Research Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

EVALUATION OF CYTOTOXIC AND ANTICANCER ACTIVITY OF PANEEYA KSHARA ON COLON CANCER CELL LINES

Gururaja D [1]*, Sneha Kumbhar [2], Sahana Acharya [3]

[1] Professor, Department of Shalya tantra, Muniyal Institute of Ayurveda Medical Sciences, Manipal, Karnataka, India

[2] PG Scholar, Department of Shalya tantra, Muniyal Institute of Ayurveda Medical Sciences, Manipal, Karnataka, India

[3] Internee, Muniyal Institute of Ayurveda Medical Sciences, Manipal, Karnataka, India

Received on: 12/01/23 Accepted on: 25/02/23

***Corresponding author**

E-mail: gurushalya@yahoo.co.in

DOI: 10.7897/2277-4343.140232

ABSTRACT

Kshara is a unique medicinal preparation mentioned in Ayurveda. It is an alkali prepared by burning different medicinal plants. Kshara has cytotoxic activity. It can be used internally and externally. Kshara is used internally as medicine and is called Paneeya kshara. Paneeya kshara is mentioned in the context of Gulma roga. Gulma is a disease with the cardinal feature of a mass per abdomen. Malignant conditions of the colon can be correlated to Gulma. So, this study was planned to prepare the Paneeya kshara mentioned in Gulma disease and study its cytotoxic activity and anticancer activity on colon cancer cell lines. The HEK 293 normal cell line was used to study the cytotoxic activity, and colon cancer cell lines COLO 205, COLO320 and HCT-15 cell lines were used to study the anticancer activity. The cytotoxic activity on HEK293 normal cell lines was good, with an IC_{50} value of 0.726 µg. IC_{50} value for COLO320 cell lines was 0.7263 µg. The effect on HCT -15 and COLO205 cell lines was insignificant, with an IC_{50} value of more than one. Paneeya kshara showed good cytotoxic action but limited anticancer activity on colon cancer cell lines. This study revealed the potentiality of Paneeya kshara in the management of colon cancer.

Keywords: Ayurveda Alkali preparation, Colon cancer, Gulma, MTT assay, Paneeya kshara

INTRODUCTION

Kshara is a unique medicinal preparation mentioned in Ayurveda. Acharya Sushruta explains in detail the importance of kshara, its preparation and indications of Kshara. The word kshara is derived from the root word kshar in the Sanskrit language, which means which cuts or destroys. Kshara helps to eliminate unwanted tissues. Kshara is considered superior to surgical instruments as it performs surgical actions like excision, incision and scraping without using sharp instruments. As kshara is prepared by burning different medicinal plants having other qualities, it pacifies three doshas (Vata, Pitta, Kapha).

There are two types of kshara, explained based on their use. Paneeya kshara *and* Pratisaraneeya kshara. Kshara, consumed internally, is called Paneeya kshara and used externally as an application over unwanted tissues is called Pratisaraneeya kshara.[1]

Paneeya kshara is indicated in gastrointestinal tract-related diseases, especially in Gulma. Gulma can be compared to different conditions presenting with mass per abdomen. In Sushruta Samhita, under the chapter Gulma chikitsa, the preparation method and indications of Paneeya kshara are mentioned.[2] This study was planned to assess the cytotoxic activity of Paneeya kshara on normal cell lines and the anticancer activity of Paneeya kshara on colon cancer cell lines. The intestine is the seat for bacterial flora. *Escherichia coli* is common bacteria present in the gut of a healthy individual. As Paneeya kshara acts in intestinal disorders, its antimicrobial activity on *E. Coli* was studied to evaluate its effect on gut flora.

