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Gossypol as an Anti-spermatogenic Agent

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ABSTRACT
In our overpopulated world, the development of men contraceptive method is require that will allow both men & women to take an active role in family planning which is mandatory. Contraception is an accepted route for control of population in world.

Hormonal contraceptives methods have focused on women. Male contraception by means of hormonal & non hormonal method is an attractive alternative. Hormonal methods of contraception using testosterone shows good results. Non-hormonal reversible methods of male contraception like inhibition of sperm is very promising. 40-45% of pregnancies across the world are unplanned. Several effective female contraceptive methods have been developed, contraceptive choices for men are still limited like condoms & vasectomy. Over the years, numerous studies have been performed to develop male hormonal & non-hormonal safe & effective contraceptives.

The present research discussed & re-evaluated the data concerning anti-spermatogenic mechanism action of gossypol as male contracepting agent highly in need to control male fertility.

Keywords: Male contraception, Hormonal contraception, Non-hormonal contraception, Contraception, Vasectomy

I. INTRODUCTION

Access to a wide range of effective methods of contraception is an important element of reproductive health.The development of new methods of contraception, such as the combined oral contraceptive pill in 1960 and more recently medicated intrauterine devices (IUD) and long term implants, has been mainly female-directed.

An oral birth control method for men has been developed by combining testosterone & progestin in a pill or by pairing an oral progestin pill with a testosterone injection. In 2019 clinical trials of oral birth control for males showed promising results for efficacy & safety with few side effects. The combination of testosterone & progestin has provided a consistent means of achieving high rates of azoospermia & low rates of pregnancy.

When healthy men used it daily for a month, it produced hormone response consistent with effective contraception. The availability of male hormonal contraceptives would givemen the chance to have control over their own fertility & to share the responsibility for family planning.

The male birth control pill could be more easy & convenient contraceptive method for men. once a daily pill that suppresses two types of male hormones- follicle stimulating (FSH) & luteinizing hormone (LH) to simultaneously decrease production of testosterone & sperm without causing symptoms of low testosterone.

This article will review the theory, the current agents in development, and the potential risks and benefits of a reversible male hormonal contraceptive.

Prospects

An ideal contraceptive for male should be easily available, cheap, easy to use, without side effects & easily reversible. The availability of male hormonal contraceptives would given men the chance to have their control on fertility. Among the different approaches to control male fertility, hormonal contraception is the closet to possible clinical applications. The prospect of clinically available hormonal male contraceptive has been considerably advanced in recent years. When the clinical trials done on contraception are examined, it is seen that the bulk of them have concentrated on female contraceptive methods. A few trials on male contraceptives have actually been withdrawn. However, the concept of hormonal and non-hormonal male contraceptive methods are highly alluring given the acceptability and potential marketing prospects if such a drug comes in to existence.

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The contraceptive methods we have now for men

Condoms

Various forms of condoms including those made from animal skin and intestines have been in use. Rubber condoms made their appearance in the 20th century and they have a dual purpose of preventing sexually transmitted diseases and acting as a contraceptive. At present, latex condoms and polyurethane condoms are available in the market. However, contraception rates when using condoms are unacceptably high (pearl index = 12).Long term compliance of patients with condom use is known to be generally poor. Condom failure may also occur secondary to condom breakage, slippage and incorrect use. Latex allergies are known tooccur with condoms and some users also describe a decrease in sexual pleasure with condom use.

Vasectomy

Vasectomy is a simple surgery performed under local anesthesia wherein the vas deferens is isolated and brought out from the scrotum through an incision followed by division and ligation. It is a safe outpatient procedure used all over the world as a male contraceptive option. Many modified techniques of vasectomy are in use. In the 'no scalpel technique', a simple scrotal puncture is made for the identification of vas which is in turn divided and occluded. The advantages of no scalpel technique include minimal blood loss and low rates of infection. The rate of unwanted pregnancies after vasectomy is generally less than 1%. However, there is delay in the development of azoospermia and effective contraception after the surgery which necessitates the use of an alternate contraceptive like condoms during this period. Another disadvantage of vasectomy is that the reversibility of procedure is not always successful. As the time elapsed from the procedure increases, the reversibility rate comes down. In fact, many patients may also develop anti sperm antibodies which may also bring down the fertility rate. Irrespective of the surgical method used, the surgical experience may be an important player in the success rate of vasectomy and its reversal. In experienced hands, complications like blood loss and infections are minimal. However, a significant number of men complain of testicular discomfort post vasectomy.

