REVIEW ARTICLE

The Potential of Gallic Acid as a Radiosensitizer on Human Prostate Cancer: A Systematic Review of Preclinical Studies

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ABSTRACT

Prostate adenocarcinoma accounts for majority of prostate cancer cases, and it was found to be highly radioresistant. Gallic acid is a phenolic acid naturally occurring in many plants, reported to exhibit biological activities in eliminating cancer cell lines and xenografts. The purpose of this study is to review gallic acid as a potential radiosensitizer agent in prostate cancer treatment. Article search was conducted in PubMed, EBSCO, and Scopus. 11 studies using different cell lines including DU145, PC-3, LNCaP, and 22Rv1 xenograft of human prostate cancer were reviewed in this paper. Gallic acid acts as a radiosensitizer mainly by increasing caspase-3 and caspase-9 activation resulting in apoptosis, while also reducing intracellular CDKs, cyclins, and cdc25 phosphatases ultimately causing G2-M cell cycle arrest. Gallic acid has a potential to be a new radiosensitizer compound in prostate cancer treatment. Additional clinical studies using gallic acid derivatives with lower hydrophilicity are needed.

Keywords: Gallic acid, Radiotherapy, Radiobiology, Apoptosis, Prostate cancer

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INTRODUCTION

Gallic acid is a type of polyphenolic acid that naturally occurs in many types of plants, including grapes, gallnuts, sumac, witch hazel, tea leaves, and oak bark (1). It has been reported to exhibit diverse biological activities, including as antioxidant, pro-oxidant, and anti-carcinogenic (2,3). Gallic acid is widely known as a substance that can induce apoptosis and inhibit the proliferation of cancer cells. Furthermore, gallic acid selectively prevents malignant transformation and in vitro cancer development without considerably affecting normal cells. Russell et al. found the LC50 of gallic acid on LnCAP human prostate cancer cell line after treatment for 72 hours to be 48,1 µg/mL compared to >80 µg/mL for PrEC normal prostate epithelial cell line (4). Kaur et al. found that gallic acid at a concentration range of 10-100 µM for 48 hours decrease DU145 human prostate cancer cell line and 22Rv1 human prostate cancer xenograft viability, but not on PWR-1E normal prostate epithelial cell line (5).

Gallic acid also act as a pro-oxidant by increasing ROS production that contributes to apoptosis, but it was dependent on the oxygen level in the cancer cells, pH, and amount of transition metal ions in the cancer cells (6,7). Hypoxic status has been found to accommodate the formation of reactive oxygen species (ROS) (8). Alkaline pH of 8-8,4 catalyzes the formation of GSH, which plays a major role in oxidant defence reaction (9). Transition metal ions also affect the pro-oxidant status by becoming the electron donor, thereby favoring the formation of ROS (8). Stirlic et al. found that gallic acid holds pro-oxidative property by promoting the formation of hydroxyl radical through iron chelation, and the pro-oxidant effect is emphasized when the molar ratio between gallic acid and iron is lower than 2 and when the pH is lower than 6 (10).

Prostate cancer is one of the biggest causes of mortality in men, with around 366.000 men died from prostate cancer every year (11). According to GLOBOCAN, there are 1,276,106 new cases of prostate cancer in 2018 (12). Irradiation is one of the standard therapy choices for low-risk prostate cancer with no accompanying

gastrointestinal and genitourinary symptoms for localized intermediate-risk and high-risk prostate cancer (13). Irradiation can also be used as an adjuvant therapy in patients who have undergone prostatectomy with pT3N0 prostate cancer, positive surgical margin, seminal vesicle invasion, and extension to extracapsular tissues (13). Other indications include prostate cancer with lymph node metastases and in patients with bone metastases in order to reduce pain and pathologic fracture incidence (13). Prostate adenocarcinoma is a histopathological subtype of prostate cancer that accounts for almost all prostate cancers, and it is known to be highly radioresistant (14,15). Radioresistance in prostate cancer depends on several factors. These factors include resistance to reactive oxygen species (ROS) and increased DNA repair activation (16). Yajun et al. found that prostate cancer might express differentially expressed gene, including INHBA, CD22, MAP2K5, and ROR1 (17). INHBA, or inhibin beta A, is a subunit of inhibin and activin, both of which are members of TGF-β family. When the isoforms of INHBA combine, it becomes activin A which correlates with lymph node metastasis and tumour invasion (18). CD22, or also known as the cluster of differentiation-22, is a glycoprotein expressed on the cell membranes of lymphocytes B. It was found to be overly expressed in prostate cancer (1,17). MAP2K5, which stands for mitogen-activated protein kinase 5 (MAP2K5), was overexpressed in prostate cancer and contributed to prostate cancer proliferation and apoptosis via the MEK5/ERK5 pathway (19).