MATERIALS AND METHODS

Preparation of Paneeya kshara

Collection of Drugs: Ingredients or useful parts of medicinal plants required to prepare the kshara were collected. Tila (*Sesamum indicum),* Sarshapa *(Brassica nigra)*, Kokilaksha *(Asteracantha longifolia),* Palasha *(Butea monosperma),* and Yavanaala (*Hordeum vulgare*) were collected from fields. Mulaka (*Raphanus sativus)* was collected from the market. All the drugs were tested by specialists from the Dravya guna department for authenticity. Drugs were dried entirely under sunlight. Dried parts of the plant were burnt separately in an open pan. On complete cooling, only white-coloured ash was collected in different containers. Drugs like Kusta *(Saussurea lappa),* Saindhava (Rock salt), Yastimadhu *(Glycyrrhiza glabra),* Nagara *(Zingiber officinale),* Vidanga *(Embelia ribes)*, Ajamoda *(Carum roxburghianum)* and sea salt were purchased from Ayurveda drug dealers and authenticated. The required quantity of gomutra and aja mutra were collected. (Table 1).

Materials required: Weighing machine, two big stainless-steel vessels for filtration, clean cotton cloth, big stainless-steel vessel for boiling, spatula, gas stove and glass bottle.

Method of preparation of *Paneeya kshara*: 80 gm of ash from each plant was collected, and a total of 480 gm of ash was mixed in a large vessel. This mixture added four parts of cow's urine (1600 ml) and goat's urine (400 ml). Stirred well and kept overnight without disturbance.

The next day, only the supernatant solution was decanted, and the sediment was discarded. The collected liquid was filtered 21 times using a fine cotton cloth. The obtained filtrate was clean and clear and had the colour of cow's urine.

Kusta, Saindhava, Yastimadhu, Nagara, Vidanga and Ajamoda were pounded well, and 8 gm of fine powder of each drug was kept ready. 80 gm of sea salt was taken.

The collected filtrate was taken in a big steel pan and boiled on low flame. On reducing to half part, fine powders of Kusta etc., drugs and Sea salt were added above said quantity. It was continued to boil under low flame and stirred using a long spatula till it attained semisolid consistency. It was allowed to cool and collected in an airtight container. A total of 400 gm of Paneeya kshara was obtained. (Photo 1)

Preliminary physiochemical analysis of Paneeya kshara: Organoleptic examination of Paneeya kshara was carried out for appearance, colour, texture, odour and taste. It was semisolid in consistency with a brownish colour. It was alkaline with a pH of 10.2. Loss on drying was estimated in a standard way. A flame test detected the presence of sodium, chloride, potassium and carbonate. (Table 2)

The cytotoxic and Anticancer activity of Paneeya kshara: Cytotoxic activity of Paneeya kshara was carried out using Human Embryonic Kidney (HEK 293), and anticancer activity of Paneeya kshara by using colon cancer (COLO 205, COLO 320 and HCT-15) cell lines.

Cell lines culture: The HEK 293, COLO 205, COLO320 and HCT-15 cell lines were procured from NCCS, Pune (CSIR lab), grown, and subcultured at Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, India. The cells were grown using RPMI 1640 with 10 % Fetal Bovine Serum (FBS) respectively.

Chemicals and Reagents: Antibiotic 100 X (A001- 100 ml), fetal bovine serum (FBS) (RM1112- 100 ml), cellulose nitrate membrane (SF98A-1× 1000NO), Cisplatin (Kemoplat®) KCl-(G12A/1012/1707/08) (SDFCL), KH_2PO_4- (61754605001730), L-glutamine (RM049-100 gm), MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) dye (TC191- 1G), Na_2HPO_4- (61795105001730), NaCl- (GRM853-500 gm), parafilm (PM-996, 380020) (TARSONS), Phosphate buffered saline (PBS), Sodium bicarbonate (RM849-500 gm), RPMI 1640 medium, Trypan blue (TC193-100 gm), Trypsin (TCL08-1×100 ml).

Instruments and equipment used: ESCO Biosafety cabinet Class-II type A2, Galaxy 170S CO_2 incubator (New Brunswick), inverted microscope (Motic AE31), multiplate reader (TECAN), water bath (ROTEK RSW 03), centrifuge (REMI R-8C), refrigerator, deep freezer (ROTEK), hemocytometer, liquid nitrogen cryocan (BA-35), pH meter (EUTeCH), autoclave (ROTEK). Cell culture flasks (T-25 and T-75 flasks), petri dishes, cell culture grade sterile 96 well plates, 2-20 µl, 20-200 µl, 100-1000 µl pipettes, easy pet, multichannel pipette, sterile tips, sterile pipette, beakers, microfuge tubes etc.), waste container, aluminium foil.

