A review of germinal cell apoptosis by herbal medicine

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ABSTRACT
Apoptosis is a mechanism of cell death with a major role in cell recycling, which occurs in several organs: Testical, placenta, prostate, breast, ovarian, and cancer cells. Apoptotic signal pathway may be used to repair reproductive health disorders. Signal pathway of germ cell usually begins with extrinsic apoptotic pathway involving Fas/Fasl molecule and mitochondrial pathway with Bcl-2 protein component. Apoptotic process in germ cell can also be triggered by exposing certain plant secondary metabolite to certain cells. Many plants have been reported successfully triggering apoptotic properties, i.e. (1) Areca catechu, (2) Carica papaya, (3) Camellia sinensis, (4) Carcuma domestica, (5) Costus speciosus, (6) Gossypium hirsutum, (7) Hibiscus spp., (8) Luffa aegyptiaca, (9) Monordica charantia, (10) Nicotiana tabacum, (11) Olea europaea, (12) Ocimum basilicum, and (13) Zingiber officinale. The metabolites may induce apoptosis through upregulating Fas/Fasl and p53 expression, Bax/Bcl-2, along with caspase-3 activation. The mentioned plants then will induce apoptosis in germ cell and may become promising candidates to treat cancer as evidenced from extensive laboratory studies.

Keywords: Herbal, Apoptosis, Germ cell.

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INTRODUCTION
Apoptosis is a cellular key mechanism which explains the fate of cells and provides further insight into the mechanism of protection against reproductive toxicity [1]. Apoptosis is also called a programmed cell death with a major role in eliminating germ cells in all stages of oogenesis and even after ovulation [153,154]. More than 99% of germ cells are eliminated in the ovaries through apoptosis through follicular atresia, while <1% peaks into oogonia [153,155]. Oogonia in the meiotic stage produce primary oocytes [154,157]. Oocytes in the diplotene stage are encircled by layers of granulosa cells in the follicle [158,159].

Cigarette smoke (CS) has a strong link with carcinogens and change in metastasis-related gen expression in human ovarian by regulating cell cycle, effect on spermatogenesis in rat, infertility in men, histologic reactions due to hypoxemia, damage to this limited pool of gametes in fertility in women [2-4]. Apoptosis of germ cells has been shown to play an important role in controlling sperm output in many species, and massive germ cell death occurs under physiological conditions during the first stages of spermatogenesis so called the constitutive apoptosis [1,148,149]. In the adult testes, germ cells strictly depend on the physical and biochemical support of Sertoli cells (SCs) and the somatic cells within the seminiferous tubules, which nourish and sustain the developing germ cells [143]. However, SCs have a limited capacity regarding the number of germ cells they can support, and it has been accepted that they play a crucial role in determining germ cell [144].

The association of death receptors and the extrinsic pathway of apoptosis with male infertility have been suggested by several studies, which showed an upregulated expression of Fasl, resulting in maturation arrest and SC-only syndrome, characterized by the absence of germ cells in the seminiferous epithelium [150-152]. Alteration of sperm DNA has been found in pre-implanted embryos and lead offspring toward greater risk of malformation, cancer, and genetic diseases that may cause apoptosis and interference in the seminiferous tubules [5,6].

Plumbum pollution can also significantly decrease sperm count, without any significant increase in the ratio of testes/100 g rat weight [7]. Heat stress may also cause DNA damage to germ cells and increase cell death (TUNEL assay), subfertility in stem cell death associated with increasing effector caspase expression (expression by caspase-3), and downregulation of protein called inhibitors caspase-activated DNase [8]. P63 can also mediate the apoptosis of male germ cells and regulate three stages of spermatogenesis transcriptionally and could provide novel targets for the diagnosis and treatment of male infertility [9]. The novel apoptosis regulators such as aven, survivin, and regucalcin have also showed an altered expression in human testes with defective spermatogenesis [145,147]. Humanin prevented stress-induced apoptosis in many cells/tissues, with ameliorated chemotherapy, cyclophosphamide, and doxorubicin (DDO)-induced germ cell apoptosis in both ex vivo and in vivo tests using cultures of seminiferous tubule [10].