Therefore, the development of new substances with anti-tumour effects that could enhance the effectiveness of radiotherapy in prostate cancer is essential. This systematic-review study was aimed to summarize evidences on gallic acid's mechanisms as a potential radiosensitizer in published preclinical studies on human prostate cancer cell lines and xenograft. The summary of these findings may provide a better look into future developmental advances in prostate cancer therapy and shape the basis for novel therapy formulations.

METHODS

This systematic review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) systematic review guidelines (20,21). This review focuses on preclinical studies since currently no clinical trial has been done on the topic. The population in this study was human prostate cancer cell lines and xenografts, including DU145, PC-3, and LnCAP as well as 22Rv1 xenograft. Intervention reviewed in this study is gallic acid administration, also known as 3,4,5-trihydroxybenzoic acid. The valid comparators include a control group that receives a placebo or another control treatment, or the use of cell lines representative of normal and healthy human cells. The principal outcome measured in this study is the inhibition of the prostate cancer cell lines and xenograft survivability, with different relevant parameters including cell viability, cell death, cell apoptosis, cell proliferation, and DNA breakage were taken into consideration. Based on these data, the PICO question was formed.

The literature search was performed on April 20, 2020 using keywords shown in Table I. The article search was conducted in PubMed, EBSCO, and Scopus. Keywords used are the combination of "gallic acid", "prostate cancer cell line", and "prostate cancer xenograft". The review protocol was reviewed internally. The keywords were based on the PICO question above and written utilizing Boolean operators. After that, screening was performed on obtained articles' titles and abstracts based on the formed PICO question.

The inclusion criteria of systematic review are: 1) written in English, 2) full-text availability, 3) in vitro or in vivo

Table I. Literature search strategy on three databases

Database	Keyword	Result
PubMed	(Gallic acid[Title/Abstract] OR 3,4,5-Trihydroxybenzoic acid [Title/Abstract]) AND (Prostate cancer cell line[Title/Abstract] OR 1013L[Title/Abstract]) OR E006AA[Title/Abstract]) OR RC-77T/E[Title/Abstract]) OR DU145[Title/Abstract]) OR DU-145[Title/Abstract]) OR DU 145[Title/Abstract]) OR LNCaP[Title/Abstract]) OR PC3[Title/Abstract]) OR PC-3[Title/Abstract]) OR Prostate cancer xenograft[Title/Abstract]) OR HPE-15[Title/Abstract]) OR C4-2B[Title/Abstract]) OR LAPC-4[Title/Abstract]) OR 22Rv1[Title/Abstract]) OR VCaP[Title/Abstract]) OR KUCaP[Title/Abstract]) OR PC346[Title/Abstract]) OR PC346C[Title/Abstract]) OR PC346B[Title/Abstract]) OR PC346BI[Title/Abstract]) OR ARCaP[Title/Abstract]) OR C4-2[Title/Abstract]) OR C4/C5[Title/Abstract]) OR DuCaP[Title/Abstract]) OR PC82[Title/Abstract]) OR PC295[Title/Abstract]) OR PC310[Title/Abstract]) OR PC324[Title/Abstract]) OR PC339[Title/Abstract]) OR PC374[Title/Abstract]) OR PC374F[Title/Abstract]) OR PC133[Title/Abstract]) OR PC-135[Title/Abstract])	51
EBSCO	AB ("gallic acid" OR "3,4,5-Trihydroxybenzoic acid") AND AB ("prostate cancer cell line" OR "1013L" OR "E006AA" OR "RC-77T/E" OR "DU145" OR "DU-145" OR "DU 145" OR "LNCaP" OR "PC3" OR "PC-3" OR "Prostate cancer xenograft" OR "HPE-15" OR "C4-2B" OR "LAPC-4" OR "22Rv1" OR "VCaP" OR "KUCaP" OR "PC346" OR "PC346C" OR "PC346B" OR "PC346BI" OR "ARCaP" OR "C4-2" OR "C4/C5" OR "DuCaP" OR "PC82" OR "PC295" OR "PC310" OR "PC324" OR "PC339" OR "PC374" OR "PC374F" OR "PC133" OR "PC-135")	65
Scopus	TITLE-ABS-KEY ("gallic acid" OR "3,4,5-Trihydroxybenzoic acid") AND TITLE-ABS-KEY ("Prostate cancer cell line" OR "1013L" OR "E006AA" OR "RC-77T/E" OR "DU145" OR "DU-145" OR "DU 145" OR "LNCaP" OR "PC3" OR "PC-3" OR "Prostate cancer xenograft" OR "HPE-15" OR "C4-2B" OR "LAPC-4" OR "22Rv1" OR "VCaP" OR "KUCaP" OR "PC346" OR "PC346C" OR "PC346B" OR "PC346BI" OR "ARCaP" OR "C4-2" OR "C4/C5" OR "DuCaP" OR "PC82" OR "PC295" OR "PC310" OR "PC324" OR "PC339" OR "PC374" OR "PC374F" OR "PC133" OR "PC-135")	113

