wang Honghua, Center of Reproductive Medicine, Wuxi Maternity and Child Care Hospital, Wuxi, PR China, E-mail: wxwhh72@163.com

ABSTRACT

Gossypol, a herbal polyphenolic compound was reported to exhibit antioxidant, anti-parasitic, lowering plasma cholesterol agent, anticancer, anti-microbial, antiviral and antifertility agent but clinical uses of gossypol and its derivatives is still attracting more attentions and the complexity of its mechanisms of action are still questionable. This review summarizes most of all results of comprehensive studies conducted on anti-spermatogenic activity of gossypol obtained from all commonly known and published scientific research websites, journals and books. The present research review discussed and re-evaluated critically the published data concerning anti-spermatogenic mechanisms of action of gossypol as promising male contraceptive agent highly in need to control male fertility, especially trusted based on its biological potentials of therapy and prevention of some human chronic and resistant diseases like cancers, and some sexually transmitted infections including Human Immunodeficiency Virus. This review is expecting to provide a new and deep understanding on pharmacological and toxicological effects of gossypol based on its mechanisms of action supporting the highest emergency need of innovative development of gossypol-based multipurpose contraceptives.

Keywords: Gossypol, Spermatozoa, Anti-spermatogenic agent, Contraceptive, Toxic effect.

INTRODUCTION

Gossypol is a polyphenolic dialdehyde extract isolated from different parts of cotton plants specifically *Gossypium* sp. of the family of Malvaceae, it was lately synthesized as 1,1'; 6,6'; 7,7'-hexahydroxy; 5,5'-disopropyl; 3,3-dimethyl; 2,2'-binaphtalenyl; 8,8'-dialdehyde (Figure 1) [1,2]. Gossypol showed two isomers and the (-)-gossypol was reported to be more biologically active than (+)-gossypol [3] but the limited use of cottonseed oil and meal known to contain some traces of gossypol as a dietary source of protein were reported to be toxic to both non-ruminant and monogastric animals [4] particularly, its increasing pregnancy loss, decreasing pregnancy rate in females and causing males infertility effects by negatively interfering with spermatogenesis in some families using cottonseed oil for cooking have been reported to be related to high free gossypol-containing diets [5].

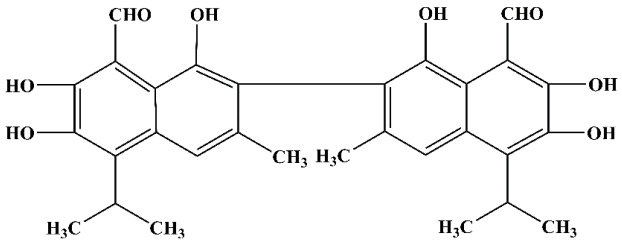

Figure 1: Chemical structure of gossypol.

The chemical properties of gossypol showed its high reactivity with either other plant compounds or any functional groups to form a complexed or bound gossypol which is actively different from free gossypol known as the most toxic form. The phenol groups of gossypol form ethers and esters; whereas the aldehyde groups are highly susceptible either to react with amine groups of amino acids, proteins by forming Schiff's bases or with organic acids compounds to form unstable heat labile products [6]. More interestingly, the multiple reactivity of gossypol is sustained by its ability of interchanging its functional groups into gossypol tautomeric forms (Figure 2) dependently to the nature of solvents; by reacting as either an aldehyde, ketonoid or hemiacetal compound [1,6].

Furthermore; many research studies related to contraception have showed pharmacological uses of gossypol, especially as an oral male contraceptive and vaginal spermicide for fertility regulation from many decades [7]. There has also been reported versatile clinical applications of gossypol and its derivatives including anticancer such as breast, prostatic cancer and endometriosis [7], antiviral including HIV [8,9] and herpes simplex [10], antimalarial effects [11,12] antioxidant [13,14], insecticidal [15] and antimicrobial agent [16] but therapeutic establishment of effective dose levels with guaranteed safety is still challenging and limiting clinical uses of gossypol.

