Gossypol Toxicity from Cottonseed Products

Ivana Cristina N. Gadelha, Nayanna Brunna S. Fonseca, Silvia Catarina S. Oloris, Marília M. Melo, and Benito Soto-Blanco

1 Programa de Pós-graduação em Ciência Animal, Universidade Federal Rural do Semi-Árido, BR 110 Km 47, 59628-360 Mossoró, RN, Brazil
2 Fundação Ezequiel Dias (FUNED), Rua Conde Pereira Carneiro 80, 30510-010 Belo Horizonte, MG, Brazil
3 Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, 30123-970 Belo Horizonte, MG, Brazil

Correspondence should be addressed to Benito Soto-Blanco; bsotoblanco@yahoo.com.br

Received 29 January 2014; Revised 4 April 2014; Accepted 16 April 2014; Published 6 May 2014

Copyright © 2014 Ivana Cristina N. Gadelha et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Gossypol is a phenolic compound produced by pigment glands in cotton stems, leaves, seeds, and flower buds (Gossypium spp.). Cottonseed meal is a by-product of cotton that is used for animal feeding because it is rich in oil and proteins. However, gossypol toxicity limits cottonseed use in animal feed. High concentrations of free gossypol may be responsible for acute clinical signs of gossypol poisoning which include respiratory distress, impaired body weight gain, anorexia, weakness, apathy, and death after several days. However, the most common toxic effects is the impairment of male and female reproduction. Another important toxic effect of gossypol is its interference with immune function, reducing an animal’s resistance to infections and impairing the efficiency of vaccines. Preventive procedures to limit gossypol toxicity involve treatment of the cottonseed product to reduce the concentration of free gossypol with the most common treatment being exposure to heat. However, free gossypol can be released from the bound form during digestion. Agronomic selection has produced cotton varieties devoid of glands producing gossypol, but these varieties are not normally grown because they are less productive and are more vulnerable to attacks by insects.

1. Introduction

Cotton (Gossypium spp.) is an arborous plant from the Malvaceae family. It is one of the earliest plants that were cultivated by man and it has been used for over 4,000 years. It is primarily cultivated for fiber used in the textile industry and the oil from the cotton seed [1]. The genus Gossypium spp. includes many species distributed throughout the world, but only four species are grown for cotton fiber: Gossypium hirsutum L., Gossypium barbadense L., Gossypium arboreum L., and Gossypium herbaceum L. The most economically important cotton species is G. hirsutum, which is grown to produce 90% of the world’s cotton [2]. Cotton fiber and oil production generate byproducts rich in fat from oil and protein which are used for animal feeding. However, this plant contains a toxic compound, gossypol [1].

2. Chemistry of Gossypol

Gossypol is a phenolic compound that was first isolated in 1899. The name is derived from the plant genus scientific name (Gossypium) combined with the ending “ol” from phenol [1]. Gossypol has a 518.55 Dalton molecular weight, has a yellow pigment, is crystalline, is insoluble in water and hexane, is soluble in acetone, chloroform, ether, and methyl ethyl ketone (butanone), and is partly soluble in crude vegetable oils. The chemical formula is C_{30}H_{30}O_{8}, and the chemical structural formula is 2,2’-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylphthalalene) (Figure 1) [1, 3, 4].

Gossypol is produced by pigment glands in cotton stems, leaves, seeds, and flower buds. The pigment glands are small black spots distributed throughout the cotton plant but their greatest concentration is in the seeds [1, 4–6]. The seed of
Gossypol promotes several toxic effects in vertebrates but provides the cotton plant with resistance to pests [1, 4–6]. The pigment glands produce additional phenolic pigments (at least 14), but they are at concentrations well below the concentration of gossypol and thus have little toxicological significance [1].

Gossypol is a mixture of two enantiomers, (−) and (+) gossypol [1, 8–11]. The (−) gossypol enantiomer is more slowly eliminated [12], although it is the most biologically active form. Consequently, it is more toxic than the (+) gossypol [11, 13]. The Gossypium species produces both enantiomers in varying proportions, which is genetically determined [1, 9, 10, 14]. For example, the (−) gossypol proportion ranges from 33.8 to 47.0% in the seeds of upland variety (G. hirsutum) [15, 16] and from 24.9 to 68.9% in the seeds of G. barbadense [1].

Two gossypol forms have been observed, free and bound [6]. The bound form is produced via covalent bonds between gossypol and the free epsilon-amino groups from lysine and arginine [1, 17, 18] through the browning or Maillard reaction [1]. However, this reaction reduces the availability of amino acids for absorption by the animal with lysine being the most affected [18].

Total gossypol production is influenced by several factors, including weather conditions and cotton species. Considering weather conditions, gossypol production is positively correlated with the rainfall rate and negatively correlated with temperature [19]. Regarding variation among cotton species, G. barbadense has higher gossypol concentrations than G. hirsutum. On the other hand, cotton storage slightly decreases the free gossypol content [1].

### 3. Gossypol in Cotton Products

The free gossypol content in whole cotton seeds varies among the many cotton varieties [6, 20]; gossypol concentrations range from 0.02 to 6.64% [21]. Cottonseed may contain concentrations greater than 14,000 mg/kg of total gossypol and 7,000 mg/kg of free gossypol [6]. However, after oil extraction from the seeds, up to 0.6% is available following solvent extraction, but approximately 0.06% is available, if the extraction process involves mechanical pressure and heat treatment [22].

In addition to its harmful effects, gossypol and its derivatives have potential therapeutic use. These compounds showed in vitro action against some viruses such as human immunodeficiency virus [23, 24] and H5N1 influenza virus [24, 25] and several bacteria and yeasts [26–29]. Gossypol is a promising treatment for leukemia [30], lymphoma [31], colon carcinoma [32], breast cancer [33, 34], myoma [35], prostate cancer [36], and other malignancies [37–43]. Furthermore, it was used in China, in 1970, to treat uterine fibroids, endometriosis, and uterine bleeding in women [35].

### 4. Toxicokinetics

The gossypol absorption rate is inversely proportional to the amount of iron in the diet [71], and dietary supplementation with ferrous sulfate inactivates free gossypol [72]. In ruminants, microbial fermentation in the rumen binds dietary free gossypol with proteins [73], but it is not known whether the bound form can be absorbed by the intestines or the microorganisms can release free gossypol from the bound form. The absorbed gossypol accumulates in the liver [74] and kidneys [75]. The primary gossypol excretion route is through bile; it is then eliminated through feces after conjugation with glucuronides and sulfates [76]. In rats dosed orally with 5 mg of both racemic forms of gossypol, 70.4% of (+) and 80.2% of (−) gossypol were excreted in the feces within five days, whereas 2.30% of (+) and 2.79% of (−) gossypol were excreted in the urine [77]. Small amounts of gossypol are also excreted in expired air [1]. Little to no gossypol is excreted in the milk [74]. The half-lives ($t_{1/2}$) of total (+) and (−) gossypol in rats range from 0.02 to 6.64% [21]. Cottonseed may contain concentrations greater than 14,000 mg/kg of total gossypol and 7,000 mg/kg of free gossypol [6]. However, after oil extraction from the seeds, up to 0.6% is available following solvent extraction, but approximately 0.06% is available, if the extraction process involves mechanical pressure and heat treatment [22].

In addition to its harmful effects, gossypol and its derivatives have potential therapeutic use. These compounds showed in vitro action against some viruses such as human immunodeficiency virus [23, 24] and H5N1 influenza virus [24, 25] and several bacteria and yeasts [26–29]. Gossypol is a promising treatment for leukemia [30], lymphoma [31], colon carcinoma [32], breast cancer [33, 34], myoma [35], prostate cancer [36], and other malignancies [37–43]. Furthermore, it was used in China, in 1970, to treat uterine fibroids, endometriosis, and uterine bleeding in women [35].

