Journal of Pharmaceutical Research International

33(47B): 653-660, 2021; Article no.JPRI.74500
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Effect of Kabasura Kudineer Extract on Inflammatory Cytokines [Interleukin-6 and Tumour Necrosis Factor-α] in Lung Cancer Cell Line A549

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i47B33167
Editor(s):
(1) Dr. Mohamed Fawzy Ramadan Hassanien, Zagazig University, Egypt.
Reviewers:
(1) Nosarieme Abey, University of Lagos, Nigeria.
(2) R. Meena, Siddha Central Research Institute, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74500

Original Research Article

Received 04 August 2021
Accepted 09 October 2021
Published 04 November 2021

ABSTRACT

Background: Kabasura kudineer is widely known for its anticancer efficiency. Kabasura kudineer is a customary formulation used by siddha practitioners for effectively managing common respiratory illness. Herbal medicines are acknowledged as a great approach to lung cancer therapy. Aim of the study is to know about the anticancer property of Kabasura kudineer extract on inflammatory cytokines IL-6 and TNF-α in lung cancer cell line (A549).
Materials and Methods: Human lung cancer cell line (A549) was purchased from National Centre for Cell Sciences (NCCS), Pune, India. Cell viability test was done by MTT assay. Gene expression analysis was done by Real Time-PCR. The obtained data were analysed statistically by one-way analysis of variance and Duncan's multiple range test with Graph Pad Prism version 5 to analyse the significance. The significance was considered at $p<0.05$ level in Duncan's test.
Results and Discussion: Kabasura kudineer caused a marked increase in cell death in dose dependent manner. AT the end of 48 hours, maximum inhibition was at 400 and 500 µg/ml. Kabasura kudineer extract reduced the expression of IL-6 and TNF-α compared to the control cells.

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Conclusion: This study concluded that Kabasura kudineer extract has anticancer activity on lung cancer cell lines (A549).

Keywords: Kabasura kudineer; IL-6 and TNF-α; lung cancer cell lines (A549); innovative technique.

1. INTRODUCTION

Cancer is a disease characterized by its uncontrolled growth and spread of abnormal cells. Increase in proliferative growth signals, insensitive to growth-inhibitory signals, apoptosis evasion, angiogenesis induction, invasion leading to metastasis are the important features of cancer (1-3). Lung cancer mortality rate has increased among cancer related deaths worldwide [1]. In 2018, GLOBOCAN estimated 2.09 million new cases (11.6% of total cancer cases) and 1.76 million deaths (18.4% of total cancer deaths), above 2012 reported rates (1.8 million new cases and 1.6 million deaths), making it the foremost frequent cancer and explanation for cancer death in men and women combined and in women, the third common cancer type and therefore the second common explanation for cancer death [2].

Traditional practice for medicine in China, Pakistan, India, Srilanka and Thailand is widespread. (Ahmad Dar, Sangwan, and Kumar 2020). Medicinal plants have anti-mutagenic, antioxidant compounds against chemicals with low cost, low side effects. (Masumeh 2006) [3,4]. Due to strong therapeutic effects, the medicinal plants have been traditionally used to treat diseases [5]. Different parts of medicinal plant have numerous nutraceutical values and are enriched with proteins, carbohydrates, vitamins, fibre, potassium, calcium and also the presence of phytoconstituents contributes to its significant medicinal property [6–8,9].

Kabasura kudineer choornam is a traditional formulation which is used by practitioners of siddha medicine for forceful management of common respiratory ailments such as the flu and cold [10]. Kabasura kudineer possesses strong anti-inflammatory, analgesics, antiviral, antioxidant and anti-bacterial [11]. Kabasura kudineer consists of 15 herbal ingredients, namely *Zingiber officinale, Andrographis paniculata,Syzygium aromaticum, Terminaila chebula, Adhatoda vasica, Coleus amboinicus, Saussurea lappa, Clerodendrum serratum, Cyperus rotundus, Tinospora cordifolia, Sida acuta, Piper longum, Hygrophila auriculata, Anacyclus pyrethrum.* The present global COVID 19 pandemic has attracted the attention of the public towards the Kabasura Kudineer and its benefits in Tamil Nadu. It has been commonly used to treat fever and respiratory ailments.