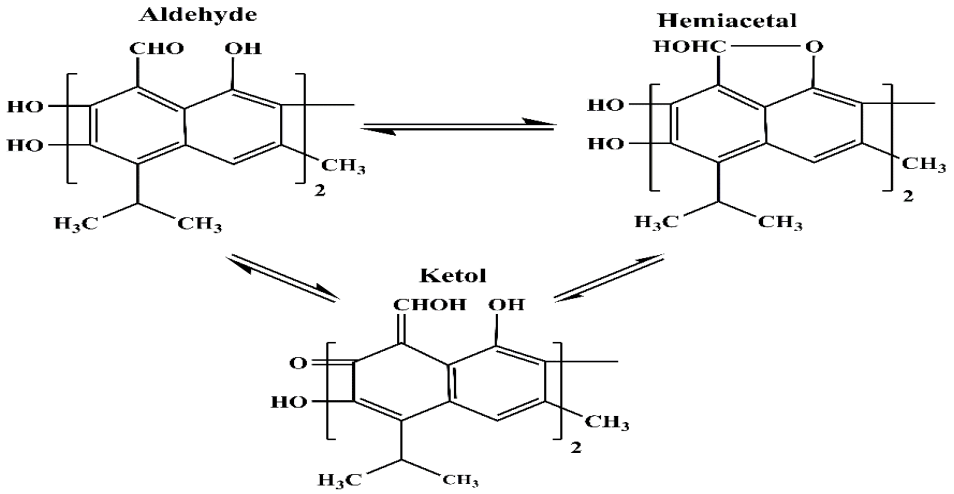

Figure 2: Tautomeric forms of gossypol.

In fact, cytotoxicity of gossypol showed to be strictly selective to certain animal cells but no genotoxic interactions were reported, and recent researches revealed antimetastatic and antiproliferative activities of gossypol and its derivatives in various human tumor cells [17] such as prostatic, colon carcinoma and leukemia cells [18], and these could be appreciably useful as multipurpose drug as male contraceptive agent with antitumor activities especially when used as anti-spermatogenic agent may reduce or prevent risks of prostatic cancers for males as it was reported to inhibit PC-3 human cancer cell lines [19]. However, toxicity of gossypol was reported to be mainly based to its high reactive ability of binding macromolecules either before or after its absorption in the body through different modes of interfering with enzymes either by binding and/or changing the ionic or enzymatic character of its reactive action sites or through reacting with the substrates and blocking the enzymatic activity.

LITERATURE REVIEW

Toxicity of Gossypol

Gossypol as a liposoluble compound is readily absorbed from the gastro-intestinal tract [20,21], due to its high affinity of binding to amine groups of amino acids or proteins, and readily to iron-containing products [20,22,23] even though the clear mechanism of action is not well known, but gossypol renders many amino acids unavailable by the formation of Schiff's base-type derivatives as well as additional protein/gossypol interactions [20,23]. It also importantly meddle in enzymatic reactions required for many biologic processes such as interfering with the cellular ability to respond to oxidative stress and inhibition of oxygen release from hemoglobin [17,21] through which make its conjugation, metabolism, and urinary excretion somehow limited, and consequently; gossypol is mostly conjugated in bile and eliminated in the feces [21,24,25].

The toxic manifestations of gossypol may affect the renal, reproductive, hepatic, cardiac and other organs [25] where cardiac necrosis is resulted from acute heart failure caused by prolonged exposure [26], and hyperkalemia associated with heart failure resulting from cardiac conduction failure can result in quick death [27]. Gossypol damages the liver cells [28], disturbs blood cells and molecules functions leading to hematologic effects 8 like stimulating the apoptosis-like erythrocyte death "eryptosis" by increasing intracellular calcium (Ca^{2+}) inducing the activation of Ca^{2+}-sensitive potassium (K^{+}) channels, hyperpolarization leading intracellular osmotic pressure and K^{+} loss (Figure 3) [29] causing to cell shrinkage while increase in

Ca^{2+} concentrations leading to cellular membrane scrambling after exposure and modification of the cellular membrane phosphatidylserine [30] possibly contributing to anemia [31]; while reproductive effects affecting spermatogenesis, sperm counts and spermatozoal motility through various mechanisms [21,22,32,33] in male animals while in females it showed promoting irregular menstruations, pregnancy or embryonic disruption through probable mechanisms including endocrine effects on the ovary as well as a direct cytotoxic effect on the uterus during embryonic implantation and development [25].

