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ANTIPROLIFERATIVE MECHANISM OF CERVICAL CANCER CELLS (HELA) TREATED WITH BIOLOGICAL-ACTIVE SUB-FRACTION FROM QUERCUS INFECTORIA EXTRACT

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LIST OF ABBREVIATIONS

BSC	Biosafety cabinet
Caov-3	Ovarian cancer cell line
CO_2	Carbon dioxide
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamineteraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FDA	Food and Drug Administration
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HeLa	Cervical cancer cell line
HPV 16	Human papillomavirus strain 16
HPV 18	Human papillomavirus strain 18
HPV	Human papillomavirus
IC_{50}	Inhibition concentration of 50% cell population
IDV	Integrated density value
MRI	Mean relative intensity
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
p53	Tumour suppressor protein
PBS	Phosphate buffer saline
pRB	Retinoblastoma protein

QI	*Quercus infectoria*
QIA	*Quercus infectoria* aqueous
RNA	Ribonucleic acid
SEM	Standard error mean
GOG	Gynecologic Oncology Group
Cis	Cisplatin

LIST OF SYMBOLS

%	Percentage
µg	Microgram
µg/ml	Microgram per millilitre
µl	Microlitre
µm	Micrometre
^{o}C	Degree celcius
cells/ml	Cells per millilitre
cm	Centimetre
cm^2	Square centimetre
g	Gram
L	Litre
mg	Milligram
mg/ml	Milligram per millilitre
ml	Millilitre
mM	Millimolar
OD	Absorbance
rpm	Revolutions per minute
V	Voltage

ABSTRAK

Kanser servik merupakan kanser keempat paling kerap berlaku dalam kalangan wanita di seluruh dunia dengan anggaran kira-kira 530,000 kes pada tahun 2012. Kanser servik juga merupakan salah satu punca utama kematian yang disebabkan oleh kanser dalam kalangan wanita di seluruh dunia. Kaedah rawatan moden dan konvensional yang digunakan sekarang didapati mendatangkan pelbagai kesan sampingan terhadap pesakit. Oleh yang demikian, pelbagai kajian telah dijalankan untuk mencari rawatan alternative yang lebih selamat dan berkesan, seperti penggunaan produk hasilan semulajadi. Kajian terdahulu melaporkan aktiviti farmakologikal manjakani atau *Quercus infectoria* (QI) termasuk aktiviti antikanser. Walaubagaimanapun, mekanisma tindak balas aktiviti antikanser masih samar dan perlu kajian mendalam. Dalam kajian ini, QI diekstrak menggunakan beberapa jenis pelarut organik sebelum diuji untuk aktiviti sitotoksik dan mekanisma tindak balas kematian sel. Kaedah asai MTT digunakan untuk menentukan aktiviti sitotoksik bagi ekstrak n-hexane (QIH), etil asetat (QIEA) dan methanol (QIM) daripada QI terhadap sel kanser servik (Hela). Aktiviti sitotoksik ekstrak QI terhadap sel fibroblast normal juga ditentukan bagi melihat kesan toksik dan sifat keselektifan ekstrak. Di samping itu, sel yang dirawat dengan DMSO bertindak sebagai kawalan negatif dan sisplatin sebagai kawalan positif. Kemudian, graf dos-tindak balas diplotkan bagi menentukan nilai IC_{50}. Pemerhatian perubahan morfologi nukleus, peratusan sel apoptotik dan pengesanan paras pengekspresan protein apoptotik juga dilakukan. Pemeriksaan kandungan sebatian fitokimia dalam ekstrak yang paling poten juga dijalankan untuk menentukan hubung kait kumpulan bioaktif dalam ekstrak. Berdasarkan keputusan eksperimen, QIEA menunjukkan aktiviti sitotoksik