Among the different types of cancer, lung cancer is considered one of the leading causes of cancer related death worldwide. High morbidity and mortality rates are seen in lung cancer [2,12]. The formation of tumour and its progression is strongly pronounced by the microenvironment surrounding the cells, which in most cases might be inflammatory (Quail DF, Joyce JA, 2013). These factors have led us to focus on pharmacological interventions to target the inflammatory pathways to inhibit growth of tumour and its progression (Lim JCW, 2012). The many of the herbs present in kabasura kudineer have been studied to possess antioxidant, anti inflammatory property. Natural antioxidants help to fight against free radicals and activated oxygen species in cancer related conditions. Since the kaba sura kudineer has been administered to treat respiratory complaints, hence the study was intended to investigate its effect on lung cancer cells. Our team has extensive knowledge and research experience that has translated into high quality publications (14-30). Our team has extensive knowledge and research experience that has translate into high quality publications [13–17]. The aim of this study was to evaluate the effect of Kabasura kudineer extract on inflammatory cytokines in the lung cancer cell line.

2. METHODS AND MATERIALS

Kabasura kudineer was purchased from Siddha pharmacy. Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Pvt Ltd, USA. Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, RPMI 1640 medium and phosphate buffered saline (PBS) were purchased from Gibco, Canada. (5,5,6,6-tetrachloro-1,1,3,3 - tetraethylbenzimidazolocarbocyanine iodide) and Real Time PCR kit was purchased TAKARA (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

2.1 Cell Lines and Cell Culture

Human lung cancer cell line (A549) was purchased from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in DMEM medium (Thermo Fisher Scientific, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, CA, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, CA, USA) at 37°C with 5% CO_2.

2.2 Cell Viability by MTT Assay

Cell viability was assayed using a modified colorimetric technique that is based on the ability of live cells to convert MTT, a tetrazolium compound into purple formazan crystals by mitochondrial reductases (Mosmann, 1983). Briefly, the cells (1 ×10^4/well) were exposed to different concentrations of Kabasura kudineer extract (100-500µg/ml) with A549 cells for 48 h. At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and incubated at 37∘C. The crystals formed were dissolved in dimethyl sulfoxide (100 µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in extract free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells] × 100.

2.3 Gene Expression Analysis by Real Time-PCR

Samples from each group were submerged in 2 ml Trizol (Invitrogen, Carlsbad, CA, USA) for RNA extraction and stored at −80°C until further processed. cDNA synthesis was performed on 2 µg RNA in a 10 µl sample volume using Superscript II reverse transcriptase (Invitrogen) as recommended by the manufacturer. Real-time PCR array analysis was performed in a total volume of 20 µl including 1 µl cDNA, 10 µl qPCR Master Mix 2x (Takara, USA) and 9 µl ddH_2O. Reactions were run on an CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) using universal thermal cycling parameters (95°C for 5 min, 40 cycles of 15 sec at 95°C, 15 sec at 60°C and 20 sec at 72°C; followed by a melting curve: 5 sec at 95°C, 60 sec at 60°C and continued melting). For quality control purposes, melting curves were acquired for all samples. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The data were analyzed by comparative CT method and the fold change is calculated by 2−ΔΔCT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

2.4 Statistical Analysis

The obtained data were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range test with a computer-based software (Graph Pad Prism version 5) to analyze the significance of individual variations among the control and experimental groups. The significance was considered at $p<0.05$ level in Duncan's test.

3. RESULTS AND DISCUSSION

3.1 Effect of Kabasura Kudineer on Cell Viability in A549 Cells

In the present study, Kabasura kudineer extract significantly increased (p<0,05) inhibiting the growth of the lung cancer cells dose-dependently compared to untreated control cells. However, 400 and 500 µg/ml concentration of the extract showed maximum inhibition of the viability of the lung cancer cells suggesting that Kabasura kudineer induces apoptosis in A549 cells (Fig. 1).