Other Non-hormonal methods of contraception

Non-hormonal targets of contraception include sperm production at the testicular level, sperm maturation at the level of epididymis and sperm motility. Obviously, the selectivity, specificity and lesser side effects compared to hormonal methods make these approaches attractive. However, many of these are experimental and in different phases of development.

Gossypol

In the 1950's gossypol was identified as the cause of male infertility in many rural communes in China where ingestion of raw cotton seed oil was common. Since, then efforts have been made by the China National Coordinating Group on Male Antifertility Agents to develop this compound as a pill for men. If successful, gossypol could be a valuable addition to the existing methods of contraception. In the late 1960's and early 1970's, studies were undertaken to determine the toxic effects of gossypol in animals and in human beings. In 1972, a multicenter study, involving 14 centers and 8806 volunteers, was carried out to gather data on the efficacy, side-effects and clinical pharmacology of gossypol when used as a male contraceptive.

Gossypol is aplant extract derived from the cotton plant. It was shown to affect both spermatogenesis and sperm motility. The studies with gossypol have been done mainly on Chinese men. Most users were able to adequately suppress the sperm concentration to levels required for contraception. However, in at least one-fifth of the patients, the effect was irreversible. Other significant dose-dependent side effects included hypokalemia and periodic paralysis.

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 the free gossypol known as the toxic form. The phenol groups of gossypol from ethers & esters, whereas the aldehyde group are highly susceptible either to react with amine groups of amino acids, proteins by forming Schiff's base or with organic acids compounds to form unstable heat label products. The multiple reactivity of gossypol is sustained by its ability of interchanging its functional groups into gossypol tautometric forms dependently to nature of solvents, by reacting as either an aldehyde, ketonoid or hemiacetal compound.

This research studies related to contraception have showed pharmacological uses of gossypol, especially as an oral contraceptive &

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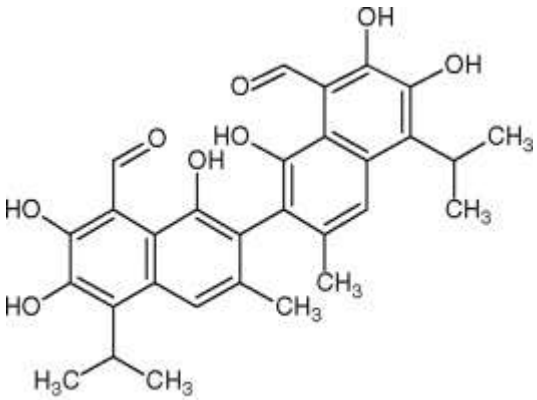
vaginal spermicide for fertility regulation from many decade. There has also been reported versatile clinical applications of gossypol & its derivatives including anticancer such as breast, prostatic cancer & endometriosis, antiviral including HIV & herpes simplex< antimalarial effects, antioxidants & antimicrobial agent but therapeutic establishment of effective dose levels with guaranteed safety is still challenging & limiting clinical uses of gossypol.



Gossypol

Chemical properties

The structure of gossypol consists of two naphthalene rings joined by a single internaphthyl bond between the 2- and 2′-carbon atoms. The presence of six phenolic hydroxyl groups and two aldehydic groups makes gossypol chemically reactive.