### 4. Toxicokinetics

The gossypol absorption rate is inversely proportional to the amount of iron in the diet [71], and dietary supplementation with ferrous sulfate inactivates free gossypol [72]. In ruminants, microbial fermentation in the rumen binds dietary free gossypol with proteins [73], but it is not known whether the bound form can be absorbed by the intestines or the microorganisms can release free gossypol from the bound form. The absorbed gossypol accumulates in the liver [74] and kidneys [75]. The primary gossypol excretion route is through bile; it is then eliminated through feces after conjugation with glucuronides and sulfates [76]. In rats dosed orally with 5 mg of both racemic forms of gossypol, 70.4% of (+) and 80.2% of (−) gossypol were excreted in the feces within five days, whereas 2.30% of (+) and 2.79% of (−) gossypol were excreted in the urine [77]. Small amounts of gossypol are also excreted in expired air [1]. Little to no gossypol is excreted in the milk [74]. The half-lives ($t_{1/2}$) of total (+) and (−) gossypol in rats
following a single intravenous dose were estimated as 25.26 hours and 10.53 hours, respectively [77].

5. Gossypol Poisoning

Cottonseed includes sufficiently high gossypol concentrations to produce acute poisoning. However, there are cumulative effects of dietary gossypol and toxicity which can occur following an ingestion period of one to three months [1, 78–81]. Gossypol poisoning has been reported in many species, including broiler chicks [82], pigs [71], dogs [83, 84], sheep [85], and goats [86]. Monogastric animals, such as pigs, birds, fish, and rodents, are more susceptible to gossypol toxicity than ruminants [5, 6, 20, 87]. Moreover, young ruminants are more sensitive to gossypol compared with adult ruminants [1] because gossypol is not bound during ruminal fermentation, as it occurs in animals with fully functional rumens. However, if the gossypol intake overwhelms the ruminal detoxification capacity, free gossypol may be absorbed at hazardous concentrations even in adult ruminant animals [88].

General signs of acute toxicity are similar among animal species and include respiratory distress, impaired body weight gain, anorexia, weakness, apathy, and death after several days [1, 6, 80, 85, 89–93]. Heart failure was reported in calves [90, 94], lambs [85], and dogs [79].

The postmortem findings in ruminants include pulmonary edema, yellowish liquid in the chest and peritoneal cavities, gastroenteritis, centrilobular liver necrosis, and hypertrophic cardiac fiber degeneration. In calves, the major pathologic findings are ascites, visceral edema, acute centrilobular hepatocyte necrosis, kidney damage, and cardiovascular lesions. Increased pneumonia has also been observed, likely due to an increased sensitivity to secondary infections [85, 90–92].

Pigs may present reduced weight gain, anorexia, respiratory distress, cardiac insufficiency, coughing, and exercise intolerance. Necropsy findings include fluid accumulation in the body cavities; edema and congestion in the liver, lung, and spleen; and cardiac hypertrophy with degenerated muscle fiber [71].

Anemia is often observed in animals fed cottonseed. In fact, gossypol is a highly reactive compound that readily binds to minerals and amino acids. Binding with iron forms a gossypol-iron complex, which inhibits the absorption of this metal. The consequent iron deficiency affects erythropoiesis. Furthermore, gossypol promotes increased erythrocyte fragility [57, 74, 87, 95]. Gossypol also stimulates the eryptosis (apoptosis-like erythrocyte death) by increasing cytosolic Ca\(^{2+}\) activity resulting in cell membrane scrambling and contraction, which contributes to anemia [96].

Gossypol also affects thyroidal metabolism [68, 97–100]. Some studies with male [98] and female [99] rats showed decreased blood concentrations of T4 and T3 after dosing with gossypol. On the other hand, gossypol dosing resulted in increased T3 serum concentrations without affecting T4 in rats [97] and sheep [68]. The histopathological evaluation of thyroid glands from male rats dosed with gossypol revealed follicular degeneration and atrophy [98]. The thyrocyte cells in the pituitary gland, which are specialized for TSH synthesis and secretion, showed hypertrophy, hyperplasia, and degranulation after gossypol dosing in rats [100].

Certain clinical signs of gossypol poisoning have been attributed to reduced antioxidants in tissues and increased reactive oxygen species formation, which produces lipid peroxidation [101–104]. At high concentrations, gossypol also impairs energy generation from oxidative metabolism by interfering with enzymatic activity in the mitochondrial electron transport chain and oxidative phosphorylation [105–107]. Furthermore, gossypol decreases the contraction force of the heart and the extent of contraction of cardiac fibers [108].

6. Liver Damage

In addition to such effects, gossypol is hepatotoxic (Table 1) [11, 44–47, 71, 109, 110]. Ascites and hepatocyte degeneration (strong cytoplasmic eosinophilia and nuclear pyknosis) were observed in rats that received a single intraperitoneal gossypol dose of 25 mg/kg BW [45] or 30 mg/kg BW [46]. Rats that received lower gossypol doses (15 mg/kg/day for four weeks or 30 mg/kg/day for two weeks) showed morphological changes in the liver, as observed through electron microscopy, which were characterized by mitochondrial vacuolation, an enlarged endoplasmic reticulum, an expanded perinuclear space, and collagen fiber proliferation in the perisinusoidal space [109]. Chickens fed a diet with 0.1% free gossypol for 21 days had increased plasma gamma glutamyltransferase activity and liver lipidosis [44]. Broilers that received a diet with 0.4% total gossypol for 20 days had greater liver weights [11].

7. Reproductive Effects

Gossypol affects male and female gametogenesis and promotes embryo lesions [81]. In the 1950s, China underwent a sharp drop in the birthrate in many rural areas where
humans were consuming cottonseed oil containing gossypol. This observation was initially associated with male infertility caused by gossypol in the cottonseed oil that they were consuming. Gossypol has been investigated for use as a male contraceptive in a number of experimental studies [1, 81, 111–115].

The gossypol toxicity for male reproduction (Table 2) was reported in several studies showing that it inhibits spermatogenesis, which decreases the sperm count and spermatozoid motility and viability [20, 47–51, 53, 55, 102, 116–120]. The male antifertility effect is dose and time dependent; in effective doses, gossypol causes infertility by inhibiting sperm motility, decreasing sperm concentrations, inducing specific mitochondrial injury to the sperm tail, and damaging the germinal epithelium [20]. However, such effects are reversible when gossypol is no longer ingested [52]. Furthermore, gossypol administration to male rats did not interfere in the embryonic and fetal development of untreated dam offspring [121].

The deleterious effects on male reproduction have not been observed for all animals fed cottonseed meal. In adult male goats [122] and sheep [123] fed a diet with 0.5 kg/animal/day cottonseed meal for 120 consecutive days, no detrimental effects on semen volume, sperm concentration, motility, and morphology.

The gossypol-mediated spermatozoid disturbance mechanism includes the inhibition of release and utilization of ATP by the sperm cells [124]. Another effect of gossypol is the reduction of cellular and microtubular β-tubular content in spermatocytes and spermatids [125]. Furthermore, gossypol inhibits calcium influx [126, 127] and Mg-ATPase and Ca-Mg-ATPase activity in spermatozoid plasmatic membranes [126]. Abnormal spermatozoids are produced because gossypol produces ultrastructural alterations in the nuclear membrane, endoplasmic reticulum, and mitochondria [119, 128–130]. In cultivated Sertoli cells from piglets, gossypol also decreases cellular oxidase activity and damages the DNA [131]. Reduced nuclear expression of androgen receptors was observed in Leydig cells, Sertoli cells, and myoid cells from rats fed gossypol-rich cottonseed flour [132].