Culture of cells and drug treatment: Around 70-80% confluent HEK 293 cell line flask was taken, and the medium from the culture flask was removed. The cells were washed twice with sterile Phosphate buffered saline (PBS) without disturbing the cells. The wash solution from the culture flask was removed. Around 50-100 µl of trypsin (0.25 %) was added to the flask and uniformly spread over the cells, and the culture flask was incubated in an incubator at the standard condition for approximately 2-5 minutes until the cell starting detached from the flask. After incubation, the excess trypsin was removed, and the flask was gently tapped and observed under an inverted microscope to check the activity of trypsin on cells. Once the cells were detached from the flask, around 1-2 ml of fresh medium (MEM with 10% fetal bovine serum) was added to the flasks. Based on the cell density, around 1 to 2 ml of medium-containing cells were transferred to a 15 ml sterile centrifuge tube and centrifuged at around 800 to 1000 rpm for 5 minutes. After centrifugation, the pellet was washed twice with PBS and re-suspended with a growth medium (MEM with 10% FBS). About 100 µl of trypan blue (0.04 %) was pipetted into a vial, and an equal volume of cell suspension was added. Both are mixed carefully, loaded onto a haemocytometer, and counted under an inverted microscope. After calculating the cells, seed the cells to 96 well plates so that each well has around 10,000 cells/well in 100 µl of the medium. After seeding, the 96 well plates were incubated in a CO_2 incubator for 24 hours. After 24 hours, the old medium from the 96 well plates were carefully discarded.

Cells were carefully washed once with PBS using a multichannel pipette. Paneeya kshara was dissolved in serum-free medium, and different concentration of Paneeya kshara (1-1,000 µg/ml) was added to different test groups and incubated for 48 hours, respectively. Control cells are supplemented with a routine growth medium. Treat the cells separately with Cisplatin (500 µg/ml) as a positive control; after completion of incubation time, 20 µL of MTT dye (5 mg/mL in PBS) was added to all wells in the dark. The plate was covered with aluminium foil and incubated in a CO_2 incubator at 37 ^{0}C for 4 hours. After 4 hours, 100 µL of 0.4 N HCl and isopropanol (1:24) was added to all the wells and mixed carefully to dissolve the crystals. Using a multi-plate reader, the absorbance was recorded at 570 nm and 640 nm reference range. The percentage of viable cells was calculated using the formula:

$$\% \text{ of viable cells} = [(\text{Test sample-blank}) / (\text{Control-blank})] \times 100$$

A similar methodology was followed to study the anticancer activity of Paneeya kshara against colon cancer COLO 205, COLO320 and HCT-15 cell lines.

Antibacterial activity

Preparation of Nutrient agar media: Beef extract (1 gm), Yeast extract (2 gm), Peptone (5 gm) and Sodium Chloride (5 gm) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2, and the volume was made up to 1000 ml. Finally, 15 gm agar was added to the media and autoclaved at 121 ^{0}C for 20 minutes.

Preparation of the inoculum: *Escherichia coli* (MTCC 42) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. A loopful of 48 hours old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well diffusion method: The media was cooled to around 45-55 ^{0}C, and around 20 ml each was poured into sterile petri plates. One ml of the inoculum was immediately added to the plate and swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37 ^{0}C and observed after 48 hours.

Table 1: Ingredients of Paneeya kshara

Content	Botanical Name	Useful part	Ash weight
Tila	*Sesamum indicum*	Dried seeds and seed cover	80 gm
Kokilaksha	*Asteracantha longifolia*	Whole plant	80 gm
Palasha	*Butea monosperma*	Stem and Branches	80 gm
Sarshapa	*Brassica nigra*	Seeds and seed cover	80 gm
Yava Naala	*Hordeum vulgare*	Seeds and seed cover	80 gm
Mulaka	*Raphanus sativus*	Fruit	80 gm
Kusta	*Saussurea lappa*	Root	8 gm
Saindhava	Rock salt	Rock salt	8 gm
Yasti madhu	*Glycyrrhiza glabra*	Root	8 gm
Naagara	*Zingiber officinale*	Root	8 gm
Vidanga	*Embelia ribes*	Seed	8 gm
Ajamoda	*Carum roxburghianum*	Seed	8 gm
Sea Salt			80 gm
Gomutra	Cow's urine		1600 ml
Ajamutra	Urine of Goat		400 ml