studies, 4) examining gallic acid as a single substance, and 5) using prostate cancer cell lines (both primary and metastatic) and/or xenograft. The title and abstract of obtained studies were screened by two reviewers (ATC and ML) adhering to the stated inclusion criteria. Only articles in English were included in this study due to language barrier and time efficiency. Older studies were also included in this systematic review since the number of publications in the last 5 years obtained from the literature search is very limited. The full text of each paper was screened further for eligibility. Data extraction from each paper was done by two reviewers (ATC and ML). The mechanisms by which gallic acid affects parameters of cancer cell survivability, dosage and treatment duration, and cell line/xenograft used in each study were noted from each paper selected for review.

RESULTS

Based on the search conducted in the three online databases, there were 51, 113, and 65 articles obtained from PubMed, Scopus, and EBSCO, respectively. The articles' title and abstract were screened adhering to the inclusion criteria. After applying the inclusion criteria, 14 studies were found to fulfill the inclusion criteria. From this result, full-article reading was performed to ascertain that these articles are aligned with the PICO question stated before. After the chosen paper was screened for duplicates, 11 articles were included in the review. The studies were carried out in the USA, Taiwan, and Iran. Figure 1 shows the schematic of the literature searching process explained above.

Each study used different types of human prostate cancer cell lines encompassing DU145, PC-3, and LnCAP. One study by Kaur et al. used both DU145 human prostate cancer cell line and 22Rv1 xenograft. The studies also

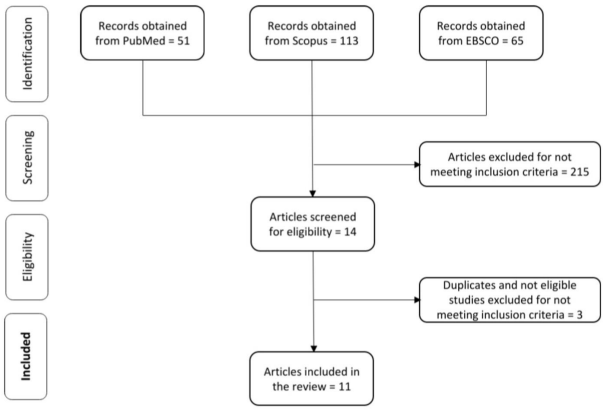

Figure 1: The schematic of study selection. A total of 51, 113, and 65 studies were obtained from PubMed, Scopus, and EBSCO respectively. The articles were then screened adhering to the inclusion criteria. A total of 14 articles were then screened further for eligibility. 3 articles were excluded because of duplicates or were deemed not eligible. In total, 11 articles were included for this systematic review.

reported the effect of gallic acid on different parameters of cancer cell survivability. Because of the difference in observed parameters and cell lines used in the studies, we focused on qualitatively describing the studies, their results, and the mechanisms that underlie the observed results. Included articles that were selected for review and the characteristics of each study can be seen in Table II.

DISCUSSION

European Prospective Investigation into Cancer and Nutrition study (EPIC study) done in 2017 found that a higher consumption of fruit significantly reduces prostate cancer risk in men aged 35-70 years old (22). Gallic acid is a form of phenolic acid which naturally occurs in many types of fruits and plant, including grapes, green tea, berries, banana, wine, and mushrooms (23). Gallic acid reduces cancer cell lines and xenograft survivability on different parameters assessed in each study.

