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Review Article

# REVIEW: GERMINAL CELL APOPTOSIS BY HERBAL MEDICINE

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**ABSTRACT**

Apoptosis is a mechanism of cell death with a main 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) *Curcuma domestica,* (5) *Costus speciosus,* (6) *Gossypium hirsutum,* (7) *Hibiscus* spp., (8) *Luffa aegyptiaca,* (9) *Momordica charantia,* (10) *Nicotiana tabacum,* (11) *Olea europaea,* (12) *Ocimum basilicum*, and (13) *Zingiber officinale*. The metabolites may induce apoptosis through upregulating Fas/Fas-L 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 metastesis-related gen expression in human ovarian by regulating celll cycle, effect on spermatogenesis in rat, infertily in men, hisologic 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 [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 (excision 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 (DOX)-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, chlorpyrifos (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 chlorpyrifos [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 interacted 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 apoptotic 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 antidiabetes, 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 pharmaceutical 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 polychromatic erythrocytes with micronuclei (MNPCE) in bone marrow cells and sperm abnormalities [22]. A study with methanol subfraction 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 *Citrullus lanatus* improved the potential of infertility in rats fed with *C. papaya* seed extract [24]. Antiproliferative effect of methylthiazolydiphenyltetrazolium bromide (MTT) 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 anti-proliferative and apoptotic induction mechanisms [25]. Other study also reported the use of extract from unripe fruit of *C. papaya* L. exhibiting an antineoplastic activity against Dalton's ascitic lymphoma cells in Swiss albino mice [26].

### *CAMELLIA SINENSIS*

*C. sinensis* contains a major component of flavonoid and its derivatives, i.e., flavan-3-ols or catechin with medicinal properties along with its enzyme flavanone 3-hydroxylase which catalyzes stereospecific (2S)-naringenin hydroxylation to form (2R, 3R)–dihydrokaempferol [27]. This plant has a strong inhibitory effect against free radicals and may exhibit potent anticancer activity [28]. *C. sinensis* extract is useful against tissue damage and hepatic dysfunction through antioxidants and anti-apoptosis in defense mechanisms [29,30]. Epigallocatechin gallate (EGCG) in this herb significantly reduced renal dysfunction, histological changes, and increasing expression of tumor necrosis factor-α, IL-1β, IL-6, Bax, and caspase [31]. Combination of green tea and vitamin E in diabetic rats increased the number of apoptotic cells in testicular tissue [32]. Green tea extract has a protective effect on the testis by inhibiting oxidation of cadmium chloride ($CdCl_2$), resulting in cell damage and apoptosis [33,34].

Green tea extract also showed some protective effects to testis based on rats' study with malathion-induced testicular oxidative [35]. The plant also affected germ cells in the placenta significantly, due to the recovery of cellular homeostasis, through effective antioxidant capacity and prevention of cell damage during stressful conditions [36]. Selenium-containing polysaccharides in tea (Se-GTPs) triggered apoptosis pathways of mitochondrial with increased Bax/Bcl-2 ratio, caspase-3, and caspase-9 in MCF-7 cells [37]. Preventive mechanisms are commonly found with catechins activity of green tea extract, used in dietary and chemoprevention of carcinogenesis [38]. The combination of three natural products such as arctigenin which is a new anti-inflammatory lignan in *Arctium lappa* seed, green tea polyphenols, EGCG, and curcumin (Cur) increased the chemopreventive potential of tLNCaP compounds in prosthetic and MCF-7 in breast cancer [39]. Purple tea extract also caused PARP cleavage, caspase-3 activation, and increasing Bax/Bcl-2 ratio in colorectal carcinoma cell proliferation [40].

### *CURCUMA DOMESTICA*

*C. domestica* contains curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin as well as volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins [41]. In India, Curcuma 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 [47]. It also improved testicular histological qualities and significantly reduced apoptotic levels by inhibiting oxidative stress and modulating cell death pathway-mediated Bax/Bcl-2 [48,49]. It inhibited proliferation of NTera-2 cells through signal pathways and caused apoptosis by reducing FasL expression and Bcl-2-to-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. domestica* and Apo2L/TRAIL ligands increased the induction of apoptotic cell death in ovarian cancer by activating extrinsic and intrinsic pathways of apoptosis [53]. *C. domestica* 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-483 cells was through p21 expression, mediated by NF-κB, 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-tocopherol, 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 *Rhaphidohora pinnata* as inhibitor of cell proliferation and apoptosis to MCF-7 cells [70].