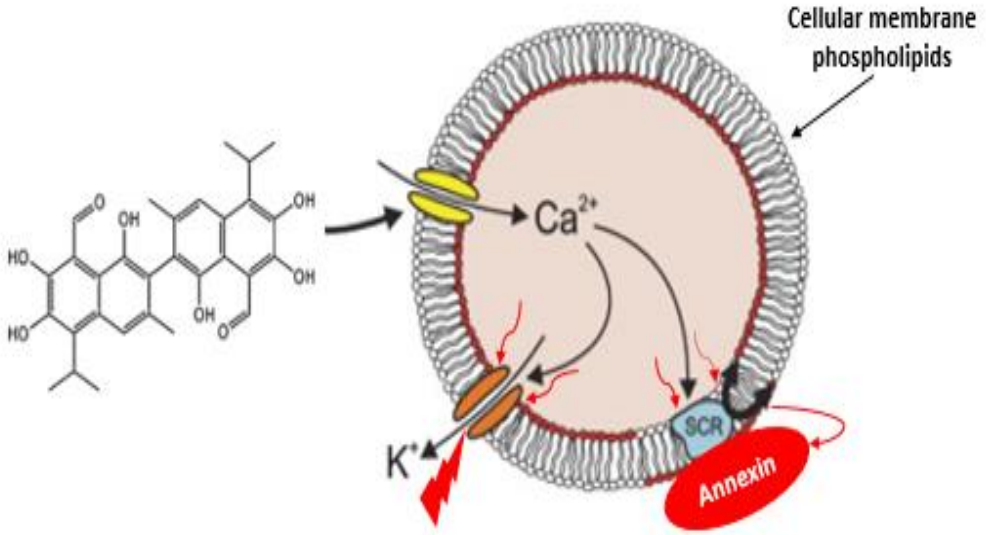

Figure 3: Mechanism of gossypol-induced suicidal erythrocyte death.

In fact, some studies reported the animals exposed to prolonged or excessive ingestion of free gossypol are characterized by increasing sensibility to stress, reduced growth and weight gain [34]. However, gastro-intestinal, pulmonary and heart failure symptoms have been frequently observed in different animals [22,25,27] but, most of toxic effects of gossypol were generally dose and species-dependent. Consequently; gossypol was reported to act as prostaglandins E (PGE) biosynthesis stimulating agent [35,36] and Na-K-ATPase inhibitor [37] leading to renal potassium loss thereby causing hypokalemic paralysis in some subjects [38,39]; as the mechanistic hypothesis of gossypol-induced hypokalemia cycle is illustrated in Figure 4 which was established after some scientific evidences [40,41] in which some studies revealed the short-term supplementation of prostaglandins synthetase inhibitors like aspirin or indomethacin to effectively normalize serum potassium and urinary PGE levels [35,37]. A very few number of cases of gossypol-induced sterility was reported to be due to a strange interruption of spermatogenesis cycle even after a period of gossypol withdrawal [41-44] but he recent clinical studies on human males with low dose gossypol concentrations did not display any life-threatening adverse drug effects [45] as the highest dosage that patients tolerated after oral ingestion has been proven to be 0.8 mg/kg of body weight/day for 6 weeks [9].

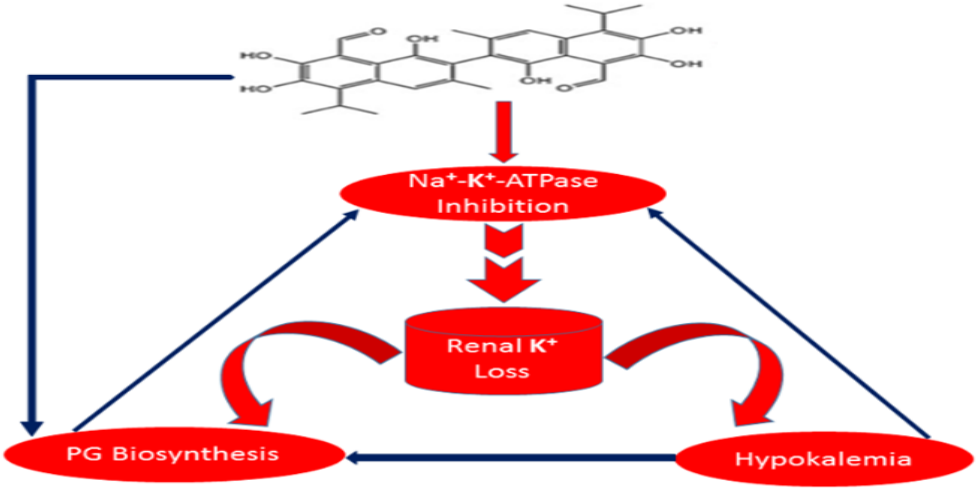

Figure 4: Mechanistic cycle of development of gossypol-induced hypokalemia.