yang lebih baik daripada QIM dan QIH dengan perencatan pertumbuhan paling poten terhadap sel Hela (6.33 ± 0.33 µg/ml) dan bersifat sitoselektif. Hasil kajian pengesanan sebatian fitokimia pula menunjukkan kehadiran tanin, alkaloid, glikosida, saponin, terpenoids, flavonoid dan sebatian fenolik dalam ekstrak QIEA. Ekstrak QIEA kemudian ditulenkan dan menghasilkan dua kompoun aktif iaitu *4-0 methylgallic acid (MGA)* dan *gallic acid (GA)*. Subfraksi MGA adalah lebih poten dengan IC_{50} 11±0.58 µg/ml. Hasil kajian juga menunjukkan sel Hela yang dirawat dengan subfraksi MGA telah mengalami apoptosis, yang mana dibuktikan melalui perubahan morfologi nukleus dengan kehadiran jasad apoptotik serta peningkatan kadar peratusan apoptosis. Tambahan pula, hasil kajian menunjukkan subfraksi MGA mencetus apoptosis melalui tapak jalan p53 . Peningkatan aras protein p53 didapati telah menurunkan paras protein Bcl-2 dan mengaktifkan kaspase-3. Kesimpulannya, dua subfraksi mengandungi terbitan asid galik MGA dan GA menunjukkan ciri aktif secara biologi terhadap sel HeLa. Subfraksi MGA merencatkan pertumbuhan sel kanser Hela secara melalui mekanisma kematian sel secara apoptosis.

ABSTRACT

Cervical cancer is placed at the fourth most frequent cancer among women worldwide, which was estimated approximately 530, 000 new cases in 2012. This type of cancer has become one of the leading causes of cancer death among women worldwide. Conventional and modern cancer treatment nowadays comes with negative and adverse side effects to the patients. Therefore, lot of studies have been conducted to search for new alternative treatment which is more safe and effective such as utilisation of natural product based medications. Previous studied revealed various pharmacological activities of Quercus infectoria (QI) galls or manjakani including anticancer activity. However, the mechanism of action lay behind was not well explained. Therefore, in this present study, QI gall was selected for the evaluation of cytotoxic activity and cell death mode of action. In this study, QI was extracted using different types of organic solvents before being tested for cytotoxicity activity and mode of cell death. MTT assay was used to determine cytotoxic activity for n-hexane (QIH), ethyl acetate (QIEA) and methanol (QIM) of QI galls extracts against cervical cancer (Hela). Cytotoxic activity of QI extracts also tested against normal fibroblast (L929) cell line to determine cytotoxic effects and cytoselective properties. Meanwhile, DMSO-treated cells served as negative control while cisplatin-treated cells served as positive control. After that, dose-response graph was plotted to determine IC_{50} values. Observation on nuclear morphology, apoptotic percentage and detection of apoptotic protein expressions were also conducted. Moreover, phytochemical screening analysis had been carried out to determine bioactive groups that present in the most potent extract. From the findings,

QIEA extract exhibited better cytotoxic activity with best growth inhibition against Hela cells (IC50 value = 6.33 ± 0.33 µg/ml) compared to other extracts and showed cytoselective property as no cytoxicity observed in the treated normal fibroblast (L929) cells. As for the phytochemical analysis of QIEA extract, it was revealed the presence of tannin, alkaloids, glycosides, saponins, terpenoids, flavonoids and phenolic compounds The QIEA extract was then purified and resulted two compounds 4-0 methylgallic acid (MGA) and gallic acid (GA). The most potent antiproliferative activity was exhibited by MGA subfraction with IC_{50} 11±0.58 µg/ml. The current study also revealed that Hela cells treated with MGA subfraction has undergone apoptosis as exerted by the alteration of nuclear morphology and the presence of apoptotic bodies as well as the increment of apoptosis rate in the treated cells. Furthermore, MGA subfraction triggered apoptosis through upregulation of tumor suppression protein p53, with down regulation of Bcl-2 expression and facilitated the apoptosis execution through caspase-3 activation. In conclusion, the purification of QIEA has resulted two biologicaly active subfraction namely MGA and GA. It was cleared that MGA subfraction reduced Hela cell growth through induction of apoptosis.