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Chemopreventive Effects of Zerumbone in Precancerous Cervical Lesions and Cervical Cancer: A Systematic Review Protocol

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ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - The review has not yet started.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 14 February 2025 and was last updated on 14 February 2025.

INTRODUCTION

Review question / Objective 1. Does zerumbone exhibits chemopreventive effects in precancerous cervical lesion and/or cervical cancer? 2. Does zerumbone has superior anticancer effects against standard chemotherapy /control(s) used for the treatment of cervical cancer ? 3. What is the molecular mechanism of zerumbone in demonstrating anticancer effects in precancerous cervical lesion and/or cervical cancer?

Rationale Despite the establishment of cervical cancer screening and vaccinations programs, cervical cancer is the second most cause of cancer among women and the third leading cause of death among women in Malaysia. In addition to surgery, the primary treatment for cervical cancer includes concurrent chemoradiotherapy. Notably, many chemotherapy drugs that are available causes side effects to patients which can be severe and debilitating. A combined therapy approach has been used in the treatment of patients which uses several anti-cancer drugs. However, these drugs do not target specific signaling pathways and are cytotoxic in their mode of action. Precancerous lesions, such as cervical intraepithelial neoplasia (CIN), represent an early stage of cervical carcinogenesis. Hence, effective interventions at this stage may prevent progression to invasive cancer. This review will explore the potential of zerumbone as a chemo preventive agent targeting both precancerous lesions and cervical cancer.

Condition being studied 1. Precancerous cervical lesion or cervical intraepithelial neoplasia (CIN). They may exist at any one of three stages: CIN1, CIN2, or CIN3. If left untreated, CIN2 or CIN3 (collectively referred to as CIN2+) can progress to cervical cancer. 2. Cervical cancer.

METHODS

Search strategy 1. Zerumbone OR 'Zingiber zerumbet' OR 'Z.zerumbet'
2. 'Cervical carcinoma' OR 'cervi* malignan' OR ' cervi* tumor' OR ' cervi* polyp' OR 'cervical neoplasia' OR 'cervical dysplasia' OR 'precancerous cervical lesion' OR 'cervical intrepithelial neoplasia' OR CIN
3. #1 AND #2.

Participant or population Inclusion:
1. Women aged 18-years above diagnosed with cervical cancer or/and precancerous lesion both pre- and menopausal. May be on conventional cancer therapy for cervical cancer.
2. Animal model implanted with cervical cancer xenograft (restricted to rodent).
3. Human cervical cancer cell lines.
Exclusion:
1. Women with immunodeficiency condition such as HIV, and/or presence of other type of cancer, women with endocrine disorders and/or other gynecological disease, pregnant women, women on hormonal therapy such as oestrogen and/or progesterone.
2. Animal model other than rodent or pregnant animal model.
3. Cervical cancer cell lines derived from sources other than human, non-cervical cancer cell lines.
4. Study that did not mention the type of cell lines/ animal model, or study population used.

Intervention
Inclusion:
1. Zerumbone as monotherapy or combined with conventional/standard cervical cancer chemotherapy drugs (e.g. cisplatin, 5-fluorouracil)
2. Zerumbone is either extracted or commercially purchased.
3. Bioactive compounds could be in any type of formulation or preparation such as tablet, powder, solution, lotion etc and administered via oral/ local (intravaginal) or other routes of administration (injection).
4. Background treatment is allowed for human study. Not restricted in terms of dose/ concentration used, and duration of treatment.
Exclusion:
1. The plant extracts (Zingiber zerumbet) without isolating zerumbone.
2. Other natural products, and or bioactive compounds were stated in the intervention.
3. Zerumbone combined with other bioactive compound(s) or drug(s) other than conventional cervical cancer chemotherapy.
4. Bioactive compounds delivery was aided by external biological factors e.g. nanocarriers, nanoparticles.
5. Biologically enhanced or conjugated bioactive compounds with cofactors/metabolite e.g. fibers, sulfate.

Comparator
Inclusion:
1. A placebo, standard chemotherapy e.g. cisplatin, 5FU, paclitaxel (as positive control), zerumbone combined with standard chemotherapy or no treatment.
2. Background treatment with conventional therapy will be considered as no treatment in human studies.
3. In in-vitro/vivo study, cells/animals that were not treated are regarded as negative control.

Exclusion:
1. Other bioactive compounds as stated in the intervention.
2. Other anticancer drugs that are not used for cervical cancer.

Study designs to be included Inclusion: 1. Human clinical trials involving a. randomized b. non-randomized c. parallel-armed studies 2. Controlled in-vitro and in-vivo studies.Exclusion: 1. Clinical trials with cross-over design, non-controlled studies, and single-arm study.2. In-silico study. 3. Observational (cohort, case-control), cross-sectional study. 4. Type of publication excluded: a. Reviewsb. Editorialc. Conference proceedingsd. Abstracts where the full texts are not available or unobtainable will also be excluded. 5. Duplicate study.

Eligibility criteria Only studies published in English will be considered due to resource limitation.