In conclusion, gossypol was only toxic once its high concentrations get into contact with the blood in circulatory system; some studies tried to counteract this toxicity either by mixing it with Selenium and Ferrous to alleviate the harmful hematological effects [46] or suppressing its aldehyde groups which stimulated much interest in its derivatives resulting from changing these groups [21,47]. Recently, some studies showed some interesting synergistic contraceptive effects when gossypol is combined with other steroidal hormones [48,49], and topical formulations have also been developed in purpose of avoiding any contact of gossypol with circulatory system for a safer use in topical drugs formulations [50,51], and concluded that gossypol incorporated into l-ascorbyl palmitate coagel does not penetrate deeper through the skin [52]. On the other hand; gossypol revealed to be neither irritant, sensitizing nor presenting any other toxicity once used topically to the monkey and humans for contraceptive purpose [21,22].

Pharmacokinetics and metabolization of gossypol

The toxicological studies in different laboratory animals [2,53] found out that systemic toxicity of gossypol is dose and species-dependent even though the ingested amount and period of exposure mostly increase its toxic effects. It was reported that the half-life ($T_{1/2}$) of a single dose of racemic gossypol in male human is about 10-11 days and (+)-gossypol takes 29 times longer than (-)-gossypol [54] whereas dogs received intravenous single injections showed elimination $T_{1/2}$ and volume of distribution of (+)-gossypol to be significantly 5-6 times more than that of (-)-gossypol [55]. After gossypol administration in rats, it was found in most major visceral organs and in the brain [56] and some studies concluded that gossypol is metabolized by various microsomal enzymes into several metabolites mainly quinones [2]; a great difference in species sensitivity to different effects in animals were assumed to be possibly due to differences in metabolism. Many animal studies reported cardiac irregularity leading to death either due to slow liberation or weak fixation of oxygen in the blood [57], and the toxicity index could not be specifically determined as it highly depends on each animal model [2].

In fact; metabolism and excretion of gossypol is complex to determine as the parameters are highly species-dependent, and its absorption starts in gastro-intestinal tract [2]. By this fact, the assessment of gastric side effects of gossypol was conducted during clinical study with gossypol-coated enteric tablets; a great difference in systemic side effects and antifertility effects were found out with non-coated tablets, the most important difference in response was apparently due to reduced gastric absorption of

the enteric-coated forms [58]. After oral or parenteral administration of gossypol or its analogues, the absorption to the systemic circulation was found to be time and species-dependent; the high molecular weight, anionic polar behavior with aromatic rings of gossypol make it soluble in biliary secretions, absorbed in intestines mainly excreted *via* fecal route [55].

Gossypol containing radioactive carbon-labeled atoms allowed deeper study of excretion through recovery and it has interestingly been showed that a large amount of gossypol recovery was obtained from expiration as CO_2 [58], and this allowed the researchers to conclude that the decarbonylation is a major route of gossypol biodegradation in rats, and estimated that the biological half-life of oral radioactive C^{14} of gossypol in their body was 48 hours but, it was noticed the binaphtalene nuclei of gossypol molecule were not degraded and only the formic carbon was metabolized to CO_2 [59]. In pigs, the metabolism of C^{14} gossypol was approximately the same as the one obtained in rats [60] but the decarboxylation was not the main degradation route of gossypol in pigs as indicated that the tissues deposit of gossypol was more than 15 times greater that its expiration recovery rate, and the researchers also reported that the higher gossypol toxicity in pigs than in rats might be related to the difference in tissues deposition and decarbonylation of gossypol in these species [59]. More interestingly, approximate results were obtained by Xue et al., but, it showed that after a single oral administration of radioactive C^{14}-labeled gossypol in rats, the biological half-life in the body was high in different organs and the maximum peak of radioactivity occurred in 4-9 days of post-administration [61,62].