Gossypol also affects female reproduction (Table 3), and ruminant females tolerate higher dietary gossypol concentrations than nonruminant females [20, 54, 118, 133], probably due to the ruminal detoxification. Female exposure to gossypol has been associated with interference with the estrous cycle, pregnancy, and early embryonic development [20, 57, 81]. Gossypol interfered with rodent estrous cycles [54, 134] and pig granulosa cell function [135]. Furthermore, ovaries from heifers fed cottonseed meal had fewer large follicles (>5 mm) than heifers fed soybean meal [57]. Gossypol affected in vitro ovarian steroidogenesis [136, 137] as well as bovine oocyte cumulus expansion and nuclear maturation [137].

Previous studies have shown that gossypol interferes with embryonic development [118, 138–141]. In fact, gossypol may reach the uterine fluids through the maternal circulation [141]. A gossypol-mediated embryotoxic effect has been observed in in vitro [118, 138, 140–142] and in vivo [57, 139, 141, 143] studies. The early pregnancy loss promoted by gossypol is not due exclusively to direct damage to embryos but also to interference with implantation of the embryo [139]. However, this compound significantly reduced the fetal body weight in pregnant mice, but no fetal abnormalities were observed [144].

The probable mechanism for gossypol embryotoxicity is through direct embryonic cytotoxicity [20, 143]. This cytotoxic effect might be promoted by (1) generation of reactive oxygen species inducing oxidative stress [102, 104, 145], (2) intercellular communication disruption [146], (3) apoptosis induction [32, 147–152], or (4) interference with ionic transport in membranes, which increases intracellular calcium [153].

### Table 2: Selected experimental studies describing effects of gossypol on male reproduction.

| Animals | Gossypol dose | Effects | Reference |
|---------|--------------|---------|-----------|
| Hamsters | 10 mg/kg BW/day | Degeneration of spermatocytes | [48] |
| Rats    | 20 mg/kg BW/day | Degeneration of spermatocytes | [48] |
| Mice    | 40 mg/kg BW/day | No degeneration | [48] |
| Rats    | 25 mg/kg BW/day | Decreased spermatogenesis, Sertoli cell, and seminiferous tubules damage | [49] |
| Rats    | 10 mg/kg BW/day | Tubular degeneration, reduced testosterone concentrations, and involutions of ventral prostate and seminal vesicles | [50] |
| Rats    | 5, 10 and 20 mg/kg BW/day | Decreased sperm count and motility, increased abnormal sperm count, and reduced serum levels of testosterone, LH, and FSH | [47] |
| Bulls   | 16.4 mg/kg BW/day | Reduced sperm production and motility and increased proportion of sperm midpiece abnormalities | [51] |
| Bulls   | 8 mg/kg BW/day | Primary and secondary sperm abnormalities and increased number of sperm with proximal droplets | [52] |
Table 3: Selected experimental studies describing effects of gossypol on female reproduction.

| Animals | Gossypol dose | Effects | Reference |
|---------|---------------|---------|-----------|
| Rats    | 5 mg/kg BW/day | Longer diestrus | [53] |
| Rats    | 25 mg/kg/day  | Lower levels of estradiol-17β | [54] |
| Rats    | 20 mg/kg/day  | Irregular and longer estrous cycles, prolonged time for mating, decreased pregnancy rate, and reduced number of viable embryos | [55] |
| Heifers | ∼51 mg/kg BW/day | No interference on cycling, first service conception rate, and ovarian morphology | [56] |
| Heifers | 5 g of free gossypol/animal/day | Reduced number of ovarian follicles > 5 mm | [57] |

8. Immunotoxicity

Gossypol may cause a reduced number of leukocytes and primarily lymphocytes, which affects the immunocompetence of the organism [154]. In vivo and in vitro mouse experiments also demonstrated that gossypol has immunosuppressive activity [155], which operates by affecting lymphocytes through inhibiting proliferation and inducing apoptosis [155, 156]. Mice that received gossypol had significantly decreased numbers of lymphocytes in the thymus and mesenteric lymph nodes [157], in the total spleen cell population [144], and in the capacity of blood and lymphatic cells to produce antibodies after sheep erythrocyte immunization [144, 157]. Furthermore, the spleen and lymph nodes from mice receiving gossypol had decreased CD4+ thymocyte populations and increased CD8+ lymphocyte populations [157].

The interference of gossypol with lymphocytes influence immune function as observed in a number of studies [157–160]. After inoculation with *Brucella abortus* smooth strain 99 (S99), specific anti-*Brucella* antibody production was impaired in lambs [159] and calves [160] fed cottonseed meal. Mice treated with gossypol had decreased IgM and IgG production after sheep erythrocyte immunization [157]. Furthermore, the spleen and lymph nodes from mice receiving gossypol had decreased CD4+ thymocyte populations and increased CD8+ lymphocyte populations [157].

The interferon of gossypol with lymphocytes influence immune function as observed in a number of studies [157–160]. After inoculation with *Brucella abortus* smooth strain 99 (S99), specific anti-*Brucella* antibody production was impaired in lambs [159] and calves [160] fed cottonseed meal. Mice treated with gossypol had decreased IgM and IgG production after sheep erythrocyte immunization [157]. Furthermore, the spleen and lymph nodes from mice receiving gossypol had decreased CD4+ thymocyte populations and increased CD8+ lymphocyte populations [157].

*In vitro* murine macrophage proliferation was inhibited by gossypol [157]. Furthermore, rat peritoneal macrophages incubated with gossypol inhibited arachidonic acid metabolism and prostaglandin E2 production [161]. On the other hand, macrophage chemotaxis induced by *Edwardsiella ictaluri* challenge was increased in channel catfish (*Ictalurus punctatus*) fed cottonseed [162] or receiving gossypol [163], but catfish were unaffected by gossypol in another study [27]. Gossypol also increased serum lysozyme activity in channel catfish following an *E. ictaluri* challenge [27, 163].

9. Preventive Procedures

The preventive procedures at this time involve the treatment of cottonseed products to decrease the concentrations of free gossypol through the use of heat and pressure in the processing of these products (Table 4). Agronomic selection has produced cotton varieties devoid of glands producing gossypol [164], but these varieties are less grown because they are not as productive and are more vulnerable to attacks by insects [1]. One alternative is the selection and use of cotton varieties containing a relatively high (+) to (−) gossypol enantiomeric ratio [13]. The directive 2002/32 of the European Union (2002L0032 - EN - 26.02.2013 - 017.001) states that the maximum free gossypol concentrations for cottonseed are 5,000 ppm and 1,200 ppm for cottonseed meal or cake and, for complete feeding stuffs, are 20 ppm for laying hens and piglets, 60 ppm for rabbits and pigs, 100 ppm for poultry and calves, and 500 ppm for cattle, sheep, and goats.

Processing including heat treatment [58, 165] and extrusion process [59] can reduce free gossypol concentrations in cottonseed. However, it is possible that the conjugate formed can release free gossypol during digestion. In fact, cows fed diets containing whole cottonseed with similar total gossypol concentrations but different free gossypol concentrations had similar total plasma gossypol [59]. Furthermore, even though the extrusion process reduced free gossypol concentration but not the total gossypol concentration; broiler chicks fed extruded cottonseed meal or feed-grade cottonseed meal showed decreased body weight gain, increased feed intake, and inefficient feed conversion rate [166].
Radiation treatment using gamma [60, 167, 168] or electron beam irradiation [60, 61] may reduce free gossypol concentrations. In fact, gossypol irradiation reduced in vitro prooxidative activity and embryotoxicity in mice [168]. The mechanism for gossypol destruction through radiation is unknown, but it has been speculated that gossypol molecule aggregation, gossypol cross-linking with other molecules, and gossypol molecule fragmentation or breakdown may produce such destruction [61]. On the other hand, ammoniation, which is a procedure that is used to reduce aflatoxin content of food, increased cottonseed meal toxicity in dairy cattle [169].