Table 2: Organoleptic examination and preliminary physiochemical analysis of Paneeya kshara

Organoleptic examination	Appearance	Semisolid
	Colour	Brown
	Texture	Fine
	Odour	Smell of Cow's Urine
	Taste	Salty, bitter
Physiochemical Analysis	pH	10.2
	Loss on Drying	18.801 gm
	Test for Sodium	Present
	Test Chloride	Present
	Test for Potassium	Present
	Test for Carbonate	Absent

Table 3: The cytotoxic activity of Paneeya kshara against human embryonic kidney (HEK 293) cell line by MTT assay)

Concentration	Mean % Viability	SD	SE
Control	100	0	0
1 µg /ml	35.45	1.47113	1.04024
5 µg /ml	30.63	0.7763	0.54893
10 µg /ml	28.07	0.85401	0.60387
20 µg /ml	26.83	1.56732	1.10826
40 µg /ml	25.77	2.01923	1.42781
50 µg /ml	25.22	2.68637	1.89955
80 µg /ml	24.42	3.20263	2.2646
100 µg /ml	23.43	2.51933	1.78143
200 µg /ml	22.64	2.17868	1.54056
400 µg /ml	22.03	2.24858	1.58998
500 µg /ml	21.75	2.03662	1.44011
800 µg /ml	21.36	2.39315	1.69222
1000 µg /ml	20.33	2.0187	1.42744
Cisplatin 500 µg /ml	4.76	0.2902	0.2052
IC_{50} = 0.726 µg			

Table 4: MTT assay of Paneeya kshara on COLO 320 cell lines

Colo 320	% Viability		
Conc. (µg /mL)	Mean	SD	SE
Control	100	0	0
1	30.635	4.419	3.124
5	27.339	4.232	2.993
10	27.320	4.050	2.864
20	27.140	0.761	0.538
40	26.875	1.054	0.745
50	21.310	1.969	1.393
100	22.089	7.784	5.504
200	16.210	0.147	0.104
400	12.488	3.500	2.475
500	8.744	4.313	3.049
800	9.554	1.917	1.356
1000	6.894	3.019	2.135
Cisplatin (500)	5.884	4.517	3.194
IC_{50} = 0.7263 µg			

Table 5: MTT assay of Paneeya kshara on COLO 205 cancer cell lines

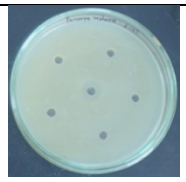
Colo 205			
Concentration	Mean % Viability	SD	SE
Control	100	0	0
1 µg /ml	76.60	16.24	11.48
5 µg /ml	55.54	17.35	12.27
10 µg /ml	52.97	16.88	11.94
20 µg /ml	45.11	11.87	8.39
40 µg /ml	43.35	13.11	9.27
50 µg /ml	41.90	14.57	10.30
80 µg /ml	37.07	11.65	8.24
100 µg /ml	35.71	12.10	8.56
200 µg /ml	34.22	12.75	9.02
400 µg /ml	33.21	12.34	8.73
500 µg /ml	31.64	12.66	8.95
800 µg /ml	30.17	12.25	8.66
1000 µg /ml	27.97	10.89	7.70
Cisplatin 500 µg /ml	2.16	0.16	0.12
IC_{50} = 13.612 µg			

Table 6: MTT assay of Paneeya kshara on HCT 15 cell lines

HCT15			
Concentration	**Mean % Viability**	**SD**	**SE**
Control	100	0	0
1 µg /ml	53.13	10.76	7.61
5 µg /ml	50.77	12.62	8.92
10 µg /ml	45.29	14.57	10.30
20 µg /ml	40.36	11.64	8.23
40 µg /ml	39.28	13.05	9.23
50 µg /ml	24.75	7.77	5.49
80 µg /ml	22.68	8.82	6.23
100 µg /ml	21.58	9.78	6.92
200 µg /ml	20.27	10.40	7.36
400 µg /ml	19.51	11.39	8.06
500 µg /ml	14.81	6.51	4.60
800 µg /ml	13.04	6.46	4.57
1000 µg /ml	10.54	4.94	3.49
Cisplatin 500 µg /ml	8.07	1.62	1.15
IC_{50} = 4.906 µg			

