Exploration of Cytotoxic, Anticancer, Antiglycation and Hypoglycemic effects of *Raphanus sativus* L. var. *caudatus*

Ishrat Younus[1], Afshan Siddiq[2*], Sadia Gousia Baig[2], Rafia Sadaf[3], Zubia Begum[4], Talha bin Fayyaz[5], Laila Anwer[1] and Noor ul ain[6]

[1]Department of Pharmacology, Faculty of Pharmacy, Hamdard University, Karachi-Pakistan.
[2]Department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Pakistan.
[3]Jinnah College of Pharmacy, Sohail University, Karachi, Pakistan
[4] Faculty of Pharmacy, Jinnah University for Women, Karachi-Pakistan.
[5]Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan
[6]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan

Corresponding author: Afshan Siddiq
Email: afshanpharma@uok.edu.p

Abstract

Raphanus caudatus belongs to the family *Brassicaceae* are pods of Radish, and are commonly known as *Mungra*. The English name for this species is Rat-tailed radish. These radish pods are unique and less explored for pharmacological effects. In this present study, the pods were evaluated for cytotoxic, anti-cancer, antiglycationand hypoglycemic potential. For cytotoxic and anticancer activity, MTT colorimetric assay method was used while for antiglycation activity, spectrophotometric methodwas utilized. Regarding in vivo, hypogly- cemic activity, biochemical testing was carried out in albino rabbits.

Raphanus caudatus interestingly revealed no cytotoxic effect against 3T3 cell line however found anticancer against different cell lines. This shows that EERC might have selectivity among cancerous and non- cancerous cell lines. The pods exhibited significant dose dependent hypoglycemic activity.

Keywords

Raphanus caudatus, cytotoxicity, anticancer, antiglycation, hypoglycemic.

1. INTRODUCTION

Vegetables play multiple important roles in the management and treatment of various diseases, as they are good sources of fibers, minerals, vitamins and etc., therefore, can be effectively used to build up and repair the body (Slavin *et al*.,2012 ; Boeing *et al*., 2012). With reference to Cruciferous vegetables, they have been famous for remarkable cardio-protective, antimicrobial, and anti-carcinogenic activity. The land and environment of Pakistan are affable for the production of vegetables. Numerous kinds of vegetables are planted in the country (Hanif *et al*.,2006).

R. sativus L. Var. *caudatus* seed pods are edible are edible and green in color. The plant is an annual and sometimes biennial, about 30cm –

90cm in height that propagate from seeds known as seed pods and can go up to 5 ft.and spread to about 2 ft.(Pocasap *et al.*, 2013). This edible part of radish is available in Pakistan in the months of November and March (Khare *et al*., 2007).

R. sativus is rich in bioactive compounds and has marvelous health importance. Therefore, the current study was planned with the purpose to investigate its in vitro and in vivo pharmacological potential of the plant which is one of the ignored and less common native vegetable belonging to radish family.

The present study was planned to discover the cytotoxic, anticancer, antiglycation as well as hypoglycemic potential of *Raphanus caudatus*

2. MATERIAL AND METHODS

2.1. Plant Material

Fresh pods of about 2.5 kg *R.sativus*L.were purchased from Karachi, Dr. Mohtashim Associate Professor of Department of Pharmacognosy, University of Karachi, performed identification of the plant and specimen with voucher #: RSP-01-14/17 which was deposited to the herbal museum of the Department of Pharmacognosy. The pods were washed and then dried in the shade and stored in air-tight containers.

2.2. Extraction

Plant material (2.5 kg) was extracted by using ethyl alcohol (Merck) (1:50, w/v) at 60 °C in a soxhlet (HMFT-5/63, England) (Khattak *et al*., 2011) , tillmaterial exhaustion about 48hlater obtained extract was filtered via autoclaved Whatmann No. 1 filter paper. In rotary evaporator obtained filtrate was dried (Buchi R-200, Switzerland) under control a pressure at 45°C to obtain semisolid mass. The percentage yield was 27.9% w/v.

2.3. IN VITRO STUDY

2.3.1. Cytotoxic and Anti-cancer activity

MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay in 96-well flat-bottomed micro plates (Pocasap *et al*., 2013) , were used to assessed EERC for its in vitro anti cancerous and cytotoxic activity. Following cell lines used which include 3T3 i.e., mouse embryonic fibroblast (normal), MCF-7 i.e., breast cancer and PC-3 i.e., prostate cancer.