Some fungus may reduce free gossypol concentrations in cottonseed meal by fermentation, including Aspergillus niger [63, 64, 170], Aspergillus oryzae [62], Candida tropicalis [63–66], Saccharomyces cerevisiae [63, 64], and Geotrichum candidum [67]. The use of fermented cottonseed meal to feed animals seems to be safe [62, 171]. However, while these microorganisms could be used to reduce free gossypol concentration in cottonseed meal, they are not currently commercially available.

Supplementation with ferric sulfate reduces free gossypol concentrations in food due to ferric sulfate binding with reactive groups from gossypol, which forms a conjugate. The recommendation for supplementation is 1 mol of gossypol for each mol of iron, which could increase the maximum concentration of gossypol from 50 to 150 ppm for laying birds and from 100 ppm to 400 ppm for pigs and poultry [1]. Additional nutrients may be used for dietary supplementation to reduce gossypol availability. Supplementing the diet with 1 mg of sodium selenite per day in adult sheep reduced the gossypol toxicity affecting semen quality [68]. Dietary vitamin E supplementation at 4000 IU/bull/day also reversed the increased erythrocyte osmotic fragility in heifers [70] promoted by feeding cottonseed meal.

Gossypol was produced as a conjugate with bovine serum albumin for vaccines. This conjugate induces antibody production against gossypol in rats, but the immunized animals were more sensitive to the acute hepatotoxic effect of gossypol [46].

10. Conclusions and Future Research Directions

The ingestion of gossypol present in cottonseed and its products (cakes and meal) may promote clinical poisoning, liver damage, male and female reproductive toxicity, and immunological impairment. The acute poisoning is not currently a significant problem but the reproductive damage causes serious economic losses to the livestock industry. Even though the male reproductive toxicity is well known, there is a need for more studies to understand the female reproductive damage promoted by gossypol. The immunotoxicity of gossypol is far from being completely elucidated, but it impacts animals by reducing their resistance to infections and by impairing the efficiency of vaccines. Extensive research is needed to develop more efficient and inexpensive technologies to reduce gossypol toxicity.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work received support for language editing by the Pró-Reitoria de Pesquisa of the Universidade Federal de Minas Gerais (Edital PRP-UFMG 03/2013).

References

[1] B. Soto-Blanco, “Gossipol e fatores antinutricionais da soja,” in Toxicologia Aplicada à Medicina Veterinária, H. S. Spinosa, S. L. Górniak, and J. P. Neto, Eds., pp. 531–545, Manoel, Barueri, Brazil, 2008.
[2] A. Borém, E. C. Freire, J. Cesar, V. Penna, and P. A. Vianna, “Considerations about cotton gene escape in Brazil: a review,” Crop Breeding and Applied Biotechnology, vol. 3, no. 4, pp. 315–332, 2003.
[3] M. B. Abou-Donia, “Physiological effects and metabolism of gossypol,” Residue Reviews, vol. 61, pp. 125–160, 1976.
[4] G. M. Rogers, M. H. Poore, and J. C. Paschal, “Feeding cotton products to cattle,” Veterinary Clinics of North America: Food Animal Practice, vol. 18, no. 2, pp. 267–294, 2002.
[5] J. A. Kenar, “Reaction chemistry of gossypol and its derivatives,” Journal of the American Oil Chemists’ Society, vol. 83, no. 4, pp. 269–302, 2006.
[6] J. Alexander, D. Benford, A. Cockburn et al., “Gossypol as undesirable substance in animal feed,” EFSA Journal, vol. 908, pp. 1–55, 2008.
[7] R. G. Percy, M. C. Calhoun, and H. L. Kim, “Seed gossypol variation within Gossypium barbadense L. cotton,” Crop Science, vol. 36, no. 1, pp. 193–197, 1996.
[8] R. J. Hron Sr., H. L. Kim, M. C. Calhoun, and G. S. Fisher, “Determination of (+)-, (-)-, and total gossypol in cottonseed by high-performance liquid chromatography,” Journal of the American Oil Chemists’ Society, vol. 76, no. 11, pp. 1351–1355, 1999.
[9] M. M. Lordelo, A. J. Davis, M. C. Calhoun, M. K. Dowd, and N. M. Dale, “Relative toxicity of gossypol enantiomers in broilers,” Poultry Science, vol. 84, no. 9, pp. 1376–1382, 2005.
[10] M. M. Lordelo, M. C. Calhoun, N. M. Dale, M. K. Dowd, and A. J. Davis, “Relative toxicity of gossypol enantiomers in laying and broiler breeder hens,” Poultry Science, vol. 86, no. 3, pp. 582–590, 2007.
[11] R. Kakani, D. A. Gamboa, M. C. Calhoun, A. U. Haq, and C. A. Bailey, “Relative toxicity of cottonseed gossypol enantiomers in broilers,” Open Toxicology Journal, vol. 4, pp. 26–31, 2010.
[12] D.-F. Wu, Y.-W. Yu, Z.-M. Tang, and M.-Z. Wang, “Pharmacokinetic of (+), (+), and (-) gossypol in humans and dogs,” Clinical Pharmacology and Therapeutics, vol. 39, no. 6, pp. 613–618, 1986.
[13] C. A. Bailey, R. D. Stipanovic, M. S. Ziehr et al., “Cottonseed with a high (+) to (-) gossypol enantiomer ratio favorable to broiler production,” Journal of Agricultural and Food Chemistry, vol. 48, no. 11, pp. 5692–5695, 2000.
[14] J. A. Scheffler and G. B. Romano, “Breeding and genetics: modifying gossypol in cotton (Gossypium hirsutum L.): a cost...
effective method for small seed samples,” *Journal of Cotton Science*, vol. 12, no. 3, pp. 202–209, 2008.

[15] M. C. Calhoun, S. K. Kuhlmann, and B. C. Balwin, “Assessing the gossypol status of cattle feed cotton products,” pp. 147A–158A, Proceedings of the Pacific Northwest Animal Nutrition Conference, 1995.

[16] R. D. Stipanovic, L. S. Puckhaber, J. Liu, and A. A. Bell, “Total and percent atropinomers of Gossypol and Gossypol-6-methyl ether in seeds from Pima cottons and accessions of *Gossypium barbadense* L,” *Journal of Agricultural and Food Chemistry*, vol. 57, no. 2, pp. 566–571, 2009.

[17] R. Bressani, R. Jarquin, and L. G. Elias, “Free and total gossypol, epsilon-amino lysine, and biological evaluation of cottonseed meals and flours in Central America,” *Journal of Agricultural and Food Chemistry*, vol. 12, no. 3, pp. 278–282, 1964.

[18] S. R. Fernandez, Y. Zhang, and C. M. Parsons, “Dietary formulation with cottonseed meal on a total amino acid versus a digestible amino acid basis,” *Poultry Science*, vol. 74, no. 7, pp. 1168–1179, 1995.

[19] W. A. Pons Jr., C. L. Hoffpauir, and T. H. Hopper, “Gossypol and cottonseed products on reproduction of mammals,” *Journal of Animal Science*, vol. 70, no. 5, pp. 1628–1632, 1992.

[20] W. D. Price, R. A. Lovell, and D. G. McChesney, “Naturally occurring toxins in feedstuffs: center for veterinary medicine perspective,” *Journal of Animal Science*, vol. 71, no. 9, pp. 2556–2562, 1993.