It has been pointed out that the distribution and excretion of gossypol in rats and monkeys were quite similar, and that in rats the half-life of gossypol in the GIT was 9.6 hours, indicating lower rate of absorption [63]. Tang et al., compared the metabolism of C^{14} gossypol in mouse, rats, dogs, and monkeys and found that the pattern of distribution of activity in various tissues following a single dose of gossypol was much alike in the four species. Among them, the specific activity of the heart was the highest in dogs, whereas in testis was the highest in the rats; the circulatory half-life was the longest in dogs, and the fecal excretion was higher in monkeys and rats than in the other two species [55]. Other researchers indicated the greater excretion and lesser absorption of gossypol in monkeys than in dogs. The two groups of authors concluded that the discrepancies of the metabolism in different species might have important bearing on their differential responses to the antifertility and toxic effects of gossypol [64].

Effects of diets on antifertility and toxic activity of gossypol

It was surprisingly found out that contraceptive doses of gossypol selectively damaged the spermatogenic cells first and left other vital organs even containing the highest concentrations of gossypol comparably to the one obtained in testis; an unaffected peaks strongly in favor of a specific vulnerability of the testicular cells to the action of gossypol [63], and co-administration of ferrous supplements with gossypol reduced tissue deposition, increased fecal excretion, and shortened the half-life in the body by accelerating the respiratory elimination of gossypol [37]; these might be explained by the postulation that iron catalyzes the decarbonylation process of gossypol [58] as iron and proteins form non-absorbable and stable complexes [6].

It may be worth to mention that magnesium-gossypol complex has been shown to be anti-spermatogenic in rats with relatively low toxicity compared with the one played by gossypol alone [65,66]. In addition, selenium [46,67] or external potassium [68] supplements showed to counteract hematological effects of gossypol while some vitamins like B6 or E may reduce gossypol-induced GIT drug adverse effects [69,70] while promoted binding of free gossypol by promoting microbial fermentation with some yeasts or fungi are also promising to reduce its toxic effects [71-74]. Even though, it is not yet well

known either if the bound gossypol can be absorbed through the intestines or can be freely released back by the microorganisms [75,76] for late biliary glucuronides and sulfates conjugation [77].

Unfortunately, it is not yet well known if consumption of cottonseed oil can provide any rate of anticancer activities of gossypol based on its cytotoxicity and antiproliferative effects as reported in some recent *in vivo* studies questioning its effectiveness against some human prostatic, breast and ovarian cancer cells [66], inhibition of apoptosis and growth of human carcinoma cells [78] through different mechanisms including oxidative stress [79] and some others which are still not clearly known but systemic toxic effects depending on ingested amount and time of exposure [80-82].

Toxicokinetics and poisoning of gossypol

The poisoning effects resulting from animal feeding with high concentration of gossypol were observed in different animals such as dogs [83,84], goats [85], chicks [86], sheep [87] and pigs [88], mostly appearing not later than 3 months of ingestion [89-93]. The animal studies showed that young animals are more susceptible to gossypol toxic effects than adults [89], and monogastric animals like rodents, pigs and birds are very sensitive to gossypol poisoning compared to ruminants animals [90,94-96]. The absorption of hazardous concentrations of gossypol showed similar general signs of acute toxicity in all animal species mostly such as respiratory distress, weakness, anorexia, reduced body weight gain, kidney and liver damage [89,92,95], death which may occur after long-term intoxication [87,97,98]; heart failure was mostly reported in lambs [87], dogs [92] and calves [98]; anemia and pneumonia were also observed in some animals.

Moreover, gossypol and its derivatives were reported to be highly hepatotoxic in different animals at different doses (Table 1) by causing liver cells degeneration and ascites [99-104]. In some cases, small concentrations of free gossypol promoted hepatic lipidosis and increase in gamma glutamyltransferase enzymatic activity [100]; other hepatic ultrastructural changes were clearly observed through microscopic studies [88,99,104]. Gossypol easily binds amino acids and minerals such as iron by which gossypol-iron complex may inhibit absorption of this ion and some other metal compounds; the iron deficiency may consequently stimulate erythropoiesis and erythrocytes fragility [75,105-107] contributing to anemia [108], tissues antioxidant effect [109-112] and decreasing cardiac muscles contractions [113].

Table 1: Some reported gossypol-induced hepatotoxicity in laboratory animals.