Mechanism and signal pathway can be utilized to improve reproductive health problems because signal pathway in germ cell usually uses an extrinsic apoptotic pathway involving Fas/Fasl molecules and intrinsic or mitochondrial pathways [11]. In placental cells, chlorpyrifrinos (organophosphorus insecticides) induced apoptosis in placental cells through independent pathways of Fas/tumor necrosis factor signaling, caspase activation, or cholinesterase inhibition due to activation of p38 mitogen-activated protein kinase (MAPK) which was an integral part of cell protection against injury exposed to chlorpyrifrinos [12]. The placental tissue contains paternal antigens, and under normal condition, the semi-allogeneic fetus and placenta are not invaded by the maternal immune system because tolerance to fetal antigen occurs in the presence of large numbers of maternal leukocytes [13]. The process of apoptosis in germ cells can also be triggered by plants that contain unique secondary metabolites.

ARECA CATECHU
A. catechu consumed by the Asian community has four major alkaloids called arecoline, arecaidine, guvacoline, and guvacine [14]. Arecoline can pass through the basal membrane of seminiferous tubules and interact with seminiferous tubular components, therefore altering gonadal function and sperm formation, and cause apoptosis in testicular tissue [15]. The plant is considered as a popular carcinogen because it contains apoptotic-inducing agents and may be used later as...
a new strategy to improve cancer therapy [16]. The effect of exposure to *A. catechu* seed water fraction has been reported to decrease sperm motility [15]. Induction of apoptosis in combination with DOX induced expression of Bax and caspase-3 proteins that mediate apoptosis based on immunocytochemistry study [17]. It also induced cytotoxicity and its genotoxicity under normal condition and may lead to the formation of larger tumors and oral squamous cell carcinoma [18]. Combination with alcohol indicated antifertility activity in the apotopic area as shown from histological study [14]. The extract with doses above 40 g/mL induced mutations in hypoxanthine so as to increase oxidative stress and genetic damage to human keratinocytes [19].

**CARICA PAPAYA**

*C. papaya* contains saponins, cardiac glycosides, anthraquinones, reducing sugars, flavonoids, alkaloids, and tannins with biological properties as antioxidants, anti-inflammatory, anticancer, and other diseases [20]. Antioxidant and anticancer potential activities of the hexane fraction from the male flower of *C. papaya* have long been known as an important source of nutraceutical and pharmacological compounds [21]. Aqueous extract from *C. papaya* leaves reduced the incidence of sperm deformity by 12.37% and 6.53%. Thus, another study reported a significant increase in the formation of luminous polychromat erythrocytes with micronuculi (MNPCs) in bone marrow cells and sperm abnormalities [22]. A study with methanol fraction from *C. papaya* seeds reduced the volume of the nucleus and cytoplasm, leading to damage of acrosome and mitochondria along with occurrence of apoptosis testis [23]. Another study reported combination of diet with *Citrus limon* improved the potential of infertility in rats fed with *C. papaya* seed extract [24]. Anti-proliferative effect of methylethylhexolodylphelletetratromelin bromide (MTH) and lipophilic extract of *C. papaya* ripening at different stages and times was exposed to breast carcinoma cell lines and did not inhibit cell proliferation of MCF-12F and MDA-MB-231, while showing a significant effect to MCF-7 cells within 72 h [25]. Percentage of MCF-7 cells exhibiting apoptosis in breast cancer exposed to *C. papaya* extract was lower than DOX, although in other case, the percentage was higher than quercetin result and thus may be considered as anticancer through antioxidants and anti-proliferative and apoptotic induction mechanisms [25]. Other study also reported the use of extract from unripe fruit of *C. papaya* L. exhibiting an antiplastic activity against Dalton’s ascitic lymphoma cells in Swiss albino mice [26].