[21] S. S. Nicholson, “Cottonseed toxicity,” in *Veterinary Toxicology: Basic and Clinical Principles*, R. C. Gupta, Ed., pp. 1161–1165, Academic Press, London, UK, 2nd edition, 2012.

[22] B. Polsky, S. J. Segal, P. A. Baron, J. W. M. Gold, H. Ueno, and D. Armstrong, “Inactivation of human immunodeficiency virus in vitro by gossypol,” *Contraception*, vol. 39, no. 6, pp. 579–587, 1989.

[23] J. Yang, F. Zhang, J. Li et al., “Synthesis and antiviral activities of novel gossypol derivatives,” *Bioorganic and Medicinal Chemistry Letters*, vol. 22, no. 3, pp. 1415–1420, 2012.

[24] J. Yang, G. Chen, L. L. Li et al., “Synthesis and anti-HSV activity of chiral gossypol derivatives and its analogs implicated by a viral entry blocking mechanism,” *Bioorganic & Medicinal Chemistry Letters*, vol. 23, no. 9, pp. 2619–2623, 2013.

[25] P. Margalith, “Inhibitory effect of gossypol on microorganisms,” *Applied Microbiology*, vol. 15, no. 4, pp. 952–953, 1967.

[26] P. Yildirim-Aksoy, C. Lim, M. K. Dowd, P. J. Wan, P. H. Klesius, and C. Shoemaker, “In vitro inhibitory effect of gossypol from gossypol-acetic acid, and (+)- and (−)-isomers of gossypol on the growth of *Edwardsiella ictaluri*,” *Journal of Applied Microbiology*, vol. 97, no. 1, pp. 87–92, 2004.

[27] E. Turco, C. Vizzuso, S. Franceschini, A. Ragazzi, and F. M. Stefanini, “The in vitro effect of gossypol and its interaction with salts on conidial germination and viability of *Fusarium oxysporum* sp. vasinfectum isolates,” *Journal of Applied Microbiology*, vol. 103, no. 6, pp. 2370–2381, 2007.

[28] Y. Anna, A. G. Medentsev, and V. I. Krupyanko, “Gossypol inhibits electron transport and stimulates ROS generation in yarrowia lipolytica mitochondria,” *Open Biochemistry Journal*, vol. 6, pp. 11–15, 2012.

[29] K. Balakrishnan, W. G. Wierda, M. J. Keating, and V. Gandhi, “Gossypol, a BH3 mimetic, induces apoptosis in chronic lymphocytic leukemia cells,” *Blood*, vol. 112, no. 5, pp. 1971–1980, 2008.

[30] P. W. M. Johnson, “New targets for lymphoma treatment,” *Annals of Oncology*, vol. 19, no. 4, pp. iv56–iv59, 2008.

[31] X. Wang, J. Wang, S. C. H. Wong et al., “Cytotoxic effect of gossypol on colon carcinoma cells,” *Life Sciences*, vol. 67, no. 22, pp. 2663–2671, 2000.

[32] C. van Poznak, A. D. Seidman, M. M. Reidenberg et al., “Oral gossypol in the treatment of patients with refractory metastatic breast cancer: a phase II clinical trial,” *Breast Cancer Research and Treatment*, vol. 66, no. 3, pp. 239–248, 2001.

[33] W. Ye, H.-L. Chang, L.-S. Wang et al., “Modulation of multidrug resistance gene expression in human breast cancer cells by (−)-gossypol-enriched cottonseed oil,” *Anticancer Research*, vol. 27, no. 1, pp. 107–116, 2007.

[34] M.-L. Han, Y.-F. Wang, M.-Y. Tang et al., “Gossypol in the treatment of endometriosis and uterine myoma,” *Contributions to Gynecology and Obstetrics*, vol. 16, pp. 268–270, 1987.

[35] J. Jiang, V. Slivova, A. Jedinak, and D. Sliva, “Gossypol inhibits growth, invasiveness, and angiogenesis in human prostate cancer cells by modulating NF-xB/AP-1 dependent and independent-signaling,” *Clinical and Experimental Metastasis*, vol. 29, no. 2, pp. 165–178, 2012.

[36] G. P. Tuszyński and G. Cossu, “Differential cytotoxic effect of gossypol on human melanoma, colon carcinoma, and other tissue culture cell lines,” *Cancer Research*, vol. 44, no. 2, pp. 768–771, 1984.

[37] Y.-W. Wu, C. L. Chik, and R. A. Knazek, “An in vitro and in vivo study of antitumor effects of gossypol on human SW-13 adrenocortical carcinoma,” *Cancer Research*, vol. 49, no. 14, pp. 3754–3758, 1989.

[38] S. Z. A. Badawy, A.-K. Soud, V. Cuenca, N. Montalfo, and E. Shue, “Gossypol inhibits proliferation of endometrioma cells in culture,” *Asian Journal of Andrology*, vol. 9, no. 3, pp. 388–393, 2007.

[39] C.-H. Ko, S.-C. Shen, L.-Y. Yang, C.-W. Lin, and Y.-C. Chen, “Gossypol reduction of tumor growth through ROS-dependent mitochondria pathway in human colorectal carcinoma cells,” *International Journal of Cancer*, vol. 121, no. 8, pp. 1670–1679, 2007.

[40] C.-C. Chien, C.-H. Ko, S.-C. Shen, L.-Y. Yang, and Y.-C. Chen, “The role of COX-2/PGE2 in gossypol-induced apoptosis of colorectal carcinoma cells,” *Journal of Cellular Physiology*, vol. 227, no. 8, pp. 3128–3137, 2012.

[41] W.-T. Hsiao, M.-D. Tsai, G.-M. Jow, L.-T. Tien, and Y.J. Lee, “Involvement of Smac, p53, and caspase pathways in induction of apoptosis by gossypol in human retinoblastoma cells,” *Molecular Vision*, vol. 18, pp. 2033–2042, 2012.

[42] F. Y. Wong, N. Liem, C. Xie et al., “Combination therapy with gossypol reveals synergism against gemcitabine resistance in cancer cells with high BCL-2 expression,” *PLoS ONE*, vol. 7, no. 12, Article ID e50786, 2012.

[43] S. Blevins, P. B. Siegel, D. J. Blodgett, M. Ehrich, G. K. Saunders, and R. M. Lewis, “Effects of silymarin on gossypol toxicosis in divergent lines of chickens,” *Poultry Science*, vol. 89, no. 9, pp. 1878–1886, 2010.

[44] D. P. Deoras, P. Young-Curtis, R. R. Dalvi, and E. E. Tippett, “Effect of gossypol on hepatic and serum γ-glutamyltransferase activity in rats,” *Veterinary Research Communications*, vol. 21, no. 5, pp. 317–323, 1997.
(±)-gossypol in male Fischer-344 rats and male B6C3F mice,” *Toxicology*, vol. 55, no. 1-2, pp. 37–51, 1989.

[77] Q. Q. Chen, H. Chen, and H. P. Lei, “Comparative study on the metabolism of optical gossypol in rats,” *Journal of Ethnopharmacology*, vol. 20, no. 1, pp. 31–37, 1987.

[78] E. Eagle, “Effect of repeated doses of gossypol on the dog,” *Archives of Biochemistry*, vol. 26, no. 1, pp. 68–71, 1950.

[79] C. S. Patton, A. M. Legendre, R. E. Gompf, and M. A. Walker, “Heart failure caused by gossypol poisoning in two dogs,” *Journal of the American Veterinary Medical Association*, vol. 187, no. 6, pp. 625–627, 1985.

[80] L. A. Kerr, “Gossypol toxicity in cattle,” *Compendium on Continuing Education for the Practising Veterinarian*, vol. 11, no. 9, pp. 1139–1146, 1989.