Kaur et al. commenced a study to understand gallic acid efficacy on prostate cancer using the DU145 cell line and 22Rv1 xenograft (5). The study found that the treatment of gallic acid reduces viability on both neoplastic cell lines and xenograft without creating the same effect on the non-neoplastic PWR-1E cell line. The study also found that gallic acid induces apoptotic death, inhibits xenograft growth, as well as demonstrates the anti-proliferative and pro-apoptotic feature in the prostate cancer cell line and xenograft. A mainly similar result was also observed in other studies using similar cell lines and xenograft (24-26). Decreased viability and growth inhibition were also observed in other cell lines, such as PC-3 (27-29). Saffari-Chaleshtori et al. also detected that gallic acid is related to PC-3 cell death in a dose-dependent manner (30). Chen et al. and Russell et al. described that the apoptosis is a consequence of mitochondrial apoptotic pathway induction, which in turn will end up in the release of cytochrome c and ultimately cause apoptosis in several different cell lines (25,26).

Veluri et al. and Agarwal et al. found that the apoptotic death of DU145 cell lines by gallic acid was contributed to the cleavages of caspase-3, caspase-9, and PARP molecule (24,31). This finding was also noticed in LnCAP cell line as delineated in a study conducted by Russell et al (32). Caspases are molecules that play major roles in the induction of apoptosis. Activation of caspase-3 resulted in the commencement of apoptosis, which then followed by the activation of PARP (31,33). The cleavage of caspase-9 further enhances the activation of caspase-3, which in turn will accelerate the process of apoptosis (33). Caspase-3 has always been considered as the main component in the apoptosis pathway, and interindividual difference of caspase-3 level is related to radiosensitivity (34).

Table II: Studies reviewed in this paper

Author	Cell Line & Xenograft Used	Treatment	Effect observed on parameters	Mechanism
Kaur et al. 2009	DU145 cell line and 22Rv1 xenograft. PWR-1E normal prostate epithelial cell line as control.	Gallic acid 10-100 µM with duration of 12-48h.	↓cell viability ↑apoptotic death ↓cancer growth ↓cancer cell proliferation ↓angiogenesis.	↓cellular PCNA level, increase apoptosis, decrease cellular proliferation ↓CD31 and decrease angiogenesis.
Veluri et al. 2006	DU145 cell line.	Gallic acid in concentration of 25-100 µg/mL for up to 72 hours. Control group was treated with DMSO.	↑apoptosis ↓cancer growth.	↑caspase-3, caspase-9, and PARP cleavages increase cell death.
Liu et al. 2011	PC-3 cell line.	Gallic acid 25-100 µM with duration of 24-48h. Control was treated with 0,5% DMSO.	↓cell viability ↓cell migration and invasion.	↓expression of MMP-2 and MMP-9 hamper cellular migration & invasion, ↓expression of protein & gene associated with cell migration.
Agarwal et al. 2006	DU145 cell line.	Gallic acid 10-50 µmol/L for 6-24 h. Control was treated with 0,1% DMSO.	↓cell growth ↑cell death ↑cell with arrested cell cycle in S and G_2-M phase.	↓CDK2, CDK4, CDK6, cyclins, cdc25 phosphatases disturbs cell cycle regulation, ↑ATM-Chk2 disturbs cell cycle regulation, ↑activation of caspase-3 and caspase-9 increase apoptotic death.
Chen et al. 2009	DU145, LnCAP, and PC-3 cell line.	Gallic acid 25-100 µg/mL for 24-48 h	↓ cell growth ↑apoptotic death ↑ cell cycle arrest in G_2-M phase ↑ DNA breakage.	↑pro-apoptotic protein Bax expression, ↓anti-apoptotic protein Bcl-xL expression. ↓cyclin B1 and Cdc25C expression, disturb cell cycle. ↓mitochondrial cytochrome c level indicates apoptosis via mitochondrial pathway. ↑ROS production activates MAPK ultimately causing apoptosis and causing double-strand breakage on DNA.
Russell et al. 2012	LNCaP cell line.	Gallic acid 10-80 µg/mL for 0-24 h. Vehicle control group treated with 0,1% DMSO Positive control group treated with 100 µM H_2O_2.	↑ROS-dependent cell death, ↑apoptosis.	↑ROS production, increase ROS-dependent cell death, ↑caspase-3 and caspase-9 activation induce apoptosis.
Russell et al. 2011	LNCaP cell line. PrEC normal prostate epithelial cells used as control.	Gallic acid 5-80 µg/mL, Triphala extract 25-500 µg/mL, or 0,1% DMSO extract for 24-96 h.	↑cell death	Direct cytotoxicity to cancer cells.
Heidarian et al. 2016	PC-3 cell line.	Gallic acid 0-120 µM incubated for 48 h.	↓cell proliferation ↓cancer cell invasion.	↓IL-6 secretion and IL-6 gene expression, inhibit cancer cell viability, survival, and metastasis. ↓pSTAT3 and pAKT levels, decrease cell proliferation and induce apoptosis.
Liu et al. 2011	PC-3 cell line.	Gallic acid 0-200 µM for 12-48 h. Control group treated with DMSO 1%.	↓cell viability	↑DNA condensation and fragmentation, result in DNA breakage, ultimately reducing cell viability. ↓DNA repair gene expression.
Saffari-Chaleshtori et al. 2017	PC-3 cell line.	Gallic acid 0-200 µM. Positive control for DNA breakage assay treated with H_2O_2 30 µM.	Cell death >50% after gallic acid treatment >35 µM.	Cell death induced by DNA breakage.
Heidarian et al. 2017	DU-145 cell line.	Gallic acid 0-100 µM for 48 hours. Control were treated with DMSO.	↓cell proliferation ↓cell invasion	↓IL-6 secretion and IL-6 gene expression reduce cell proliferation and invasion. ↓ pSTAT3, pAKT, and pERK1/2 inhibits cell growth and increase apoptosis.