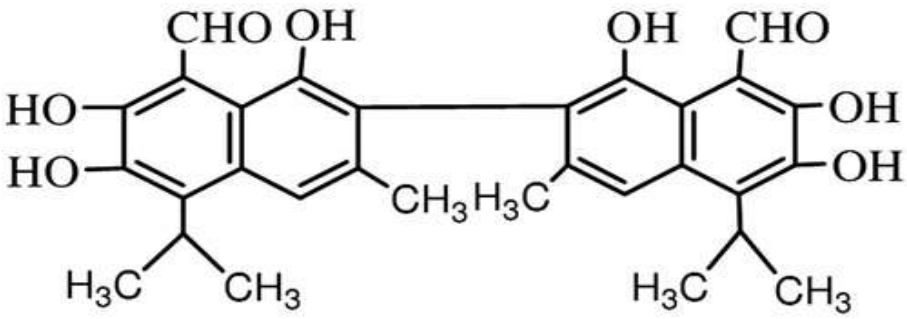


Figure 1: Chemical structure of gossypol.

Gossypol is a polyphenolic bissesquiterpene that has been isolated as a racemic mixture from cottonseed. Gossypol exists as (+) and (−) enantiomers because of hindered rotation around the binaphthyl bond.

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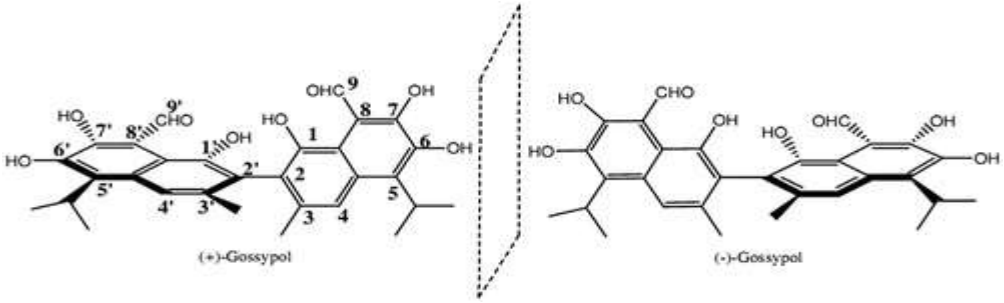

Figure 2: Structure of gossypol enantiomers

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Jaroszewski et al.1992 investigated the gossypol racemization energy barrier using a molecular mechanics program and found that racemization of gossypol requires inaccessibly high energy and, thus, the individual enantiomers are optically stable under normal conditions (e.g., ambient temperature and neutral pH).

Gossypol has a complicated reaction chemistry that stems from its different tautomeric forms. Adams ET al.1960 proposed three tautomeric forms – aldehyde, ketone (quinoid), and lactol (hemiacetal) – to explain some of these reactions and their properties and degradation products.

ToxicityofGossypol

Gossypol as a liposoluble compound is readily absorbed from the gastro-intestinal tract due to its high affinityof binding to amine groups of amino acids or proteins, and readily to iron-containing products even though the clearmechanism 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. It also importantly meddle in enzymatic reactionsrequired for many biologic processes such as interfering with the cellular ability to respond to oxidative stress and inhibition ofoxygen release from haemoglobinthrough which make its conjugation, metabolism, and urinary excretion somehowlimited, and consequently; gossypolis mostlyconjugatedinbileand eliminatedin thefeces.

The toxic manifestations of gossypol may affect the renal, reproductive, hepatic, cardiac and other organs wherecardiac necrosis is resulted from acute heart failure caused by prolonged exposure, and hyperkalaemia associated with heartfailure resulting from cardiac conduction failure can result in quick death. Gossypol damages the liver cells, disturbsblood cells and moleculesfunctions leading to hematologic effects 8 like stimulating the apoptosis-like erythrocytedeath"eryptosis"byincreasingintracellularcalcium(Ca^{2+})inducingtheactivationofCa^{2+}-sensitivepotassium(K^{+})channels,hyperpolarizationleadingintracellularosmoticpressureandK^{+}loss(Fig.3)causingtocellshrinkagewhileincrease Ca^{2+}concentrationsleadingtocellularmembranescramblingafterexposureandmodificationofthecellularmembranephosphatidylserine possibly contributing to anaemia, while reproductive effects affecting spermatogenesis, sperm countsand spermatozoal motility through various mechanisms in male animals while in females it showed promotingirregular menstruations, pregnancy or embryonic disruption through probable mechanisms including endocrine effects on theovaryaswell asadirectcytotoxiceffectontheuterus duringembryonic implantation anddevelopment.