[81] I. C. N. Gadelha, A. H. do Nascimento Rangel, A. R. Silva, and B. Soto-Blanco, “Efeitos do gossipol na reprodução animal,” *Acta Veterinaria Brasilia*, vol. 5, no. 2, pp. 129–135, 2011.

[82] M. H. Henry, G. M. Pesti, and T. P. Brown, “Pathology and histopathology of gossypol toxicity in broiler chicks,” *Avian Diseases*, vol. 45, no. 3, pp. 598–604, 2001.

[83] J. L. West, “Lesions of gossypol poisoning in the dog,” *Journal of the American Veterinary Medical Association*, vol. 96, pp. 74–76, 1940.

[84] F. A. Uzal, B. Puschner, J. M. Tahara, and R. W. Nordhausen, “Gossypol toxicity in a dog consequent to ingestion of cottonseed bedding,” *Journal of Veterinary Diagnostic Investigation*, vol. 17, no. 6, pp. 626–629, 2005.

[85] S. Morgan, E. L. Stair, T. Martin, W. C. Edwards, and G. L. Morgan, “Clinical, clinicopathologic, pathologic, and toxicologic alterations associated with gossypol toxicity in feeder lambs,” *American Journal of Veterinary Research*, vol. 49, no. 4, pp. 493–499, 1988.

[86] N. E. East, M. Anderson, and L. J. Lowenstein, “Apparent gossypol-induced toxicity in adult dairy goats,” *Journal of the American Veterinary Medical Association*, vol. 204, no. 4, pp. 642–643, 1994.

[87] W.-J. Zhang, Z.-R. Xu, X.-L. Pan, X.-H. Yan, and Y.-B. Wang, “Advances in gossypol toxicity and processing effects of whole cottonseed in dairy cows feeding,” *Livestock Science*, vol. 111, no. 1-2, pp. 1–9, 2007.

[88] S. T. Willard, D. A. Neuendorff, A. W. Lewis, and R. D. Randel, “Effects of free gossypol in the diet of pregnant and postpartum Brahman cows on calf development and cow performance,” *Journal of Animal Science*, vol. 73, no. 2, pp. 496–507, 1995.

[89] P. A. M. Rogers, T. P. Henaghan, and B. Wheeler, “Gossypol poisoning in young calves,” *Irish Veterinary Journal*, vol. 29, no. 1, pp. 9–13, 1975.

[90] C. A. Holmberg, L. D. Weaver, W. M. Gutterbock, J. Genes, and P. Montgomery, “Pathological and pathological studies of calves fed a high concentration cotton seed meal diet,” *Veterinary Pathology*, vol. 25, no. 2, pp. 147–153, 1988.

[91] C. A. Risco, C. A. Holmberg, and A. Kuches, “Effect of graded concentrations of gossypol on calf performance: toxicological and pathological considerations,” *Journal of Dairy Science*, vol. 75, no. 10, pp. 2787–2798, 1992.

[92] R. Z. Zelski, J. T. Rothwell, R. E. Moore, and D. J. Kennedy, “Gossypol toxicity in prerninant calves,” *Australian Veterinary Journal*, vol. 72, no. 10, pp. 394–398, 1995.

[93] R. B. Fombad and M. J. Bryant, “An evaluation of the use of cottonseed cake in the diet of growing pigs,” *Tropical Animal Health and Production*, vol. 36, no. 3, pp. 295–305, 2004.

[94] L. M. Hudson, L. A. Kerr, and W. R. Maslin, “Gossypol toxicosis in a herd of beef calves,” *Journal of the American Veterinary Medical Association*, vol. 192, no. 9, pp. 1303–1305, 1988.

[95] H. Mena, J. E. P. Santos, J. T. Huber, M. Tarazon, and M. C. Calhoun, “The effects of varying gossypol intake from whole cottonseed and cottonseed meal on lactation and blood parameters in lactating dairy cows,” *Journal of Dairy Science*, vol. 87, no. 8, pp. 2506–2518, 2004.

[96] M. Zbidah, A. Lupescu, N. Shaik, and F. Lang, “Gossypol-induced suicidal erythrocyte death,” *Toxicology*, vol. 302, no. 2-3, pp. 101–105, 2012.

[97] F. Tang and P. Y. D. Wong, “Serum potassium and aldosterone levels in gossypol-treated rats,” *International Journal of Andrology*, vol. 7, no. 2, pp. 149–153, 1984.

[98] Y. Rikihisa and Y. C. Lin, “Effect of gossypol on the thyroid in young rats,” *Journal of Comparative Pathology*, vol. 100, no. 4, pp. 411–417, 1989.

[99] Y. C. Lin, M. Chitcharoenuth, and Y. Rikihisa, “Effect of gossypol on thyroid hormones in young female rats,” *Contraception*, vol. 41, no. 4, pp. 431–440, 1990.

[100] P. Udoh, D. R. Patil, and M. K. Deshpande, “Histopathological and biochemical effects of gossypol acetate on putitary-gonadal axis of male albino rats,” *Contraception*, vol. 45, no. 5, pp. 493–509, 1992.

[101] D. R. Janero and B. Burghardt, “Protection of rat myocardial phospholipid against peroxidative injury through superoxide-(xanthine oxidase)-dependent, iron-promoted fenton chemistry by the male contraceptive gossypol,” *Biochemical Pharmacology*, vol. 37, no. 17, pp. 3335–3342, 1988.

[102] M. W. Fornes, A. M. Barbieri, and M. H. Burgos, “Sperm motility loss induced by gossypol: relation with OH scavengers, motile stimulators and malondialdehyde production,” *Biochemical and Biophysical Research Communications*, vol. 195, no. 3, pp. 1289–1293, 1993.

[103] A. de Peyster, A. Quintanilha, L. Packer, and M. T. Smith, “Oxygen radical formation induced by gossypol in rat liver microsomes and human sperm,” *Biochemical and Biophysical Research Communications*, vol. 118, no. 2, pp. 573–579, 1984.

[104] P. Kovaci, “Mechanism of drug and toxic actions of gossypol: focus on reactive oxygen species and electron transfer,” *Current Medicinal Chemistry*, vol. 10, no. 24, pp. 2711–2718, 2003.

[105] L. A. Meksongse, A. J. Clawson, and F. H. Smith, “The in vivo effect of gossypol on cytochrome oxidase, succinoxidase, and succinic dehydrogenase in animal tissues,” *Journal of Agricultural and Food Chemistry*, vol. 18, no. 5, pp. 917–920, 1970.

[106] M. B. Abou Donia and J. W. Dieckert, “Gossypol: uncoupling of respiratory chain and oxidative phosphorylation,” *Life Sciences*, vol. 14, no. 10, pp. 1955–1963, 1974.

[107] W. W. Tso and C. S. Lee, “Gossypol uncoupling of respiratory chain and oxidative phosphorylation in ejaculated boar spermatozoa,” *Contraception*, vol. 25, no. 6, pp. 649–655, 1982.

[108] W. M. Huang and F. Urthaler, “The direct negative inotropic effect of gossypol,” *Journal of Ethnopharmacology*, vol. 17, no. 1, pp. 31–36, 1986.

[109] Y. Wang and H.-P. Lei, “Hepatotoxicity of gossypol in rats,” *Journal of Ethnopharmacology*, vol. 20, no. 1, pp. 53–64, 1987.

[110] S. Manabe, D. C. Nuber, and Y. C. Lin, “Zone-specific hepatotoxicity of gossypol in perfused rat liver,” *Toxicicon*, vol. 29, no. 6, pp. 787–790, 1991.