Tested animal	Free gossypol dose	Administration route	Treatment duration	Reference
Rats	5-10 mg/kg/BW	Intraperitoneal	10 days	[103]
Rats	20 mg/kg/BW	Intraperitoneal	10 days	[60,103]
Rats	25 mg/kg/BW	Intraperitoneal	Single dose	[101]
Rats	25 mg/kg/BW	Intraperitoneal	Single dose	[102]
Chickens	0.1% in feedstuff	oral	21 days	[100]
Broilers	0.4% in feedstuff	oral	21 days	[99]
Dogs	4 mg/kg/BW	oral	<10 days	[64]
Monkeys	4 mg/kg/BW	oral	24 months	[64,114]

Consequently; through studies with different human males [115] and other animal species showed great differences in their sensitivity to the antifertility effects of gossypol, and it decreases in order starting from hamsters, rats, pigs, monkeys to dogs while rabbits and mice seems to be insensitive [56,64,116-119]. The effective dose for hamsters was about 5-10 mg/kg per day for 6-12 weeks, fertility recovery occurred 4-14 weeks after drug withdrawal [117-119] while the effective dose for rats was 10-30 mg/kg per day for 3-10 weeks, infertility onset and fertility recovery have showed to be dose-dependently ranging between 3 to 12 weeks [56,116,120,121]. In rats, 5 mg/kg per day for 6 weeks [116] led to infertility but 6 mg/kg per day for 5 weeks mostly showed to be ineffective with some individual variations where long-term treatment might cause complete atrophy of the testicles seminiferous tissues leading to sterility [122,123] whereas in dogs antifertility doses caused direct death [64,124].

The rabbits are hardly affected by antifertility effects of gossypol up to 10 mg/kg of body weight per day for 14 weeks and even when the same dose prolonged treatment up to 77-250 days did not induce any antifertility effect, neither sperms concentration, motility for males nor the pregnancy rate, implantation site for female rabbits were affected but severe toxicity resulting in eventual death occurred [119]; the same case, an oral dose of 15-30 mg/kg of body weight per day did not play anti-spermatogenic effects in male mice but it inhibited pregnancy within 15 days of gestation in female mice and rats [116,118]. Monkeys are moderately sensitive to the anti-spermatogenic effects of gossypol, where only a dose of 4 mg/kg per day for 24 months or 10 mg/kg per day for 6 months completely inhibited spermatogenesis through decreasing the sperms count and motility but in reversible way dependently to dose and duration of exposure [54,125] (Table 2).

Table 2: Reported gossypol-induced toxic effects from different animal studies.

Tested animal	Dose (mg/kg/d)	Treatment duration	Toxic effects	References
Rats	10-20	6-14 weeks	-Liver cells damage & necrosis -Digestive troubles	[116,118,126]
Rats	25	26 weeks	-Liver damage -Body weight loss	[127]
Rabbits	10-16	14-41 days	-Deep weight loss -Weight loss and death	[128]
Rabbits	20-80	8-84 days	-Pulmonary and hepatic congestion -Limbs paralysis & Death	[119]
Dogs	1.5-5	50-140 days	-Hepatic and renal congestion -Pulmonary edema and dyspnea -Heart failure causing death	[124]
Dogs	30	18-28 days	-Severe anorexia and vomiting -Cachexia and anemia causing death	[124]
Monkeys	4-12	4-14 months	-Weight loss -Liver swelling	[129]
Monkeys	05-Oct	4 months	-No clinico-pathological side effects	[64]
Humans	15-50	≥ 6 months	-Hypokalemia around 0.75%	[56]

In fact; among laboratory animals tested, dogs and rabbits showed to be the least tolerant to gossypol toxicity while the monkeys and rats are the most tolerant even though deep studies reported the hamsters to be less tolerant than rats but female

hamsters are eventually less tolerant to gossypol compared to males; monkeys tolerate toxicity of gossypol but they are generally sensitive to its anti-spermatogenic effects [117,130]. Rats are normally sensitive to anti-spermatogenic activity of gossypol and the ordinary antifertility dose of 7.5 mg/kg/day for 12 weeks is non-toxic to rats up to its 5-9 times around 30 mg/kg per day for 16 weeks reported to cause some hepatic lesions leading to minor cardiac and renal toxic manifestations in few subjects [117]. More interestingly; rabbits and dogs are very sensitive to toxic effects of gossypol but not to its anti-spermatogenic dose, most of rabbits are still fertile up to their death while for dogs even the small doses induced serious renal, cardiac and hepatic failures causing fatal cachexia leading to direct death [64,118,124,128].