**CAMELLIA SINENSIS**

*C. sinensis* contains curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin as well as volatile oil (tumerone, atlantone, and zingiberone), sugars, proteins, and resins [41]. In India, *C. sinensis* undergoes genetic variation in its population due to a wide range of ecological conditions and distribution [167]. The secondary metabolite, namely, curcumin hindered the synthesis of aflatoxin by *Aspergillus flavus* [42]. Demethoxycurcumin and bisdemethoxycurcumin in curcuminoid were found to be varied in two samples regarding the pharmacological activities [43]. Curcumin decreased MDA levels in pre-eclampsia by lowering blood pressure and protein levels in the urine [44]. Curcumin also decreased depolarization of mitochondrial membranes and expression of apoptotic proteins [45]. In male germ cells, *C. domestica* showed protective effect despite its also damages to testes [46]. Treatment of *C. domestica* reduced apoptosis in the testes and decreased expression of Fas, Bax, and cleaved-caspase-3 and also increased expression of Bcl-xl. It also improved testicular histological qualities and significantly reduced apoptotic levels by inhibiting oxidative stress and modulating cell death pathway-mediated Bax/Bcl-2 [46,49]. It inhibited proliferation of NTEa-2 cells through signal pathways and caused apoptosis by reducing FasL expression and Bcl-2/Bax ratio and activating caspases 9, 8, and 3 [48,50]. However, when caspase 3 active in germ cells like spermatogonia, spermatocytes and spermatids, encourage of decreasing spermatozoa concentration [51].

Curcumin inhibited cell growth by inducing apoptosis on cancer cells but not on normal cells, and PGV-1 cell showed the strongest apoptosis induction effect on cancer cell lines [52]. Combination of *C. sinensis* and Apo-2L/TRAIL ligands increased the induction of apoptotic cell death in ovarian cancer by activating extrinsic and intrinsic pathways of apoptosis [53]. *C. sinensis* also induced G2/M phase cells in CR cells by increasing phosphorylation of p53 and apoptosis through caspase-3 activation and PARP degradation [54]. Combination of arsenic and chlorpyrifos triggered apoptosis, but *C. domestica* simultaneously inhibited apoptosis because of its antioxidant properties [55]. A study showed that *C. domestica* inhibited the proliferation of cancer cells through molecular mechanisms [56]. The molecular mechanism of *C. domestica* for breast cancer by inhibiting proliferation of MDA-MB-231 and BT-474 cells was through p21 expression, mediated by NF-xB, cyclin D, and MMP-1 regulation [57,58]. *C. domestica* also altered cellular response through a p53-dependent pathway, in which Bax acted as p53 effector [59]. The plant significantly reduced tumor size and its proliferation with a combination of *C. domestica*-paclitaxel for the treatment of breast cancer [60]. Other mechanism reported was the inhibition to migration and invasion of N18 cells that acted as preventive agent in metastatic cancer [45]. *C. domestica* also significantly inhibited apoptosis of ESC-B5 cells in mice and blastocysts induced by methylglyoxal [61]. The chromatography technique, namely, reverse-phase high-performance liquid chromatography was very useful in quantifying and controlling the quality of extracted curcumin [166].

**COSTUS SPECIOSUS**

*C. speciosus* contains ascorbic acid, beta-carotene, alpha-tocoferol, glutathione, phenols, flavonoids, alkaloids and terpenoids, and flavonoids [62]. *C. speciosus* possessed potential antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* which commonly used and served as therapy in traditional medicine [63]. This plant exhibits biochemical effect on serum glucose, serum cholesterol (Chol), carcinoembryonic
antigen, and carbohydrate antigens [64]. Diosgenin isolated from C. speciosus inhibits cell viability, generating induction in cell proliferation, and significantly increased caspase-3 in MCF-7 cells [65]. Diosgenin with taurine decreased the number of forming spermatogonia, spermatocytes, and spermatid cells [66]. Combination of DOX and hesperidin also led to increasing MC-7 cell apoptosis and was developed as chemotherapy agent to breast cancer [67]. Other components, namely, costunolide in this plant induced apoptosis of breast cancer cells [68]. The costunolide acted with SOD, catalase, and GPx as potential antioxidant agents in human breast cancer cells through apoptosis mechanism [69]. It also synergized with the use of Rhaphidophora pinnata as inhibitor of cell proliferation and apoptosis in MCF-7 cells [70].