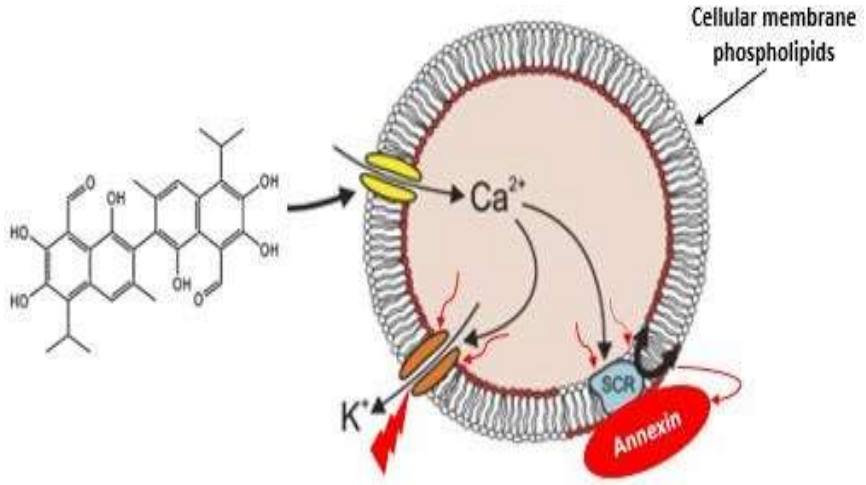

Figure3: Mechanismofgossypol-induced suicidalerythrocytedeath.

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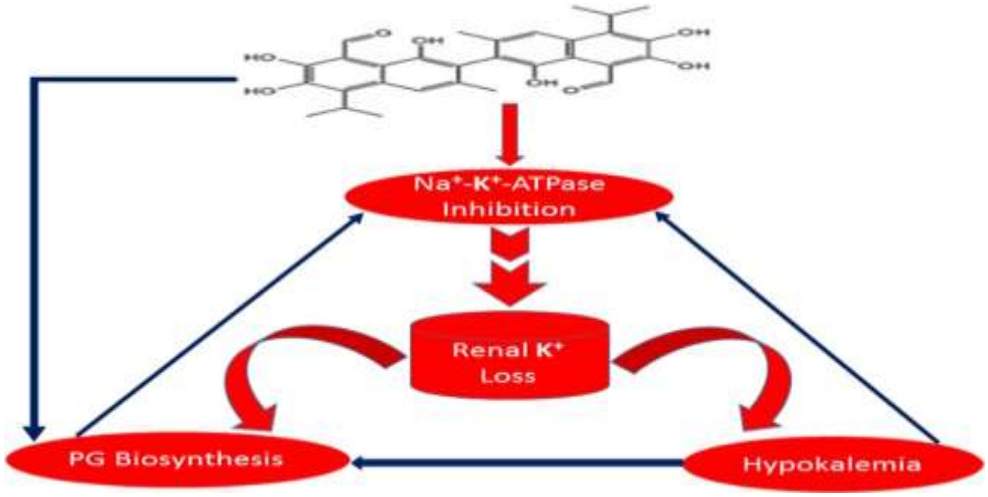

Figure4: Mechanisticcycleofdevelopmentofgossypol-inducedhypokalemia.

Pharmacokineticsand Metabolizationofgossypol

The toxicological studies in different laboratory found out that systemic toxicity of gossypol is dose andspecies-dependent even though the ingested amount and period of exposure mostly increase its toxic effects. It was reported thatthe 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 longerthan (-)-gossypol. After gossypol administration in rats, it was foundin most major visceral organs and in the brain and some studies concluded that gossypol is metabolized by variousmicrosomal enzymes into several metabolites mainly quinone's, a great difference in species sensitivity to different effects inanimals were assumed to be possibly due to differences in metabolism. Many animal studies reported cardiac irregularity leadingto death either due to slow liberation or weak fixation of oxygen in the blood, and the toxicity index could not be specificallydetermined as ithighlydependsoneachanimalmodel.