Anti-spermatogenic mechanisms and site of action of gossypol

It was firstly in 1978, the Chinese researchers investigated effects of gossypol on spermatogenesis and clinical trials of its effectiveness as first oral male contraceptive were undertaken [56]. Some emerging investigations on pharmacological effective doses were competitively conducted in different parts of the world mainly in china [131-134], Japan [135], and Brazil [136,137] as oral male contraceptive agent and intensively studied *in vivo* as vaginal spermicide in Finland [16]. Through highlighting the possible interactions of gossypol with sperms or spermatozoal genetic material as potential direct or indirect genotoxic effects involving in male germ cells to clearly understand the mechanistic hypotheses of antifertility effects on males [131] without interference with hormonal mechanisms but through direct interactions with sperm cells maturation and production [138] especially decreasing sperms motility, counts, and spermatids maturation rather than damaging Sertoli and leyding cells [100,138], following by increase in number of abnormal sperms head and morphologies without causing significant hormonal disturbances as reported in animal studies and clinical trials [129,137-139].

The ultrastructure studies have shown that spermatozoa mid-piece is one of the important targets of gossypol as it is one of the cellular part rich in mitochondria [9,96,140,141], and this also supported the fact that potential mechanism of gossypol is strongly based on mitochondrial uncoupled oxidative phosphorylation by altering sperm cells' ATP generation cycle and inhibiting enzymes involving in acrosomal reaction during spermatozoa maturation [142-144]. More interestingly; *in vitro* studies with low concentrations of gossypol inhibited sperm cells specific enzymes involved not only in oxidative respiratory metabolism, glycolysis and spermatozoal capacitation [145] but also binding to tubulin; damaging spermatozoal flagella inhibiting its anti-motility [146].

Furthermore, during the uncoupled oxidative phosphorylation caused by gossypol through spermatozoal respiratory inhibition by reducing ATP production [145], the mitochondria was showed to be the most damaged as target cellular organelle [37,141,147-152]. Lately, different studies postulated the hypothetic facts of anti-spermatogenic mechanism of gossypol were interestingly developed based on the facts that gossypol inhibits Na-K-ATPase activity and lactate dehydrogenase-X (LDH-X) which are suggested to be the main molecular and enzymatic sites of gossypol-induced hypokalemia [153] and low incidence of irreversible infertility [154] as both of these enzymes exist in mitochondria [152] the same as pyruvate dehydrogenase which is also susceptible to inhibition of gossypol and its derivatives [155], and it was also reported to interfere with the synthesis of nuclear histones from lysine to arginine-rich transition mechanism during the maturation of late spermatids into spermatozoa as an indispensable last step of spermatogenesis [156].

Some important evidence-based factors contributing to mechanism of action of gossypol were defined by the facts that gossypol lowers K^+ content of the spermatozoal membrane resulting from inhibition of Na-K-ATPase [37,157], testicular ATPase and spermatozoal acrosin [158] and testis-specific LDH-X [149-151], succinyl-CoA synthetase and NAD-isocitrate

dehydrogenase [159,160] activities, strong inhibition of utilization of spermatozoal fructose [161] and its cytotoxicity *in vitro* [162] were convincing and allowed to confirm the proposition of Qian et al., by which prostaglandins might be one of the strongest substances participating in the anti-spermatogenic mechanism of gossypol [37] and its effects of inducing hypokalemia in some subjects after it has been demonstrated that prostaglandin synthetase inhibitors such as aspirin or indomethacin showed a strong reversing power of interfering with either anti-spermatogenic, toxic effects or K deficiency caused by gossypol administration in few subjects [36,37] which is considered as an individual or species sensitivity (Table 3).

Table 3: Some mechanisms of action on anti-spermatogenic effects of gossypol.

Mechanism of action	**References**
1. Uncoupling mitochondrial oxidative phosphorylation leading to reduced spermatozoal ATP production cycle.	[49,52,77,84]
2. Inhibition of testicular and spermatozoal specific LDH-X, pyruvate DH, succinyl-CoA synthetase and NAD-isocitrate DH enzymes.	[156,155,159,163]
3. Impairing spermatozoal and testicular ATPases activity.	[160]
4. Inhibition of spermatozoal acrosin and acrosomal proteinases.	[143,158]
5. Inhibition of nuclear histones synthesis during transition mechanism for spermatids maturation and capacitation	[78,156]
6. Inhibition of spermatozoal fructose utilization and modifying spermatozoal membrane permeability.	[39,161,164]
7. Inhibition of spermatozoal metabolism and respiration through increasing cAMP/cGMP ratio.	[164]
8. Promoting Sertoli cells damages by decreasing formation of androgen-binding proteins.	[165]
9. Inhibiting spermatozoal motility and damaging cellular flagella by binding tubulin.	[146,161,166]
10. Increasing renal, plasma and testicular prostaglandins levels.	[35,167]