For cell culturing Dulbecco's Modified Eagle Medium (DMEM)was used, which includes 5% fetal bovine serum (FBS), penicillin (100 IU/ml) and streptomycin (100 µg/ml) kept at 37ºC with 5% CO_2 incubation in 75 cm^2 flasks. Growing cells were gatheredand counted viahemocytometer. In a certain medium all cells were diluted. 100 µl/well of cell culture in concentration of $1x10^5$ cells/ml was place in 96-well plates and incubate for overnight. Later, media was decanted and fresh media of 200 µl was poured with several concentrations of EERC (50 ìg/ml was first prepared in DMSO and later diluted serially). After 72 hours, 50 µl of MTT (2 mg/ml) dye was added into each well and incubated it for further 4 hours. Later on, 100 µl of DMSO was decanted in each well of the plates and then incubated for 2 hours at 37°C. Optical density (OD) of each plate was assessed by taking the absorbance at a wave length of 570 nm, with the help of micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The assay method was directed in the triplicate.

Cytotoxic effect was determined as IC_{50} value, the concentration providing 50% growth inhibition. For PC-3and MCF-7 cell lines, Doxorubicin was used as positive control whereas for 3T3 cell line Cyclohexidine was used as positive control; however, Phosphate-buffered saline (PBS) used as a negative control.

The formula for percent inhibition
% Inhibition = 100-[(A-B) / (C-B)] * 100]

Where,

A, B and C indicate mean OD of EERC, control and standard respectively.

From % inhibition results, parallel IC_{50} values were proved by Soft- Max Pro software (Molecular Device, USA).

2.3.2. Anti-glycation activity by Spectrophotometer

The anti-glycation assay was conducted by the method explained by (Khare, *et al.,* 2007)) after slight alteration in which bovine serum albumin solution of 10 mg/ml was mixed in phosphate buffer (pH 7.4, 100 mM) including sodium azide (3 mM) as anti-microbial agent. To prepare methylglyoxal solution 14 mMthe same buffer used. In DMSO bothRutin (standard) and EERC (sample) with a concentration were prepared. In the presentinvestigation, all96-well plate contained a total reaction volume of 200 µl (50 µl methylglyoxal,20 µl of standard or test, 80 µl of phosphate buffer and 50 µl of BSA). Conversely, control comprisedof 20 µl of DMSO relatively than test compound. For 9 days the mixture was incubated at 37 ^{p}C. Once incubation is completed, rutin and EERC were noticedfor the emergenceof specific fluorescence i.e.,emission 420 nm &excitation 330 nm, versus blank using a microplate reader (Spectramax M2 Devices, CA, USA). All methods were executed in triplicate and results were described in terms of IC_{50}.
Given formula to calculate % inhibition of AGE formation is:
% Inhibition = 100-[A/B * 100]
Where,
A and B indicates Fluorescence of EERC and control respectively.

2.4. IN VIVO STUDY

2.4.1. Experimental Animals

The albino rabbits (both sexes) weight 1200 to 1800 g were used to observe the effects on blood glucose level.The rabbits were housed individually in transparent cages at 23°C temperature, humidity (50 - 60 %) in an alternate 12/12h light and dark cycle, with proper access to water and diet *ad labitum*. Animals were examinedfor adaptation to the laboratory atmosphere and to eliminate any undercurrent infection one week before the beginning of the study.

2.4.2. Dosing

In case of in vivo estimation, hypoglycemic activity of pods was identified in albino rabbits. Animals were divided in to four different groups (n = 10 each) as follows (table 1):

Table 1. Dosing for *in vivo* Analysis

Groups		Treatment	Dose (mg/kg) p.o
Control		Normal saline	1 ml/kg
Test group	I	EERC I	250
	II	EERC II	500
	III	EERC III	1000

All doses were given via oral route after dissolving in distilled water.

Effect on Glucose (sugar) Level

GOD-PAP enzymatic colorimetric technique was used to evaluate serum glucose (Barham *et al*.,1972).

3. RESULTS AND DISCUSSION

3.1. INVITRO STUDY

3.1.1. Cytotoxic and Anti-cancer effect

EERC does not produce cytotoxic effects against NIH 3T3 mouse embryonic fibroblasts. As standard we use Cyclohexidine which indicate 50 percent inhibitory concentration of 0.67±0.8 ìM against a similar cell line.Likewise, EERC revealed no cytotoxicity activity against PC-3 cell line, converselydoxorubicin which was used as positive control showed IC_{50}=0.31±0.02 ìM against malignant PC3 cell line.

However, IC_{50} values obtained against MCF-7 (breast cancer cell line) by EERC and doxorubicin were 26.37±1.2 ìg/ml and 0.92±0.05 ìM respectively thus corresponding to positive anti-proliferative activity against MCF7 cell line in our study.

3.1.2. Anti-glycation effect

Ethanol extract of R.*caudatus* did not reveal in vitro anti-glycation effect. But, the standard Rutin revealed significant anti-glycationeffect with an IC_{50} value of 0.20 ± 0.001 ìM.