Metabolism and excretion of gossypol is complex to determine as the parameters are highly species-dependent,and its absorption starts in gastro-intestinal tract. By this fact, the assessment of gastric side effects of gossypol was conductedduring clinical study with gossypol-coated enteric tablets; a great difference in systemic side effects and antifertility effects werefoundoutwithnon-coatedtablets,themostimportantdifferenceinresponsewasapparently duetoreducedgastricabsorptionof the enteric-coated forms. After oral or parenteral administration of gossypol or its analogues, the absorption to the systemiccirculation was found to be time and species-dependent; the high molecular weight, anionic polar behaviour with aromatic rings ofgossypolmakeitsoluble inbiliarysecretions, absorbed inintestinesmainlyexcretedviafaecalroute.

Effectsofdietsonantifertilityandtoxicactivityofgossypol

It was found out that contraceptive doses of gossypol selectively damaged the spermatogenic cells first andleft other vital organs even containing the highest concentrations of gossypol comparably to the one obtained in testis, anunaffected peaks strongly in favour of a specific vulnerability of the testicular cells to the action of gossypol, and co-administration of ferrous supplements with gossypol reduced tissue deposition, increased faecal excretion, and shortened the half-life in the body by accelerating the respiratory elimination of gossypol, these might be explained by the postulation that ironcatalysesthedecarboxylationprocessofgossypolasironandproteinsformnon-absorbableandstablecomplex.

It may be worth to mention that magnesium-gossypol complex has been shown to be anti-spermatogenic withrelatively low toxicity compared with the one played by gossypol alone. In addition, selenium or externalpotassiumsupplementsshowedtocounteract haematologicaleffectsofgossypolwhilesomevitaminslikeB6orEmayreduce gossypol-induced GIT drug adverse effects while promoted binding of free gossypol by promoting microbialfermentationwithsomeyeastsorfungiareals

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opromisingtoreduceitstoxiceffects.Eventhough,itisn otyetwell knowneitheriftheboundgossypolcanbeabsorbedthro ughtheintestinesorcanbefreelyreleasedbackbythemi croorganismsfor latebiliaryglucuronides andsulphatesconjugation.

Toxicokineticsandpoisoningofgossypol

The poisoning effects resulting from animal feeding with high concentration of gossypol were observed in differentanimals such as dogs, goats, chicks, sheep and pigs, mostly appearing not later than 3 months ofingestion. The animal studies showed that young animals are more susceptible to gossypol toxic effects than adults,and monogastric animals like rodents, pigs and birds are very sensitive to gossypol poisoning compared to ruminants animals. The absorption of hazardous concentrations of gossypol showed similar general signs of acute toxicity in all animalspecies mostly such as respiratory distress, weakness, anorexia, reduced body weight gain, kidney and liver damage,death which may occur after long-term intoxication, heart failure was mostly reported in lambs, dogs andcalves, anaemia and pneumonia werealsoobserved insomeanimals.

Table1: Somereported gossypol-inducedhepatotoxicityinlaboratoryanimals.

Testedanimal	**Freegossypoldose**	**Administrationroute**	**Treatmentduration**
Rats	5-10mg/kg/BW	Intraperitoneal	10days
Rats	20mg/kg/BW	Intraperitoneal	10days
Rats	25mg/kg/BW	Intraperitoneal	Singledose
Rats	25mg/kg/BW	Intraperitoneal	Singledose
Chickens	0.1%in feedstuff	oral	21days
Broilers	0.4%in feedstuff	oral	21days
Dogs	4mg/kg/BW	oral	<10days
Monkeys	4mg/kg/BW	oral	24 months

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Table2: Reportedgossypol-inducedtoxiceffects fromdifferentanimalstudies.