**GOSSYPIUM HIRSUTUM**

G. hirsutum contains phenolic compounds and hydrolytic enzymes including β-glucosidase, carboxylesterase, and glutathione-S-transferase [71]. Rice treated with gossypol acetic acid exhibit a SC toxicity and seminiferous tubular degeneration in sperm with increasing activity of 17β-hydroxysteroid dehydrogenase and 17-ketosteroids [72]. Combination of gossypol with methyltestosterone and ethinylestradiol was reported to act as contraceptive agents by involving the system of Fas, Bax, caspase, and apoptotic in intrinsic and extrinsic pathways [73]. G. hirsutum induced a decrease in pro-survival regulation of Bcl-xl and Mcl-1 proteins that increased the sensitivity of urinary cancer cells toward carboplatin and gemcitabine [74]. Carboplatin from G. hirsutum at a concentration of 380 mg/mL inhibited ovarian cancerous and tumorous cells [75]. G. hirsutum also disrupted estrous cycle by reducing number of follicles in female reproduction [76]. It affects granulosa cell activity in vitro and might recover fertility in pigs [77]. Combination with zeolidronic acid produced a synergistic cytotoxicity and direct inhibition to cell proliferation, although it also possessed some side effects to certain molecules in angiogenesis [78].

**HIBISCUS SABDARIFFA**

Hibiscus spp. contains triterpene, flossteroid, phenolic, and flavonoids that are capable of preventing the proliferation of malignant cells in the development of new anticancer drugs [79,80]. Purified H. sabdariffa with ellagic acid compounds exhibited apoptotic properties through intrinsic and extrinsic apoptotic pathways that inhibited the growth of LNCaP cells in prostate cancer [81]. However, a study reported treatment using Hibiscus which caused a reversible suppression of spermatozoa membrane cholesterol levels, glucose levels and changes in the seminiferous tubules, and seminal epithelial density [82]. Combination of H. sabdariffa and Zingiber officinale increased antioxidant level and enzyme activity in the testes as to restore the motility of rat spermatozoa treated with cisplatin [1]. Aqueous H. sabdariffa extract inhibited the growth of MCF-7 cells [57]. However, anthocyanins isolated from H. sabdariffa failed to stimulate proteins associated with apoptosis Bcl-2, Bax, and AMP-activated kinase [83]. H. sabdariffa also inhibited the proliferation of serum stimulatory smooth muscle cells and triggered apoptosis through the activating protein kinase P38 (MAPK) pathway [82].

**LUFFA AEGYPTIACA**

L. aegyptiaca contains alkaloids and saponins with known antimicrobial activities against E. coli, S. aureus, Salmonella typhi, and Bacillus subtilis [165]. The extract from Luffa aegyptiaca also possessed potential antioxidant and anthelmintic activity [84]. Caspase-3 may be activated by caspase-8 so as to trigger germ cell apoptosis. Combination of testosterone undecanoate (TU) with L. aegyptiaca (Blustru) extract increased the activity of caspase-3 molecules, triggering the occurrence of germinal cell (apoptotic) fragmentation through decreasing testosterone and thereby decreasing the quality and quantity of spermatogonia. Combination of hormones increased apoptosis in germ cells, causing azoospermia [164]. Other Luffa species, namely, L. cylindrical seed extract contains Luffin-a which is a single Type I protein inactivation (the most toxic in the Luffin family) possessed antitumor activity by inhibiting protein synthesis in rabbit reticuloocyte while inducing apoptosis [85]. In addition to germ cell apoptosis, L. aegyptiaca (cylindrica) also possessed great potential to remove heavy metals in water, although still not standardized as a drinking water media filter [86].