Moreover, the spectrophotometric analysis of DNA of sperm cells from men treated with gossypol revealed no detectable genetic changes in overall DNA content [168]; but some highly sensitive techniques have showed that gossypol inhibits synthesis of some nuclear proteins and histones which are involved in maintaining the three-dimensional structure of sperm-cell DNA and genetic stability [169], also the suspicious possibility of sperm cells' DNA damages resulting from unbalance of ratio between nuclear proteins and DNA was raised even though the influences of these effects are not yet clearly understood in human sperm cells [27]; some other mechanisms which may involve in indirect interactions of gossypol with DNA such as the ability of gossypol to interfere with cell division by causing alterations of either sperm cells nucleic acids [161] or microtubules function [157], and some others processes which may be suspected to alter sperm function and structure have not yet been studied for clinical implications.

Difficulties and perspectives of using gossypol as potential male contraceptive agent

The different studies reported a considerable contraceptive efficacy of gossypol to be higher than 99% even though some subjects experienced some adverse drug effects such as hypokalemia, transient muscle fatigue, slight disturbance of FSH,

liver and renal impairments but few users claimed permanent infertility which may occur after long-term or high doses use of gossypol [139,170]; and the development studies of a non-steroidal male contraceptive agent with lower side effects based on gossypol and its derivatives is still going on mostly focusing on low dosage formulations to minimize its toxicity [138,171].

The general antifertility dose of gossypol has been estimated to be 20 mg daily for more than 2 months [56] but the significant variations in lag-times for gossypol to achieve a significant and stable antifertility effect of maintaining human males' semen parameters below the infertility levels. More interestingly; this deep analysis was reported after some studies on Chinese men where 30 days was the shortest lag-time [172] and 1.5-4 months [173], and then around 4 months were enough to produce the same effects in non-Chinese users [137] while globally, a strange range of 2-9 months was required using low doses of gossypol formulations [45]. However, many factors may be contributed to the differences in lag-time, by taking into considerations that even though the same doses of gossypol were used but mostly either the body weight of the users or the used forms of gossypol which could supply to the body unequal amounts of pure gossypol based on their different molecular weights.

Furthermore, late studies showed that dietary oil enhance gossypol effects as gossypol is liposoluble may be easily absorbed by the target tissues while the both forms of chelated gossypol to ferrous cations and bound to proteins counteract the antifertility of gossypol [45,174]. Therefore, some difficulties of using gossypol as a male contraceptive in effective and safe way lies on the facts that it would be required to determine an appropriate dose of gossypol to be administered to each individual and to be thoroughly adjusted according to individual proteins intake as same dose and form of gossypol may produce safe antifertility effects to one person taking high proteins and may cause harmful effects once given to another one under low protein diet. Consequently, individual dose adjustment and high monitoring of sperms' fertility parameters such as sperm counts and motility, and simultaneous follow up of other vital parameters after each gossypol administration must be closely regulated for each user.

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

The numerous studies outlined above show that gossypol has preventive and therapeutic potentialities as multipurpose contraceptive without hormonal perturbation effects but by mainly by acting on spermatogenic cells, and more interestingly acting on various human cancers as a global health threat even though much more further studies focusing on development of its appropriate and efficient dosage forms for a trustworthy advanced clinical uses are still in needed. Moreover, more clinical trials which involve combinations of gossypol with other hormonal contraceptives or chemotherapeutic agents for synergistic activities are encouraged; the application of nanotechnology for gossypol-based drug dosage formulations such as advanced encapsulated forms using nano-carriers like nano-liposomes or nano-micelles would be of great significance to improve its potential activities better than it was reported before from *in vitro* to *in vivo* studies. In fact, even though the male reproductive toxicity is well known, there is a huge need of more studies to understand more of its effects on females, and extensive researches are still required to develop more efficient and inexpensive technologies to reduce gossypol toxicity for clinical human use.

DISCLOSURE OF INTEREST

The authors report no conflict of interest.

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