Testedanimal	Dose (mg/kg/d)	Treatment duration	Toxiceffects
Rats	10-20	6-14weeks	-Livercellsdamage&necrosis -Digestivetroubles
Rats	25	26weeks	-Liverdamage -Bodyweightloss
Rabbits	10-16	14-41days	-Deepweightloss -Weightlossanddeath
Rabbits	20-80	8-84days	-Pulmonaryandhepaticcongestion -Limbsparalysis&Death
Dogs	1.5-5	50-140days	-Hepaticandrenalcongestion -Pulmonaryedemaanddyspnea -Heartfailurecausingdeath
Dogs	30	18-28days	-Severeanorexiaandvomiting -Cachexiaandanemiacausingdeath
Monkeys	4-12	4-14months	-Weightloss -Liverswelling
Monkeys	05-Oct	4months	-Noclinic-pathologicalsideeffects
Humans	15-50	≥ 6months	-Hypokalemiaaround0.75%

Mechanism action on Anti-spermatogenic effects of gossypol

1. Uncoupling mitochondrial oxidative phosphorylation leading to reduced spermatozoal ATP productive cycle.
2. Inhibition of testicular & spermatozoal specific LDH-X, pyruvate DH, succinyl-CoA synthatse & NAD-isocitrate DH enzymes.
3. Impairing spermatozoal & testicular ATP's activity.
4. Inhibition of spermatozoal acrosome & acrosomal proteinases.
5. Inhibition of nuclear histones synthesis during transition mechanism for spermatids maturation & capacitation.
6. Inhibition of spermatozoal fructose utilization & modifying spermatozoal membrane.
7. Inhibition of spermatozoal metabolism & respiration through increasing cAMP / cGMP ratio.
8. Promoting sertoli cells damage by decreasing formation of androgen-binding proteins.
9. Inhibiting spermatozoal motility & damaging cellular flagella by binding tubulin.
10. Increasing renal, plasma & testicular prostaglandins levels.

Difficultiesandperspectives ofusinggossypolaspotentialmalecontraceptiveagent

The different studies reported a considerable contraceptive efficacy of gossypol to be higher than 99% even thoughsomesubjectsexperiencedsomeadversedrugeffectssuchashypokalemia,transientmusclefatigue,slightdisturbanceofFSH, liver and renal impairments but few users claimed permanent infertility which may occur after long-term or high doses use ofgossypol and the development studies of a non-steroidal male contraceptive agent with lower side effects based ongossypolanditsderivativesisstillgoingon mostlyfocusingon lowdosage formulationsto minimizeitstoxicity.

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The general antifertility dose of gossypol has been estimated to be 20 mg daily for more than 2 months but thesignificant 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 Chinesemenwhere30dayswastheshortestlag-timeand1.5-4months,andthenaround4monthswereenoughtoproducethe same effects in non-Chinese users while globally, a strange range of 2-9 months was required using low doses ofgossypolformulations.However,manyfactorsmay becontributedtothedifferencesinlag-time,bytakingintoconsiderations that even though the same doses of gossypol were used but mostly either the body weight of the users or the usedformsofgossypolwhich could supplyto thebodyunequalamountsofpuregossypolbased on theirdifferentmolecularweights.

These studies showed that dietary oil enhance gossypol effects as gossypol is liposoluble may be easilyabsorbed by the target tissues while the both forms of chelated gossypol to ferrous cations and bound to proteins counteract theantifertility of gossypol. Therefore, some difficulties of using gossypol as a male contraceptive in effective and safe waylies on the facts that it would be required to determine an appropriate dose of gossypol to be administered to each individual andtobethoroughly adjustedaccordingtoindividualproteinsintakeassame doseandform ofgossypolmay producesafeantifertility effects to one person taking high proteins and may cause harmful effects once given to another one under low proteindiet. Consequently, individual dose adjustment and high monitoring of sperms' fertility parameters such as sperm counts andmotility, and simultaneous follow up of other vital parameters after each gossypol administration must be closely regulated foreach user.

Drug interactions

- **Digoxin (Lanoxin) interacts with gossypol**

Large amounts of gossypol can decrease potassium levels in the body. Low potassium levels can increase the side effects of digoxin (Lanoxin).

- **NSAIDs (Nonsteroidal anti-inflammatory drugs) interacts with gossypol**

NSAIDs are anti-inflammatory medications used for decreasing pain and swelling. NSAIDs can cause irritation to the stomach and intestines. Gossypol can also cause irritation to the stomach and intestines. Taking NSAIDs along with gossypol might increase the chances of adverse effects. Avoid taking NSAIDs and gossypol together. Some NSAIDs include ibuprofen (Advil, Motrin, Nuprin, others), indomethacin (Indocin), naproxen (Aleve, Anaprox, Naprelan, Naprosyn), piroxicam (Feldene), aspirin, and others.