**MOMORDICA CHARANTIA**

M. charantica contains two classes of saponins, namely, cucurbitane and oleanane types that can reduce cell viability and reduce lipid accumulation [87]. The presence of high total phenolic acid content in 50% ethanol extract of this plant may act as an antitumor agent [88]. The high dose of M. charantia extract caused infertility in the seminiferous tubules and testosterone levels that affected sperm motility and acrosome membranes [89]. M. charantia was also reported as pharmacological and phytochemical extract with antidiabetic activity [90]. Methane extract of M. charantia seed + depot medroxyprogesterone acetate (DMPA) can be used as a male contraceptive tool in the future, as it accelerated the decrease of progesterone production (precursor testosterone) and reduced the quantity and quality of sperm in rats [91]. DMPA reduced sperm viability due to its terpenoid effects that trigger pro-apoptotic proteins such as Bax, Bid, and p53 in which later increased the incidence of apoptosis [92]. This plant induced caspase-3 cleavage, DFF-45, and PARP activation, which caused DNA fragmentation by triggering apoptosis through the path of caspases and mitochondrial pathways in cancer cells [93]. It also significantly decreased the formation of micronucleus, inhibited chromosomal aberrations, and increased the mitotic index [94]. Methane extract of M. charantia seed and DMPA affected stem cell activity in signal transduction as shown by histological image of mice cerebellum [95,96]. Administration of ethanol extract induced apoptosis in rat testes with increasing expression of Fas/Fas-L and p53, regulation of Bax/Bcl-2 ratio, cytochrome c translocation with caspase-3 activation, and glutathione depletion [97]. MCF-7 cells treated with M. charantia during G2/M phase increased expression of p53 and p21, pChk1/2 inhibited the expression of cyclin B1 and cyclin D1 by involving regulation of cell cycle, thus inhibiting the growth of breast cancer cells [98]. Combination of M. charantia and C. domestica prevented cell damage and provided significant protection against changes in malondialdehyde (MDA), conjugated diene, and defense antioxidants [99]. Tahitian noni dietary supplements can also improve testicular toxicity supplemented with high doses of M. charantia extract [100].

**NICOTIANA TABACUM**

N. tabacum contains alkaloids, fatty acids, nitrogen, fluorine, sulfur, and oxygen-containing compounds [101]. N. tabacum may pass its compounds through the placenta and then causing apoptosis, affecting sex hormone secretion, germ cell extension, and infertility in men [102]. Fetal membranes exposed to N. tabacum were experiencing apoptosis in a non-inflammatory pathway on pRROM that increased proteolysis resulting in membrane weakening and rupture [103]. Exposure of N. tabacum during pregnancy and lactation can lead to transient structural changes in the male fetal testes and epididymis and the number of germ cells and somatic embryos [104,105]. Micro-RNA/miRNA exposed to cigarette smoke compared with controls was highly responsive to the exposure on placenta [106]. The fate of fetus may be critical, when germ cells resolved proliferation, in which their germ cells were multiplied by 2%, although bud cell apoptosis was unaffected in germ cells within oocytes, expressing AhRat the stage of proliferative development (metiotic stage) [107]. N. tabacum also acted by suppressing testosterone biosynthesis, reducing mRNA levels, Bcl-2 protein, regulating p53, caspase-3 mRNA, and protein levels that may affect spermatogenesis [108]. Aqueous extract of N. tabacum was considered as a potential endocrine disruptor that may affect the micro-anatomical form and testicular function [109]. However, N. tabacum showed a slight effect on body weight although this effect was significantly low [110].
OLEA EUROPAEA