Table 2. Results of in vitro assessment

CYTOTOXICITY AND ANTI-CANCER EFFECT			
IC_{50} ± SEM (µM)			
	3T3	**PC3**	**MCF-7**
EERC	-	-	26.37±1.2 µg/ml
Cyclohexidine	0.67±0.8	-	-
Doxorubicin	-	0.31±0.02	0.92±0.05
ANTI-GLYCATION EFFECT			
IC_{50} ± SEM (µM)			
EERC		Inactive	
Rutin		0.20 ± 0.001	

Number of experiments (n) = 3, Values are IC_{50} ± Std. Error of Mean

EERC: Ethanol Extract of R.caudatus

3.2. INVIVO STUDY

3.2.1. Serum Glucose Level

Table 3 indicates EERC effect after 60 days of treatment at several doses on blood glucose levels. Investigated by ANOVA with post hoc Tukey HSD presented significant lowering effect ($p<0.005$) on blood glucose level in rabbits treated with EERC-III i.e., 77±4.4 mg/dl as compare to control i.e., 107 ± 2.72 mg/dl.On the other hand, EERC-I and II treated rabbits shown non -significant change on blood glucose level as compared to control.

Table 3. Effect of EERC on Glucose Level in Blood

Groups	Blood Glucose (mg/dl)
Control	107±2.72
EERC-I (250 mg/kg)	101±2.37
EERC- II (500 mg/kg)	98 ±3.3
EERC-III (1000 mg/kg)	77±4.4**

Number of animals (n) =10 per group, Values are Mean ± Std. Error of Mean, *P ≤ 0.05 significant versus control, **P ≤ 0.005 highly significant versus control

EERC: Ethanol Extract of R.*caudatus*

In the current study, EERC was found to be inactive in the Antiglycation assay. According to research it is estimated that compounds which exhibit antioxidant effectsalso have anti-glycation activity(Mosmann *et al*., 1983). In another research, antioxidative effectswithantiglycation effects was also insignificant (Barham *et al*., 1972). According to our results, it can be recommended that other zealous mechanisms were involved in the anti-glycationeffect of plant extract.

In the current investigation for cytotoxicity testing, MTT colorimetric assay was used. MTT assay isreproducible, sensitive and simple technique. MTT is a tetrazolium yellow color salt which reducesto formazan crystals of violet color in metabolically active cellsby help of enzyme succinate dehydrogenase which shows mitochondrial activity. MTT assay has multiple application like cell growth, cell viability, cell toxicity, cell activation and sensitivity of drug conversely generallyused for the purpose of compounds having cytotoxic effect (Barham *et al*., 1972). According to our results, EERC showed minorgrowth inhibitory effect against cancerous cell line (MCF-7) by using MTT assay. Remarkablyour extract shown no cytotoxic effect against cell line (3T3) which is normal (non – cancerous) cell line. Thus, EERC may have selectivity among cancerous and non- cancerous cell lines.

Isothiocyanatecompounds found abundance in radish (Wu *et al*., 2015 ; Ramkissoon *et al*., 2013) are known for its cytotoxic and anticancer effect. In earlierresearch,sulforaphene and sulforaphane were separatedfrom the extract of *R. caudatus* and indicated remarkable activity against colon cancer cell line (HCT116). These compounds were also observed for its anticancer potential against colon cancer cell lines (HT29) (Kuang *et al*., 2013). Correspondingly, Phenethyl isothiocyanate had been proven against lung cancer as a chemopreventive agent (Sangthong *et al*., 2017).

Anti-cancer effects of Raphanus have been explainedagainst variouscancer cell lines like SK-MEL-2, HCT-15, A549, SK-OV-3, MCF-7, HeLa, PC-3, HEPG2 and A-549. As stated by

Edra *et al* (2016), *R. sativus*contain noticeable anticancer activity against breast cancer.

Several more bioactive compounds like oxazolidine, alkaloidsnandindoles were separated from *R. caudatus*have considerable cytotoxicity against colon cancer cell line (HCT116) in vitro (Kim *et al*.,2014). The proposed growth inhibitory mechanism of sulforaphane is associated to inhibition of phase I and stimulation of Phase II enzymes. In contrast, this compound can stimulate apoptosis and depression of anti-apoptotic protein expression . In a recent research on sulforaphene, another major isothiocyanate of radish, haspotent cytotoxic activity against breast cancer due to mitochondrial dysfunction, oxidative stress and cell death (Kim *et al*.,2014).

After chronic dosing, our observations revealed hypoglycemic effects of EERC at dose of 1000 mg/kg. The observed hypoglycemic effect of this variety of radish in the present research could be due to enhanced synthesis/secretion or increased peripheral utilization of glucose. However, the finding remains hypothesis and extract may be explored in diabetic model with possible mechanism of action.

4. CONCLUSION

The results from recent research reveal that *R.caudatus* potential in vitro. At dose of 1000 mg/kg hypo-glycemic effects of EERC were noted. It is highly recommended to study the mechanistic action of observed anti-cancer and hypoglycemic potential.

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