Information sources Electronic databases: PubMed, Scopus, Science Direct, CENTRAL.
Other sources: Manual screening of eligible studies references. We will search the references lists of all relevant articles for further studies.

Main outcome(s) 1. In human study: reduction in tumour or/and cervical lesion size by imaging tests, reduction in tumor activity through hematological changes testing, and regression of lesion through histological evaluation.
2. In in-vitro/vivo study: The anticancer activity and cytotoxicity effects assessed by observation of reduction in cell viability and cell proliferation, cell cycle growth, rate of apoptosis, volume of tumor, or histology changes via standard procedures e.g.

MTT assay, Sulforhadamine B assay, apoptosis assay, flow cytometric assay.

Additional outcome(s) 1. Clinical human study: Reduction in cervical dysplasia by p16INK4a and ki-67 key markers. 2. In-vitro/vivo studies: Protein expression activity, gene expression activity via standard procedures e.g. western blotting, immunohistochemistry and RT-PCR after treatment with zerumbone.

Data management Procedure for study selection:
The study selection process will involve two independent reviewers (TAN and NA) conducting the search, screening results for eligibility, performing data extraction, and assessing study quality. A third reviewer (RZF) will resolve any conflicts that arise during these stages. The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram will be employed to transparently present the study selection process.
Method for data extraction:
Two review authors working independently will extract data using a standardized data extraction sheet. Any disagreements will be resolved by consensus or a third reviewer will be consulted.
The following data will be extracted:
a) Study characteristics: Name of first author, year of publication, country, study design
b) Human clinical trials: Total number of participants, age, menopausal status if applicable, background therapy, stage of cancer, dose and duration of intervention, randomized, non-randomized and number of interventions arm, adverse effect reported.
c) Animal study: Number of animal (s) used per treatment arm (n) and total N, type of rodent, sex and age of rodent and type of tumor xenograft, duration of treatment, dose of treatment and route of administration, comparator used (positive control).
d) Cervical cancer cell lines: type of cell line used, media used, concentration of intervention used, duration of treatment, comparator used (positive control) or negative control used.
e) Intervention of interest: Source of zerumbone, dose, concentration, timing, duration of treatment, route of administration
f) Primary outcome(s): Percentage of cell viability, stage of cell cycle inhibited, rate of apoptosis, volume of tumor, or histology changes, and procedures used to measure specified outcomes.
In human study, reduction in tumour size by imaging tests, reduction in tumor activity through hematological changes testing.
Secondary outcome(s):
i. Molecular mechanisms of active compound including pathway(s) involved in anticancer effect, specific protein expression activity (reduced or increased), specific gene expression activity (reduced or increased).
ii. Adverse effects of treatment with zerumbone observed in human study (number of incidence (s) of specific AE).

Quality assessment / Risk of bias analysis 1. The Cochrane Risk of Bias (RoB) 2.0 tool will be used to assess the quality of clinical trials in human participants.
2. The SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool will be used to assess the quality of animal study.
3. For in-vitro study, a customised risk of bias tool will be used based on a study conducted by Abdull Rahim et al., (2023) (low, moderate and high risk). Low risk of bias when study reached more than 70% score 'yes', moderate with 50-69% score 'yes', and high with 49% and less score 'yes'. The following components will be evaluated for risk of bias in a study using cell culture:
i. Was the cervical cancer cell line used reported?
ii. Was the concentration on the cancer cell culture used reported?
iii. Was the duration of exposure to the cell line reported?
iv. Were standard culture media used for control treatment?
v. Were reliable tools used to assess the outcomes?
vi. Were experiments performed in triplicates?
x. Was more than one independent experiment performed?

Strategy of data synthesis Characteristics of studies and variables of interest will be tabulated and described narratively according to themes.
Quality results will be summarized in a table and presented in a traffic-light plot so that trend can be seen.
If a meta-analysis is conducted, the following parameters will be presented:
Dichotomous data: Relatives risk and Odds ratio will be calculated with CI set to 95%.
Continuous data: Mean Difference will be calculated with CI set to 95%.
Effect models:
The fixed-effect model will be used if the number of included studies is small. The random-effect model will be used if the included studies are not homogenous.
Heterogeneity:
Heterogeneity e.g. Chi Square and Higgin's I2 test, and sensitivity analyses will be conducted to assess variability across studies.

Publication bias will only be assessed if there are 10 or more included studies in the meta-analysis using funnel plot.

Subgroup analysis Where applicable, subgroup analysis will be conducted based on the following characteristic:
1. Age of study population
2. Stage of cancer
3. Source of intervention
4. Type of formulation
5. Dose/ concentration of intervention
6. Type of comparator used
7. Duration of treatment
8. Type of cell used
9. Precancerous cervical lesion.

Sensitivity analysis Sensitivity analysis will be conducted by exclusion/and inclusion of studies accordingly.

Language restriction Only studies published in English will be considered due to resource limitation.

Country(ies) involved Malaysia.

Keywords zerumbone; anticancer; cervical cancer; precancerous cervical lesion; systematic review protocol.

Dissemination plans The completed review will be published in an indexed journal. Preliminary findings may be presented at conference and published in a conference proceeding.

Contributions of each author

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