Table 7: Antibacterial activity of Paneeya kshara

Sample	Volume	Zone of inhibition – (Radius in mm)	
Paneeya kshara (1 mg/ml)	25 µl	0	0
	50 µl	0	0
	100 µl	0	0
	200 µl	0	0
Control (distilled water)	50 µl	0	0
Standard (*Ampicillin)* 1 mg/ml	50 µl	11	11
Paneeya kshara (10 mg/ml)	25 µl	0	0
	50 µl	0	0
	100 µl	0	0
	200 µl	0	0
Control (distilled water)	50 µl	0	0
Standard (*Ampicillin)* 1 mg/ml	50 µl	12	11



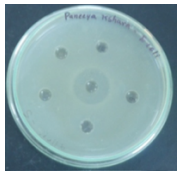


Photo 1: Paneeya kshara

RESULTS

Cytotoxic activity of Paneeya kshara on HEK 293 cell lines was measured, and the mean percentage of cell viability was 35.45% at 1 µg /ml and 21.75 % at 500 µg /ml concentration, while standard drug cisplatin showed 4.76% of viability at 500 µg /ml (Table 3). IC_{50} value (half maximal inhibitory concentration) was 0.726 µg.

Anticancer activity Paneeya kshara on COLO 320 cell lines: At a lower concentration of 1µg /ml mean viability of cells was 30.63%, and at 500 µg /ml concentration, it was 8.744%, while that of standard drug cisplatin showed 5.884% viability at 500 µg /ml. IC_{50} value was 0.7263 µg. COLO 205 cell lines showed 76.60% viability at 1 µg/ml and 31.64% of viability at 500 µg/ml, while the standard drug Cisplatin showed 2.16% at 500 µg/ml. IC_{50} was 13.612 µg. Cell lines HCT 15 showed 53.13% and 10.54% viability at lower and higher concentrations of drugs against standard drugs showed 8.07% at 500 µg/ml. IC_{50} value was 4.906 µg (Table 4-6). Anticancer activity increased with an increase in the concentration of the drug.

Antimicrobial effect was not seen during 48 hours of incubation at different concentrations of Paneeya kshara against *E. coli* colonies. (Table 7)

DISCUSSION

Kshara is an alkaline preparation with a pH varying between 8-11. Kshara word itself means a substance which is having cytotoxic activity. Studies have shown that acidic pH is responsible for tumour growth, and changing this into alkaline media is effective in controlling the tumour growth; it is known as the "Warburg effect".[3] It has been demonstrated that treatment of cancer is possible by manipulating intra and extracellular pH ratio in the tumour.[4] Nowadays, an alkaline or acid-ash diet is being promoted based on the claims that the modern diet acidifies the body. Contrary to these, studies have shown that an alkaline diet can alter urine pH but not blood pH/ systemic pH.[5] As Paneeya kshara is a potent medicine with alkaline pH, it may act on colon cells by changing the colonic pH.

Plants which are used in the preparation of the kshara have specific disease indications. Paneeya kshara, explained in the context of Gulma, contains the plants indicated in intestinal disorders. Most of these drugs are the ingredients of South Indian cuisine. South Indian dish sambar has shown protective action against colon cancer.[6] Plants used in preparing Paneeya kshara like *Asteracantha longifolia*,[7] *Butea monosperma*, [8]*Hordeum vulgare*,[9] *Raphanus sativus*,[10] *Saussurea lappa*,[11] *Sesamum indicum*,[12] *Glycyrrhiza glabra*,[13] *Brassica nigra*,[14] *Embelia ribes*,[15] and *Zingiber officinale*[16] have shown anticancer activities. Liquid media used for preparation were cow urine and goat urine. The urine of cows has antitumor action, and it acts as a bio-enhancer for chemotherapeutic agents.[17] two types of salts, rock salt and sea salt, were used in major portions. High salt inhibits tumour growth by enhancing antitumor immunity.[18]