- **Stimulant laxatives interacts with gossypol**

Stimulant laxatives speed up the bowels. Overuse of stimulant laxatives can cause low minerals in the body. Gossypol can also decrease minerals in the body. Do not take gossypol along with stimulant laxatives.

- **Theophylline interacts with gossypol**

Theophylline might decrease some of the effects of gossypol.

- **Water pills (Diuretic drugs) interacts with gossypol**

Large amounts of gossypol can decrease potassium levels in the body. "Water pills" can also decrease potassium in the body. Taking gossypol along with "water pills" might decrease potassium in the body too much. Some "water pills" that can deplete potassium include chlorothiazide (Diuril), chlorthalidone (Thalitone), furosemide (Lasix), hydrochlorothiazide (HCTZ, HydroDiuril, Microzide), and others.

- **Warfarin (Coumadin) interacts with gossypol**

Gossypol can work as a laxative. In some people gossypol can cause diarrhea. Diarrhea can increase the effects of warfarin and increase the risk of bleeding. If you take warfarin do not to take excessive amounts of gossypol.

Side effects

- **When taken by mouth**: Gossypol is possiblysafe when used in daily doses of 20 mg or less for up to 1 year. At these doses, gossypol can cause nausea, vomiting, diarrhoea, and low potassium levels. Gossypol is possibly unsafe when used at higher doses or for longer than 1 year without close supervision by a healthcare professional. When men take gossypol by mouth for more than one year, the effects on sperm are unpredictable and might cause permanent inability to father a child (sterility). High doses of gossypol can also cause

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malnutrition, gastrointestinal (GI) bleeding, heart failure, and liver problems.

- **When applied to the skin**: There isn't enough reliable information to know if gossypol is safe or what the side effects might be.

Dose

For male birth control: 15-20 mg is used daily for 12-16 weeks, followed by a maintenance dose of 7.5-10 mg per day. Treatment should be carefully monitored by a healthcare professional, because the effects of gossypol are unpredictable and might lead to permanent loss of the ability to father a child

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Use

- Birth control. Taking gossypol by mouth seems to reduce sperm count and function in 60% to 100% of men. In 50% to 77% of men, sperm recover within 3-24 months after treatment is stopped. In about 10% of men, sperm counts remain very low for over 4.5 years. In some men, continual use of gossypol can cause permanent loss of the ability to father a child.

II. CONCLUSION

Nowadays, despite increases in female contraceptive options,40–45% of pregnancies across the world are effective, reversible, and safe male contraceptive methods. Numerous studies have been performed to develop male hormonal and non-hormonal safe and effective contraceptives, however progress in research in the last decade has been slow and commercialization is not on the horizon. A variety of new molecules are still under development as oral or transdermal hormonal contraceptives for men demonstrating few side effects. The goal for the future is the development and commercialization of a male contraceptive method that will allow both men and women to take an active role in family planning.

Gossypol has preventive and therapeutic potentialities as multipurposecontraceptivewithouthormonalperturbationeffectsbutbymainlybyactingonspermatogeniccells,andmoreinterestinglyacting on various human cancers as a global health threat even though much further studies focusing on development of itsappropriate and efficient dosage forms for a trustworthy advanced clinical uses are still in needed. Moreover, more clinical trialswhich involve combinations of gossypol with other hormonal contraceptives or chemotherapeutic agents for synergistic activitiesare encouraged; the application of nanotechnology for gossypol-based drug dosage formulations such as advanced encapsulatedforms using nano-carriers like nano-liposomes or nano-micelles would be of great significance to improve its potential activitiesbetter than it was reported before from in vitro to in vivo studies. In fact, even though the male reproductive toxicity is wellknown, there is a huge need of more studies to understand more of its effects on females, and extensive researches are stillrequiredtodevelopmoreefficientandinexpensivetechnologiestoreducegossypol toxicityforclinical human use.

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