Akt = Protein-kinase B; ATM = Ataxia-telangiectasia mutated; Bcl-xL = B-cell lymphoma-extra large; CDK = Cyclin-dependent kinase; CD31 = Cluster of differentiation 31; DMSO = Dimethyl sulfoxide; ERK = extracellular signal-regulated kinase; ; IL-6 = interleukin-6; MAPK = mitogen-activated protein kinase; MMP = matrix metalloproteinase; PARP = Poly (ADP-ribose) polymerase; PCNA = Proliferating Cell Nuclear Antigen; ROS = Reactive oxygen species; STAT3 = Signal transducer and activator of transcription 3.

Agarwal et al. found that the populations' cell cycle was primarily arrested at S and G2-M phases in the DU145 cell line (24). However, another study by Chen et al. found that the administration of gallic acid may only affect cell cycle arrest in G2-M phase (25). With longer duration of gallic acid treatment on the cell line, it was found that arrest only happened at G2-M phase at 24-h duration, compared with the arrest at both S and G2-M cycle at treatment durations of 6 h and 12 h. Cell cycle arrest was one of the action mechanisms of radiosensitizers, and it was found that cells in the G2-M phase are more sensitive to radiotherapy compared to cells in other phases of cell cycle (35,36). The mechanism by which gallic acid affects cell cycle is apparently mediated by its role in affecting the level of CDKs and cyclins proteins. CDKs are a group of highly homologous serine/threonine kinases, some of which are CDK-4 and CDK-6. Both CDK-4 and CDK-6 mediate the progression of cell cycle by phosphorylating RB1 protein, which allows E2F transcription factors to activate many different genes involved in cell cycle progression (37). Another study found that the administration of a

highly selective antibody to CDK4 and CDK6 resulted in radiosensitization of cancer (38). Other means by which gallic acid affects cell cycle also include its capability in affecting cdc25 phosphatases. Downregulation of cdc25 phosphatases caused an increase in the apoptosis rate (24,39). cdc25s in human consists of three different isoforms, which are cdc25A, cdc25B, and cdc25C that serves as checkpoint components and dephosphorylating CDK, thereby activating it (40).

The underlying mechanism of gallic acid anti-proliferative and pro-apoptotic properties noted in a study by Kaur et al. may be caused by reduced Proliferating Cell Nuclear Antigen (PCNA) (5). PCNA is a protein that plays an important role in DNA repair and chromatin remodeling, and it was found that PCNA phosphorylation is commonly observed in prostate cancer (41). DNA damage repair mediated by PCNA was found to contribute to radioresistance of glioma cells, and downregulation of PCNA arrest cell cycle in the G0/G1 phase, resulting in increased radiosensitivity (42,43).