The preparation method of Paneeya kshara was also unique. Herbal drugs were burnt; only white-coloured, properly burnt ash was collected. It was dissolved in the urine of cows and goats. Cow urine was used in a major proportion. A total of 400 gm of kshara was obtained. It was semisolid in consistency and brownish in colour. The taste of the kshara was salty and bitter, possibly because the proportion of salt was more. Basic physiochemical analysis showed that Paneeya kshara had a pH of 10.2. Salts like sodium, potassium and chloride were present. Studies conducted on physiochemical analysis on different Ayurveda alkalizer or kshara also showed high pH and different salts, which matches the present study[19].

The cytotoxic activity on HEK293 cell lines was good, as the IC_{50} value was below one. The anticancer activity of Paneeya kshara was more in COLO 320 cell lines with IC_{50} value below one compared to HCT and COLO 205, where IC_{50} values were more than one. Compared to standard drugs, the results were at par in normal HEK and COLO320 cell lines but ineffective on other cancer cell lines. Paneeya kshara showed an increase in cytotoxic and anticancer activity with increasing doses. The results of this study can be attributed to alkaline pH, high salts and anticancer properties of the drugs used in the preparation. A similar result was obtained in Apamarga kshara on HeLa and SiHa cervical cancer cell lines, but it was also comparatively less potent than the standard drug.[20] An ideal anticancer drug should possess less cytotoxic activity on normal cell lines. Kshara, by nature, has more cytotoxic activity and anticancer activity, but to make it more effective in cancer management, it needs further studies to minimize cytotoxic activity and improve its anticancer activity. Paneeya kshara did not show any antibacterial activity against *E. coli* colonies. Contrary to this, Pratisaraneeya kshara prepared by *Achyranthes aspera* plant showed good antibacterial actions.[21] Thus, it confirms that Paneeyakshara acts at the cellular level rather than the microbial level or kshara acts without affecting the gut flora. There is a scope to study further to improve its potency by adding suitable prakshepaka dravyas or additives to get the necessary action.

CONCLUSION

Ingredients and properties of Paneeya kshara point towards its potential cytotoxic and anticancer action. Paneeya kshara showed good cytotoxic action on normal cell lines. Anticancer activity on colon cancer cell lines was not uniform on different cancer cell lines. This study revealed the potentiality of Paneeya kshara in the management of colon cancer.

ACKNOWLEDGEMENT

This study was funded under a UG research grant by Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India.

REFERENCES

1. Jadavji TA, editor. Sushruta Samhita of Acharya Sushruta, with Nibandha samgraha commentary, Sutra Sthana, Ksharapaaka vidhi Adhyaya, chapter 11, verses 3-13. Varanasi: Chaukhamba Sanskrit Sansthan; 2019. P 45-48.
2. Jadavji TA, editor. Sushruta Samhita of Acharya Sushruta, with Nibandha samgraha commentary, Uttara tantra, Gulma pratisheda, chapter 42, verse 40-44. Varanasi: Chaukhamba Sanskrit Sansthan; 2019. P 720.
3. Kato *et al.* Acidic extracellular microenvironment and cancer, Cancer Cell International, Bio Med Central. 2013;13:89.
4. Guanyu Hao, Zhi Ping Xu and Li L, Manipulating extracellular tumor pH: An effective target for cancer therapy, Royal Society of Chemistry Adv., 2018, 8, 22182-22192. DOI: 10.1039/c8ra02095g.
5. Fenton TR, Huang T. Systematic review of the association between dietary acid load, alkaline water and cancer. BMJ Open, 2016;6:e010438. DOI:10.1136/bmjopen-2015-010438
6. Vutturu Ganga Prasad, Neetinkumar Reddy. Sambar, an Indian Dish Prevents the Development of Dimethyl Hydrazine–Induced Colon Cancer: A Preclinical Study, Pharmacogn Mag. 2016 Jul; 12(Suppl 4): S441–S445, PMCID: PMC5068121