3.2 Effect of Kabasura Kudineer on Interleukin - 6 mRNA Expression in A549 Cells

In untreated control cells,interleukin - 6 and tumour necrosis factor - α expression was found to be decreased . Treatment with 400 and 500 µg/ml concentration of extract Kabasura kudineer reduced the expression of interleukin - 6 when compared to control cells (p<0,05) (Fig. 2).

3.3 Effect of Kabasura Kudineer on TNF-α mRNA Expression in A549 Cells

In untreated control cells, interleukin - 6 and tumour necrosis factor - α expression was found to be significantly decreased. Treatment with 400 and 500 µg/ml concentration of Kabasura kudineer extract reduced the expression of TNF - α when compared to control cells (p<0.05) (Fig. 3).

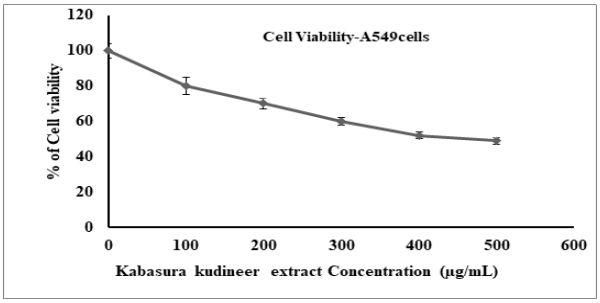

Fig. 1. Effect kabasura kudineer extract on cell viability in A549 cells. Each bar represents a mean ± SEM of 6 observations. The X-axis represents different concentrations of kabasura kudineer and the Y-axis represents the percentage of cell viability. There is a statistically significant difference between the control and treated groups with p value < 0.05

Natural products rich in phytochemicals have been studied to evaluate its anti-cancer and cancer chemo-preventive activity, in vitro and in vivo. There is limited information on the medicinal value of kabasura kudineer, particularly its cytotoxicity effect against cancer cell lines. Hence the current study was done to evaluate the effect of Kabasura kudineer extract on inhibition of cell proliferation which could serve a better understanding of the underlying mechanisms of their anticancer potential of the extract [18].

Kabasura kudineer which is an important plant well known for its medicinal properties [19]. It is traditionally used for the treatment of respiratory ailments; recently it is used in covid-19 treatment [20,21,22]. The present study was carried out to investigate the role of Kabasura kudineer on A549 lung cancer cell line [23]. Accumulating evidence suggests that traditional medicines possess the potential as chemotherapeutic agents to prevent and treat cancer [24]. The great advantage of focusing on natural compounds to be considered for research is that they possess low toxicity or no few adverse side effects [25].

There are numerous studies which have reported that nuclear factor NFκB, a pro-inflammatory transcription factor, could favour tumorigenesis when activated by carcinogens, inflammatory agents, and tumor promoters [26,27]. It was found that Kabasura kudineer extract significantly increased inhibiting the growth of the lung cancer cells dose-dependently compared to untreated control cells [28]. Targeting the NFkB pathway in anti cancer treatment could serve a better route in the treatment against the cancer cells. NF-κB is a transcription factor playing a significant role in inflammatory pathways [29]. In our study, the effect of kabasura kudineer on TNF alpha was determined and found that on treatment of cancer cell lines with the plant extract at the concentration of 400 and 500 µg/ml, downregulated the mRNA expression of TNF alpha and IL 6 which showed that the plant extract has anti cancer effect through inhibition of mRNA expression of TNF alpha and IL 6. Recently, some medicinals have been studied for their possible action as inhibitors of the pro-inflammatory cytokines [30]. Ginger (*Zingiber officinale)* is widely used all over the world as a spice and condiment in daily cooking and this is one of the important components of kabasura kudineer. Various compounds are isolated from the substance like *Zingiber officinale* which can be used to study the anti lung cancer activity [31].