[111] S. Z. Qian and Z. G. Wang, “Gossypol: a potential antifertility agent for males,” *Annual Review of Pharmacology and Toxicology*, vol. 24, pp. 329–360, 1984.
Y. Y. Yuan and Q. X. Shi, “Inhibition of hamster sperm

H. Breitbart, S. Rubinstein, and L. Nass-Arden, “Effect of
diets containing gossypol

J. Arshami and J. L. Ruttle,” Effects of diets containing gossypol on spermatogenic tissues of young bulls,” Theriogenology, vol. 30, no. 3, pp. 507–516, 1988.

C. S. Teng, “Reversible changes in the content of cellular and microtubular tubulin in spermatogenic cells after gossypol treatment,” Contraception, vol. 55, no. 1, pp. 41–46, 1997.

H. Breitbart, A. Mayevsky, and L. Nass-Arden, “Molecular mechanisms of gossypol action on sperm motility,” International Journal of Biochemistry, vol. 21, no. 10, pp. 1097–1102, 1989.

A. P. Hoffer, “Effects of gossypol on the seminiferous epithelium in the rat: a light and electron microscope study,” Biology of Reproduction, vol. 28, no. 4, pp. 1007–1020, 1983.

Z.-H. Yu and H. C. Chan, “Gossypol as a male antifertility agent—why studies should have been continued,” International Journal of Andrology, vol. 21, no. 1, pp. 2–7, 1998.

E. M. Coutinho, “Gossypol: a contraceptive for men,” Contraception, vol. 65, no. 4, pp. 259–263, 2002.

K. Dodou, “Investigations on gossypol: past and present developments," Expert Opinion on Investigational Drugs, vol. 14, no. 11, pp. 1419–1434, 2005.

Q. Chang, Z. Liu, W.-Z. Ma et al., “Drug synergistic antifertility effect of combined administration of low-dose gossypol with steroid hormones in rats,” Chinese Medical Journal, vol. 124, no. 11, pp. 1678–1682, 2011.

S. Chongthammakun, C. Ekavipat, B. Sanitwongse, and K. Pavasuthipaisit, “Effects of gossypol on human and monkey sperm motility in vitro,” Contraception, vol. 34, no. 3, pp. 323–331, 1986.

C. Y. Hong, J. J. Huang, and P. Wu, “The inhibitory effect of gossypol on human sperm motility: relationship with time, temperature and concentration,” Human Toxicology, vol. 8, no. 1, pp. 49–51, 1989.

C. Brocas, R. M. Rivera, F. F. Paula-Lopes et al., “Deleterious actions of gossypol on bovine spermatozoa, oocytes, and embryos,” Biology of Reproduction, vol. 57, no. 4, pp. 901–907, 1997.

P. J. Chenothers, C. C. Chase Jr., C. A. Risco, and R. E. Larsen, “Characterization of gossypol-induced sperm abnormalities in bulls,” Theriogenology, vol. 53, no. 5, pp. 1193–1203, 2000.

Y. Y. Yuan and Q. X. Shi, “Inhibition of hamster sperm acrosomal enzyme by gossypol is closely associated with the decrease in fertilization capacity,” Contraception, vol. 62, no. 4, pp. 203–209, 2000.

A. R. Beaudoin, “A developmental toxicity evaluation of gossypol,” Contraception, vol. 37, no. 2, pp. 197–219, 1988.

F. D. C. R. Nunes, D. A. F. de Araujo, M. B. Bezerra, and B. Soto-Blanco, “Effects of gossypol present in cottonseed cake on the spermatogenesis of goats,” Journal of Animal and Veterinary Advances, vol. 9, no. 1, pp. 75–78, 2010.

F. C. B. Guedes and B. Soto-Blanco, “Sperm quality of sheep fed cottonseed cake,” Acta Scientiae Veterinariae, vol. 38, no. 4, pp. 415–418, 2010.

H. Ueno, M. K. Sahni, S. J. Segal, and S. S. Koide, “Interaction of gossypol with sperm macromolecules and enzymes,” Contraception, vol. 37, no. 3, pp. 333–341, 1988.

G. M. Sein, “The embryotoxic and immunodepressive effectsof gossypol with spermmacromolecules and enzymes,” International Journal of Andrology, vol. 23, no. 2, pp. 220–228, 2002.

M. Zhang, H. Yuan, Z. He et al., “DNA damage and decrease of cellular oxidase activity in piglet semitubular cells exposed to gossypol,” African Journal of Biotechnology, vol. 10, no. 14, pp. 2797–2802, 2011.

N. Timurkaan, F. Yilmaz, and S. Timurkaan, “Effects of cottonseed flour on immunohistochemical localization of androgen receptors (AR) in rat testes,” Revue de Medecine Veterinaire, vol. 162, no. 1, pp. 13–17, 2011.

M. L. Gray, L. W. Greene, and G. L. Williams, “Effects of dietary gossypol consumption on metabolic homeostasis and reproductive endocrine function in beef heifers and cows,” Journal of Animal Science, vol. 71, no. 11, pp. 3052–3059, 1993.

G. O. Adeyemo, O. G. Longe, and D. O. Adejumo, “The reproducive performance of breeder cocks fed cottonseed cake-based diets,” International Journal of Poultry Science, vol. 6, no. 2, pp. 140–144, 2007.

G. Basini, S. Bussolati, L. Baioni, and F. Grasselli, “Gossypol, a polyphenolic aldehyde from cotton plant, interferes with swine granulosa cell function,” Domestic Animal Endocrinology, vol. 37, no. 1, pp. 30–36, 2009.

Y. Gu, Y. C. Lin, and Y. Rikihisa, “Inhibitory effect of gossypol on steroidogenic pathways in cultured bovine luteal cells,” Biochemical and Biophysical Research Communications, vol. 169, no. 2, pp. 455–461, 1990.

Y. C. Lin, S. Coskun, and A. Sanbuissko, “Effects of gossypol on in vitro bovine oocyte maturation and steroidogenesis in bovine granulosa cells,” Theriogenology, vol. 41, no. 8, pp. 1601–1611, 1994.

S. M. Zirkle, Y. C. Lin, F. C. Gwazauskas, and R. S. Canseco, “Effect of gossypol on bovine embryo development during the preimplantation period,” Theriogenology, vol. 30, no. 3, pp. 575–582, 1988.

Y. C. Lin, P. Rajamahendran, and Y. Rikihisa, “Inhibition of rat embryo implantation in the gossypol-treated uterine horn,” Theriogenology, vol. 35, no. 4, pp. 769–777, 1991.

J. Hernández-Cerón, F. D. Jousan, P. Soto, and P. J. Hansen, “Timing of inhibitory actions of gossypol on cultured bovine embryos,” Journal of Dairy Science, vol. 88, no. 3, pp. 922–928, 2005.

M. Villaseñor, A. C. Coscioni, K. N. Galvão, R. C. Chebel, and J. E. P. Santos, “Gossypol disrupts embryo development in heifers,” Journal of Dairy Science, vol. 91, no. 8, pp. 3015–3024, 2008.

Y. C. Lin, A. Sanbuissko, S. Coskun, and Y. Rikihisa, “Inhibition of intravitro fertilization and early embryonic development in hamsters by gossypol,” Life Sciences, vol. 55, no. 14, pp. 1139–1145, 1994.

Y. F. Li, G. M. Booth, and R. E. Seegmiller, “Evidence for embryotoxicity of gossypol in mice and chicks with no evidence of mutagenic activity in the Ames test,” Reproductive Toxicology, vol. 3, no. 1, pp. 59–62, 1989.

G. M. Sein, “The embryotoxic and immunodepressive effects of gossypol,” American Journal of Chinese Medicine, vol. 14, no. 3–4, pp. 110–115, 1986.