7. S Ahmed, A Rahman, M Mathur, M Athar, S Sultana. Antitumor promoting activity of *Asteracantha longifolia* against experimental hepatocarcinogenesis in rats, Food Chem Toxicol, 2001 Jan;39(1):19-28. PMID: 11259848.
8. Tenzin Choedon 1, Surendra Kumar Shukla, Vijay Kumar. Chemopreventive and anticancer properties of the aqueous extract of flowers of *Butea monosperma*, J Ethnopharmacol. 2010 May 27;129(2):208-13. DOI: 10.1016/j.jep.2010. 03.011. Epub 2010 Mar 20, PMID: 20307637.
9. Czerwonka A, Kawka K, Cykier K, Lemieszek MK, Rzeski W. Evaluation of anticancer activity of water and juice extracts of young *Hordeum vulgare* in human cancer cell lines HT-29 and A549. Ann Agric Environ Med. 2017; 24(2): 345–349.
10. Omar MN, Fahd AN, *et al*. Comparative study of antioxidant and anticancer activities and HPTLC quantification of rutin in white radish (*Raphanus sativus* L.) leaves and root extracts grown in Saudi Arabia. Open Chemistry. 2021; 19:408-416.
11. Patel AA, Amanullah M, Eissa M, Elsaid FG, Soliman T. A Study on Anticancer Properties of *Saussurea lappa* (Asteraceae) Against Breast and Colonic Cancer Cell Lines. Clin Oncol. 2020; 5: 1702
12. Amin F Majdalawieh, Mariam Massri. A comprehensive review on the anticancer properties and mechanisms of action of sesamin, a lignan in sesame seeds (*Sesamum indicum*), Eur J Pharmacol. 2017 Nov 15; 815:512-521.PMID: 29032105
13. Seyed MN, Alireza N *et al*. Effect of Root Extracts of Medicinal Herb *Glycyrrhiza glabra* on HSP90 Gene Expression and Apoptosis in the HT-29 Colon Cancer Cell Line. Asian Pac J Cancer Prev. 2015;16(18): 8563-8566.
14. Asmaa Gamal Ahmed, Usama Khamis Hussein. Mustard Seed (*Brassica nigra*) Extract Exhibits Antiproliferative Effect against Human Lung Cancer Cells through Differential Regulation of apoptosis, Cell Cycle, Migration, and Invasion, Molecules 2020;25: 2069.
15. Kaur V, Hallan SS. Isolation of embelin from and evaluation of its anticancer potential in *Embelia ribes*, breast cancer, Asian Journal of Pharmacy and Pharmacology 2015; 1(1):33-39.
16. Ranjani Ramakrishnan. Anticancer properties of *Zingiber officinale* – ginger: A review, International Journal of Medicine and Pharmaceutical Sciences, 2013;3(5):11-20.
17. Randhwa GK, Sharma R. Chemotherapeutic potential of cow urine: A review. J Intercult Ethnopharmacol. Apr-Jun 2015; 4(2).
18. Willebrand R, Hamad I *et al*. High Salt Inhibits Tumor Growth by Enhancing Anti-tumor Immunity. Front. Immunol. 2019; 10:1141.
19. Singh Vivek *et al.* Need of Ayurvedic Alkalizer (Antah-Parimajana Kshara) In Present Time: An Experimental Study. Unique Journal of Ayurvedic and Herbal Medicines. 2014;2(1)
20. Mrudula KS, Mamatha KV. Efficacy of apamarga kshara in cervical cancer cell lines, International Ayurvedic Medical Journal, July 2017;5:7.
21. Kumara JP, Jayaratne DL, Anthony DJ. Antibacterial activity of pratisaraniya kshara (*Achyranthes aspera*) in the management of the fistula in ano, International Journal of Ayurvedic and Herbal Medicine 2016;6(3):2220 –2228.

Cite this article as:

Gururaja D, Sneha Kumbhar and Sahana Acharya. Evaluation of cytotoxic and anticancer activity of Paneeya kshara on colon cancer cell lines. Int. J. Res. Ayurveda Pharm. 2023;14(2):12-17 DOI: http://dx.doi.org/10.7897/2277-4343.140232

Source of support: Nil, Conflict of interest: None Declared