Ginger extract have been proved to possess anticancer potential against liver cancer cell lines. Hence present study was focused to identify the anti cancer effect of kabasura kudineer against lung cancer cell kines. The ginger being natural compounds are rich in active phenolic compounds such as shogaol and gingerol, and it has been shown to have anti-cancer and antioxidant effects [32]. The previous studies have reported the anti-cancer effect exhibited by ginger on liver cancer cells is mediated by inflammatory markers NFκB and TNF-α [33-35]. This is in accordance with our present study where ginger is one of the components of kabasura kudineer.

Piper longum which is one of the formulations of kabasura kudineer have been investigated for its anti cancer potential against prostate cancer cell lines in previous studies and have been proved to be effective against prostate cancer cells. This is in accordance to the present study where the piper longum, one of the formulations of kabasura kudineer have been found effective against lung cancer cells [36].

IL-6 mRNA expression (Fold change over control)

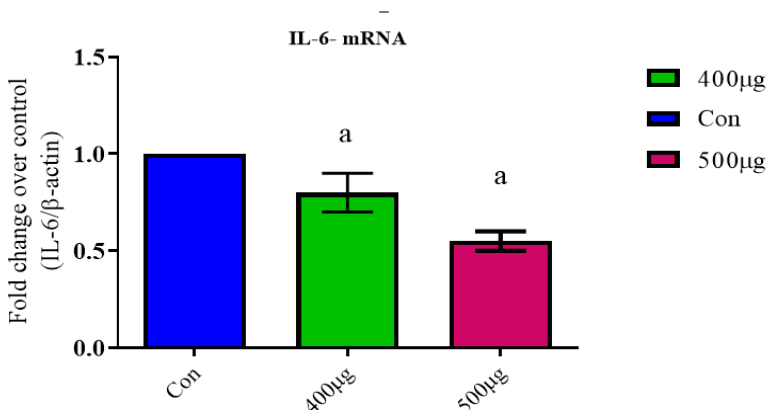

Fig. 2. Effect of kabasura kudineer extract on IL-6 mRNA expression in A549 cells. Each bar represents a mean ± SEM of 6 observations. The X-axis represents different concentrations of kabasura kudineer and the Y-axis represents the percentage of fold change over control. There is a statistically significant difference between the control and treated groups with p value < 0.05. a-compared with untreated control cells

TNF-α- mRNA Expression (Fold Change over Control)

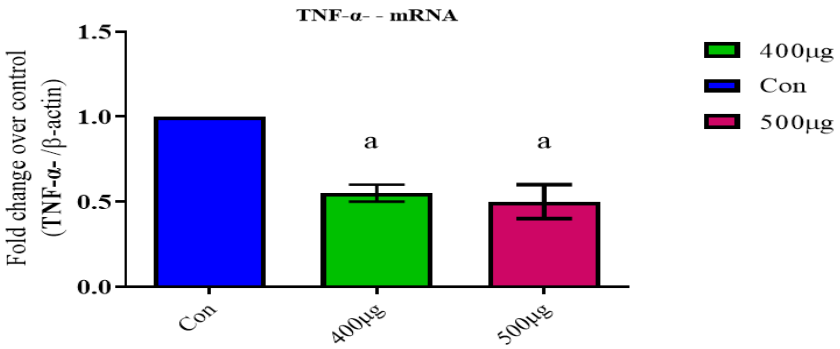

Fig. 3. Effect of Kabasura kudineer extract on TNF alpha mRNA expression in a549 cells. Each bar represents a mean ± SEM of 6 observations. The X-axis represents different concentrations of kabasura kudineer and the Y-axis represents the percentage of fold change over control. There is a statistically significant difference between the control and treated groups with p value < 0.05. a-compared with untreated control cells

4. CONCLUSION

The present study has concluded that Kabasura kudineer extract has significantly down-regulated the pro-inflammatory cytokines [Interleukin-6 and tumour necrosis factor-α] in lung cancer cell line exhibiting its anticancer property. This study showed that Kabasura kudineer extract has an anticancer potential against lung cancer cells. The anticancer effect of kabasura kudineer could be attributed to the presence of phytochemicals present in the ingredients of kabasura kudineer.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

FUNDING

This study is funded by The International Association of Lions club, Madurai. Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science, Saveetha University.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74500