O. europaea contains exogenous antioxidants with various benefits. Olive leaves were compared to Extra virgin olive oil (EVOO) possessed an improved anti-inflammatory, and anticancer properties [111]. EVOO was reported to be able to control the induction of Hsp70 serum levels, thereby reducing growth of cell due to fetal complications in pre-eclampsia [160]. Induction of Hsp 70 may cause germ cell damage, cancerous cells, and apoptosis in mouse cochlea by cooperating with caspase-3 [112-114]. Provision of serelaxin improved the pathophysiology of placental ischemia in pre-eclampsia rats [115]. The ability of polyphenols in EVOO to inhibit HER2 activity significantly affected breast cancer cell proliferation, although no significant effect was observed on the metastasis of gene expression in HT115 cells through molecular mechanisms [116-118]. Depending on its structure, some polyphenols [e.g., flavonoids] modulated tyrosine HER2 receptor kinase in human breast epithelial cells based on in vitro transformation study [119]. Effects of oleuropein on breast cancer cell death was also reported from O. europaea and suggested the specific cytotoxicity in breast cancer cells, with a higher effect on MDA-MB-231 cells [120]. Combination of EVOO and high supplementation of corn oil provided modulation effects on breast cancer through a combination of different signaling pathways [121]. In addition, EVOO also possessed neuroprotective activity that may counter the oxidative damage to the brain caused by 2,4-D [122].

OCIMUM BASILICUM

O. basilicum contains alkaloids, phytoestrogens, resins, flavonoids, tannins, diterpenes, and protein in the seed extract [124]. O. basilicum possessed anti-proliferative activity in testicular apoptosis because Cd increased Bax and decreased Bcl-2 in germ cells [123]. Ocimum was also known for its antioxidant activity while may also trigger testicular apoptosis occurred due to decrease in cell proliferation and Ki-67 expression [125]. In addition, the plant caused elevation of glucose levels in the testes and epididymis and served as a substrate for gluconeogenesis [127]. Methanol extract of O. basilicum possessed a considerable anti-proliferative activity against the MCF-7 cells [161]. The purified essential oil of the plant inhibited the proliferation of 170 mg/mL of Michigan-7 cancer stem cells (MCF-7) that later induced apoptosis and acted as a pro-apoptotic inducer or acted as an antioxidant agent [37]. And up-regulated the expression of apoptotic gene and as well increased the bax/bcl2 ratio [126]. The plant constituents also increased choline acetyltransferase (ChAT) expression and restored ChAT expression due to deteriorating human cerebral microvascular endothelial cells in nerve cells [128]. O. basilicum may also reduce electromagnetic exposure to testicular histologic apoptosis [162]. As from other study, there was a clear evidence of potential triggering effect of uterine apoptosis using electromagnetic fields’ laboratory technique [129].

Z. OFFICINALE

Z. officinale contains several bioactive constituents with the highest total amount of phenolics and flavonoids in the rhizomes and stems [130]. Treatment with Z. officinale improved histological changes, reduced apoptosis in rat testes, and caused a decrease in the percentage of apoptotic cells in positive Bax cells [131]. The extract repaired cells induced by Sodium arsenite as apoptotic inducing, contraceptive inducing agents, and treatment of ovarian cancer; (7) Hibiscus spp., as apoptotic inducing spermatogenesis; (8) L. egypptica, as apoptotic inducing in germinal cell, through decreasing testosterone; (9) M. charantia, as apoptotic inducing in rat testis, and inhibiting the growth of breast cancer cells; (10) N. tabacum, as apoptotic inducing, affecting sex hormone secretion, germ cell extension, infertility in men, and increase proteolysis in membrane weakening and rupture in fetal; (11) O. europaea, as apoptotic inducing on breast cancer and reducing growth of cell due to fetal in pre-eclampsia; (12) O. basilicum, as apoptotic inducing, pro-apoptotic inducer by electromagnetic exposure; (13) Z. officinale, as apoptotic inducing in endometrial cancer and promising anticancer through apoptotic.

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CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

AUTHORS’ CONTRIBUTION

Putri Cahaya Situmorang undertook the most part during preparation of manuscript and Syafrudin Ilyas acted as corresponding author with responsibility to review the content and English grammar.

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Amedu and Idoko (2015) found that *Carica papaya* extract can inhibit the motility of human spermatozoa with a concentration of 1:100. They also demonstrated that the extract can inhibit the motility of human spermatozoa with a concentration of 1:100.

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