Chen et al. observed that the administration of gallic acid upregulated the expression of the pro-apoptotic protein Bax while downregulated the anti-apoptotic protein Bcl-xL (25). Apoptosis is the process of gene' activations affected by environmental stimulus ultimately resulting in cell death (44). Bax is an apoptotic effector protein of the Bcl-2 protein family which plays a role in mitochondrial outer membrane permeabilization (MOMP) by forming large protein-conducting pores in the outer membrane of mitochondria (45). Bcl-xL is another protein of the Bcl-2 protein family that prevents apoptosis by binding to pro-apoptotic Bcl-2 proteins including BAX, sequestering it and hindering the activity of the proapoptotic proteins (46). The proteins are involved in the apoptosis pathway by contributing to the formation of conducting channels in mitochondria outer membrane, which will result in the release of cytochrome c (47). Bcl-xL was also found to promote metastasis, signifying its role in cancer pathogenesis (48). Higher expression of Bcl-xL in cancerous cells was associated with lower radiosensitivity, thus responding more poorly to radiotherapy (49). Bax/Bcl-2 ratio as a criterion to determine radiosensitivity has been proposed as early as in 1999 (50). In metastatic prostate cancer, low Bax level is correlated with high resistance to apoptosis (51).

Administration of gallic acid was also found capable of inhibiting matrix metalloproteinase (MMP) in PC-3 human prostate cancer cell line (27). MMP is a group of zinc-dependent proteases, and MMP-2 together with MMP-9 are mainly secreted by tumour cells in the form of zymogens (52). Activation of MMP-2 and MMP-9 leads to degradation of type IV collagen in basement membrane which will facilitate metastasis and tumour invasion (53). The reduction of the enzymes' activities happened in a dose-dependent manner after the provision of gallic acid. Expression of MMP-2 and MMP-9 in cancer cells will facilitate cancer cells' migration, invasion, and adhesion by modulating ECM in the local tissue, which contributes to treatment resistance (54,55). Specific inhibition of MMP-2 and MMP-9 resulted in increased radiotherapy efficacy (56). Gallic acid decreases the expression of MMP-2 and MMP-9 by inhibiting the ERK1/2 and AKT pathway. (27).

Chen et al. reported an increase in radiation-induced formation of ROS after application of gallic acid, which results in apoptosis and double-strand breakage (25). Irradiation also directly contributes to the formation of double-strand breakage (57). Increased ROS formation was also observed in LnCAP cell line as assessed by Russell et al (32). Chen et al. also reported that administration of gallic acid stimulates time-dependent creation of ROS in prostate cancer cells (25).

An interesting discovery by Liu et al. is that gallic acid also restrains the expression of genes related to DNA repair (29). In the study, the expression of DNA repair genes including ATM, ATR, MGMT, DNA-PK, and p53 was suppressed following the administration of gallic acid. ATM and ATR helped DNA repair by functioning as a checkpoint kinase in double-strand break (58). MGMT is an anti-mutation DNA repair protein that amplifies cancer cells' radioresistance by undoing DNA methylation in cancer cells (59). Recruitment of DNA-PK will lead to DNA ligation after double-strand breaks (60).

Gallic acid treatment feasibly causes decreased IL-6 secretion and downregulation of IL-6 gene expression in PC-3 prostate cancer cell line according to Heidarian et al (28). Reduced IL-6 secretion culminates in decreased PC-3 viability and survival as outlined in the previous study (61). IL-6 is an inflammatory marker capable of preventing radiation-induced death by enhancing the activation of both STAT3 and the Nrf2-antioxidant pathways, which causes upregulation in Mn-SOD and ultimately reduces oxidative stress and associated DNA damage (62). The association between IL-6 and STAT3 was coherent with the finding obtained from another study conducted by Heidarian et al, in which gallic acid treatment decreases intracellular pSTAT3 (28). STAT3 is a transcription factor responding to upstream receptors including IL-6, and its activation upregulates cyclin D1, survivin, and Bcl-xL transcription (63). Another pathway that was spotted to be inhibited by gallic acid is the AKT pathway, manifesting in pAKT level reduction. pAKT is the phosphorylated form of AKT and is commonly detected in higher concentrations in cancer cells (28). AKT was remarked to regulate cellular proliferation and apoptosis suppression in prostate cancer cells (64). Akt is also coined as "survival kinase" in another study because of its potential to interact with a different cellular signaling pathway to promote the cell resistance

against radiotherapy (65).