H. Morales, P. Tilquin, J. F. Rees, A. Massip, F. Desvy, and A. van Langendonckt, “Pyruvate prevents peroxide-induced injury of in vitro preimplantation bovine embryos,” Molecular Reproduction and Development, vol. 52, no. 2, pp. 149–157, 1999.
[146] J.-C. Hervé, F. Pluciennik, B. Bastide et al., “Contraceptive gossypol blocks cell-to-cell communication in human and rat cells,” European Journal of Pharmacology, vol. 313, no. 3, pp. 243–255, 1996.

[147] E. Yurtcu, M. A. Ergun, and A. Meneve, “Apoptotic effect of gossypol on human lymphocytes,” Cell Biology International, vol. 27, no. 9, pp. 791–794, 2003.

[148] G.-H. Cui, Z.-L. Xu, Z.-J. Yang, Y.-Y. Xu, and S.-P. Xue, “A combined regimen of gossypol plus methylestosterone and ethinylestradiol as a contraceptive induces germ cell apoptosis and expression of its related genes in rats,” Contraception, vol. 70, no. 4, pp. 335–342, 2004.

[149] M. A. Ergun, E. Konac, D. Erbas, and A. Ekmekci, “Apoptosis and nitric oxide release induced by thalidomide, gossypol and dexamethasone in cultured human chronic myelogenous leukemic K-562 cells,” Cell Biology International, vol. 28, no. 3, pp. 237–242, 2004.

[150] D.-O. Moon, M.-O. Kim, J.-D. Lee, and G.-Y. Kim, “Gossypol suppresses NF-κB activity and NF-κB-related gene expression in human leukemia U937 cells,” Cancer Letters, vol. 264, no. 2, pp. 192–200, 2008.

[151] E. Cengiz, B. Karaca, Y. Kucukzeybek et al., “Overcoming drug resistance in hormone-and drug-refractory prostate cancer cell line, PC-3 by docetaxel and gossypol combination,” Molecular Biology Reports, vol. 37, no. 3, pp. 1269–1277, 2010.

[152] D.-O. Moon, Y. H. Choi, S.-K. Moon, W.-J. Kim, and G.-Y. Kim, “Gossypol decreases tumor necrosis factor-α-induced intercellular adhesion molecule-1 expression via suppression of NF-κB activity,” Food and Chemical Toxicology, vol. 49, no. 4, pp. 999–1005, 2011.

[153] J.-S. Cheng, Y.-K. Lo, J.-H. Yeh et al., “Effect of gossypol on intracellular Ca²⁺ regulation in human hepatoma cells,” Chinese Journal of Physiology, vol. 46, no. 3, pp. 117–122, 2003.

[154] A. P. Braga, M. V. Maciel, D. G. E. Guerra, I. S. A. S. Maia, S. C. S. Oloris, and B. Soto-Blanco, “Extruded-expelled cottonseed meal decreases lymphocyte counts in male sheep,” Revue de Medecine Vetrinaire, vol. 163, no. 3, pp. 147–152, 2012.

[155] W.-B. Xu, L.-H. Xu, H.-S. Lu et al., “The immunosuppressive effect of gossypol in mice is mediated by inhibition of lymphocyte proliferation and by induction of cell apoptosis,” Acta Pharmacologica Sinica, vol. 30, no. 5, pp. 597–604, 2009.

[156] P. J. E. Quintana, A. de Peyster, S. Klatzke, and H. J. Park, “Gossypol-induced DNA breaks in rat lymphocytes are secondary to cytotoxicity,” Toxicology Letters, vol. 117, no. 1-2, pp. 85–94, 2000.

[157] D. Sijun, A. Pawlak, B. Pożniak et al., “Effects of gossypol acetic acid on cellular and humoral immune response in non-immunized and SRBC-immunized mice,” Central-European Journal of Immunology, vol. 37, no. 1, pp. 11–19, 2012.

[158] D. Xu, W.-J. Cai, B.-H. Zhu, C.-J. Dong, Z.-C. Zheng, and Z.-Q. Gao, “Clinical safety of long-term administration of gossypol in 32 cases,” Contraception, vol. 37, no. 2, pp. 129–135, 1988.

[159] D. Nagalakshimi, V. R. B.Sastry, D. K. Agrawal, and R. C. Katiyar, “Haematological and immunological response in lambs fed on raw and variously processed cottonseed meal,” Asian Australasian Journal of Animal Sciences, vol. 14, no. 1, pp. 21–29, 2001.

[160] A. K. Pattanaik, V. R. B. Sastry, D. K. Singh, T. K. Goswami, and D. N. Mohanty, “Effect of gossypol from cottonseed meal diets on some clinico-biochemical parameters and humoral immune response of crossbred calves fed barley or sorghum,” Asian Australasian Journal of Animal Sciences, vol. 16, no. 9, pp. 1291–1296, 2003.

[161] K. Ohuchi, M. Watanabe, N. Hirasawa, S. Tsurufuji, T. Ozeki, and H. Fuji, “Inhibition by gossypol of tumor promoter-induced arachidonic acid metabolism in rat peritoneal macrophages,” Biochimica et Biophysica Acta: Molecular Cell Research, vol. 971, no. 1, pp. 85–91, 1988.

[162] M. M. Barros, C. Lim, and P. H. Klesius, “Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of channel catfish (Ictalurus punctatus) to Edwardsiella ictaluri challenge,” Aquaculture, vol. 207, no. 3–4, pp. 263–279, 2002.

[163] M. Yildirim, C. Lim, P. J. Wan, and P. H. Klesius, “Growth performance and immune response of channel catfish (Ictalurus punctatus) fed diets containing graded levels of gossypol-acetic acid,” Aquaculture, vol. 219, no. 1–4, pp. 751–768, 2003.

[164] G. S. Fisher, A. W. Frank, and J. P. Cherry, “Total gossypol content of glandless cottonseed,” Journal of Agricultural and Food Chemistry, vol. 36, no. 1, pp. 42–44, 1988.

[165] G. A. Broderick and W. M. Craig, “Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein,” Journal of Nutrition, vol. 110, no. 12, pp. 2381–2389, 1980.

[166] M. H. Henry, G. M. Pesti, R. Bakalli et al., “The performance of broiler chicks fed diets containing extruded cottonseed meal supplemented with lysine,” Poultry Science, vol. 80, no. 6, pp. 762–768, 2001.

[167] H. Jaddou, M. Al Hakim, L. Z. Al Adamy, and M. T. Mhaisen, “Effect of gamma-radiation on gossypol in cottonseed meal,” Journal of Food Science, vol. 48, no. 3, pp. 988–989, 1983.

[168] C. Jo, H. S. Youk, M. S. Lee et al., “Irradiation effects on embryotoxicity and oxidative properties of gossypol dissolved in methanol,” Food and Chemical Toxicology, vol. 41, no. 10, pp. 1329–1336, 2003.

[169] S. A. Smalley and E. J. Bicknell, “Gossypol toxicity in dairy cattle,” Compendium on Continuing Education for the Practising Veterinarian, vol. 4, no. 9, pp. S378–S381, 1982.

[170] X. Yang, J.-Y. Sun, J.-L. Guo, and X.-Y. Weng, “Identification and proteomic analysis of a novel gossypol-degrading fungal strain,” Journal of the Science of Food and Agriculture, vol. 92, no. 4, pp. 943–951, 2012.

[171] H. Sun, J. W. Tang, C. L. Fang et al., “Molecular analysis of intestinal bacterial microbiota of broiler chickens fed diets containing fermented cottonseed meal,” Poultry Science, vol. 92, no. 2, pp. 392–401, 2013.