### *GOSSYPIUM HIRSUTUM*

*G. hirsutum* contains phenolic compounds and hydrolytic enzymes including β-glucosidase, carboxylesterase, and glutathione-S-transferase [71]. Mice 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 zoledronic 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, fitosteroid, 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 spermatogenesis, cholesterol levels, glucose levels and changes in the seminiferous tubules, and germinal 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 spermatozoa. 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 reticulocyte 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. charantia* 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 anticancer 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 pPROM 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 germinal cells resolved proliferation, in which their germ cells were multiplied by 29%, although bud cell apoptosis was unaffected in germ cells within ovaries, expressing AhR at the stage of proliferative development (meiotic 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. Olives converted to Extra virgin olive oil (Evoo) possessed analgesic, anti-inflammatory, and anticancer properties [111]. EVOO was reported to 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, phytosterols, resin, 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 ($IC_{50}$ = 170 µg.mL$^{-1}$) of Michigan-7 cancer stem cells (MCF-7) that later induced apoptosis and acted as a pro-apoptotic inducer or acted as an anticancer 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 deterioration of 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 arsenite which caused oxidative stress that contributed to reproductive damage in male rats [132]. The reproductive system of mice exposed to formalin 10% significantly induced oxidative stress [133]. Oral administration improved weight, decreased antioxidant enzyme activity, and increased MDA and serum total homocysteine [134]. *Z. officinale* also improved testosterone and luteinizing hormone levels due to exposure of mancozeb-fungicide induction [135]. The plant may become a promising candidate for the treatment of breast carcinoma because it decreases the expression of pro-survival genes, such as NF-κB, Bcl-X, Mcl-1, survivin, and cell cycle regulatory proteins including cyclin D1 and cyclin-dependent kinase-4 [163]. The extract may also improve the effects of $CdCl_2$ as a toxin that induces changes in the uterus and ovaries [136]. Treatment of steam distilled extract of ginger mediated apoptosis by activating p53 and other agents in the treatment of endometrial cancer [137]. Ginger varieties with high $CO_2$ concentrations can be used against two cancer cell lines in humans (MCF-7 and MDA-MB-231), especially breast cell lines [138].Compounds like 6-gingerol in this plants, and combination with PI-3K inhibitors and cisplatin was considered safe and has potential in Cervical cell lines and anti-tumor activity in human cancer cells [139]. Combination of *Z. officinale* and *Piper retrofractum* induced myeloma and WiDr cells with apoptotic activity and increased expression of p53 [140]. ZER-HPβCD complex isolated from *Z. officinale* acted as a promising anticancer agent through apoptotic mechanism [141]. The plant also possessed an ameliorative effect on kidney damage induced by metalaxyl [142].

## CONCLUSION

Apoptosis caused by incorporation of plant extracts into several in vitro studies has shown promising result. The plants who successfully triggering apoptotic i.e (1) *A. catechu*, as apoptotic inducing agents and as a new strategy to improve cancer therapy; (2) *C. papaya*, as apoptotic inducing in testis and breast cancer; (3) *C. sinensis*, as apoptotic inducing in testicular tissue, affected germ cells in the placenta, and potent anticancer activity; (4) *C. domestica*, as apoptotic inducing in ovarian cancer; (5) *C. speciosus*, as apoptotic inducing in breast cancer cells and decreased the number of spermatogonia, spermatocytes, and spermatid cells. (6) *G. hirsutum*, as apoptotic inducing, contraceptive agents, and treatment of ovarian cancer; (7) *Hibiscus spp*., as apoptotic inducing spermatogenesis; (8) *L. aegyptiaca*, 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 Syafruddin Ilyas acted as corresponding author with responsibility to review the content and English grammar.

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