Radiation-induced cell death can be classified into three categories, which are apoptosis, necroptosis (necrosis), and autophagy-dependent cell death (66). Apoptosis is thought to be the main mechanism of radiation-induced cell death. Dysregulation of apoptosis is thought to play an important role in the development of cancer (67). Radiotherapy may cause apoptosis through the intrinsic pathway, extrinsic pathway, and membrane stress pathway, with radiotherapy primarily acts via the intrinsic pathway (68). The intrinsic pathway of apoptosis is initiated by the existence of single-strand breaks and double-strand breaks, which will activate ATM and ATR (68,69). ATM and ATR will phosphorylate CDK1 and CDK2, which will stimulate p53 activation (70). The events of p53 activations will lead to p53 accumulation, causing transactivation of pro-apoptotic genes including Noxa, Bax, and PUMA (71).

Single-strand breaks and damages to DNA base is repaired by the base excision repair mechanism (57,72). In double-strand breaks, DNA repair mechanism may fail, which will cause disruption in the cell cycle and ultimately ends in the respective cell's death (72,73). It is known that cells in G2-M phase are more prone to radiation-induced damage and cell death when compared to cells on the other phases of cell cycle (35,36,74).

Radiosensitivity is a function of the tumour intrinsic radiosensitivity, tumour reoxygenation capacity, tumour repair capability, tumour tissue repopulation, tumour vasculature damage process, tumour immunity, and tumour cell cycle's redistribution (75). A radiosensitizer must have the capability to affect one of these characteristics without affecting the toxicity of the treatments. The combination between radiotherapy and radiosensitizer depict the potential to enhance the effectiveness of radiotherapy while also minimizing side effects and damages to normal tissues at the same time (76). Gallic acid has many specifications required for radio-sensitizing effects. Figure 2 illustrates summary of the radiosensitization mechanism by gallic acid reviewed in this study. The anti-cancer capability of gallic acid reflected its potential as a novel radiosensitizer agent. The manifold properties of gallic acid discussed in this systematic review make it a promising novel candidate as a radiosensitizer agent.

This study has some limitations. Gallic acid was known to be highly polar with low lipophilicity, resulting in low bioavailability and cellular penetration (77). The lack of lipophilicity in gallic acid may be resolved by creating its derivatives designed to increase its hydrophobicity (78). The scarcity of gallic acid randomized clinical trials in cancer patients is another factor hampering the clinical use of gallic acid in cancer treatment. Additional studies using gallic acid derivatives with lower hydrophilicity

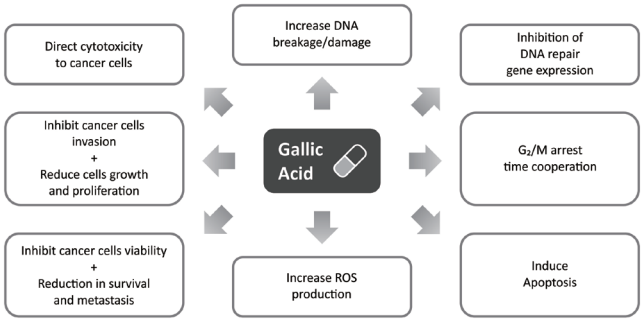

Figure 2: Summary of the radiosensitization mechanism by gallic acid reviewed in this study. Gallic acid exerts its radiosensitizer characteristic using several mechanisms, including increasing DNA breakage, inhibiting the expression of DNA repair gene expression, causing cell cycle arrest in G2-M phase, inducing apoptosis, increasing ROS production, decreasing cancer cell viability and growth, and by causing direct cytotoxicity to cancer cells.

are needed.

CONCLUSION

Gallic acid has a potential as a radiosensitizer by imposing various mechanism pathways. The main mechanisms of gallic acid affecting human prostate cancer cell lines and xenograft appear to be increasing cellular apoptosis by increasing caspase-3, caspase-9 activation and inhibits cell cycle progression by reducing CDKs, cyclins, and cdc25 phosphatases level inside the cancer cells. Gallic acid also has low toxicity on normal cells as shown in some of the reviewed studies. The function of gallic acid as a radiosensitizer still needs to be verified further since it may act as both antioxidant and pro-oxidant. This may be achieved by modifying the environment that enhance the pro-radiation characteristic of gallic acid to optimize the radiosensitization effect of gallic acid. Further clinical studies and research using gallic acid derivatives with lower hydrophilicity are still needed to determine the clinical application of gallic acid as a radiosensitizer in prostate cancer.

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