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Cytotoxic Effect of Ethanol Extract of *Abrus precatorius* Leaves on Raw 264.7 and SK-N-SH Cell lines

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study was aimed to investigate the cytotoxic effect of ethanol extract of *Abrus precatorius.* leaves on Raw 264.7 and SK-N-SH cell lines.
Methodology: Soxhlet extraction was carried out using absolute alcohol and subsequently, the profiling of phytoconstituents of ethanol extract was performed by LC-MS analysis.
Results: The results showed that the presence of anthocyanin, phenolic acid, carboxylic acid, amino acid and monoester in ethanol extract of *Abrus precatorius*. The phytoconstituents such as picolinic acid, N-Acetyl-DL-tryptophan, 3-Hydroxybenzoic acid, kuromanin, aflatoxin G2, monobutyl Phthalate, laurolactam, 4-Dodecylbenzenesulfonic acid, 4-Methoxycinnamic acid, caffeic acid and octyl decyl phthalate were found in ethanol extract. In addition to this, the cytotoxic effect of the ethanol extract was tested on Raw 264.7 and SK-N-SH cell lines using MTT assay. The cytotoxic study revealed that the ethanol extract of *Abrus precatorius* was non-toxic to Raw 264.7 cell, but it showed a toxic effect on human neuroblastoma cell line, SK-N-SH.
Conclusion: In this study, it was observed that the ethanol extract of *Abrus precatorius* was non-toxic to Raw 264.7 cell line, but it exhibited strong inhibition on the viability of SK-N-SH cell line.

Keywords: Abrus precatorius; anthocyanin; phenolic acid; cytotoxic; Raw 264.7 and SK-N-SH cell lines.

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1. INTRODUCTION

The plants of Fabaceae family possess numerous phytoconstituents which make them effective therapeutic agents for various diseases. *Abrus precatorius* is a thin, much branched, deciduous, climbing shrub that twines around the tree [1] and it is found in subtropical areas of the world [2]. *Abrus precatorius* has glabrous branchlets, even-pinnate leaves with 9-12 pairs of leaflets arranged in a manner such that opposite to each other. The leaflets are generally hairless and are in the size of 5-25 mm long and 2-8 mm wide, oblong shape [3,4,5]. *Abrus precatorius* leaves habitually called as C*hanoti*, have been used as folk remedies by tribes for over many years [6].

The roots and leaves are preferred as astringent, anthelmintic as well as alexiteric besides useful in conditions of cough, pharyngodynia and pectoralgia. [6]. The seeds are bitter in taste, used as abortifacient and aphrodisiac. The paste of the seeds are applied on the wounds, skin disease, sciatica and also to treat ulcers, asthma, stomatitis, vitiligo, hyperdipsia and hair loss [7]. The seed is poisonous due to the presence of "abrin", a glycoprotein is a most potent toxins [8]. The presence of Glycyrrhizin is responsible for the sweet taste of the leaves of *Abrus precatorius*. In addition, triterpenes, abrusgenic acid, abruslactone A, and methyl abrusgenate [9,10,11] and the nitrogen-containing compounds like precatorine and trigonelline are also the constituents of the leaves [12].

Liquid chromatographic profiling is capable of classifying the constituents present in the plant extract or any other mixture and the analysis of components mainly depends on the detector coupled with HPLC or UHPLC. A mass spectrometer can function as a highly specific chromatographic detector and a high-resolution mass spectrometer even more so [13]. Liquid chromatographic method is more suitable for the complex mixtures like plant extracts due to its ionization properties i.e., some of the analytes may undergo positive ionization and the remaining is detectable in the process of negative ionization. These positive and negative modes will cover maximum number of analytes in the mixture. In addition to these, mass spectral libraries also facilitate to identify the compounds. It is therefore, the LC-MS study has been carried out to identify the phytoconstituents present in the leaves extract of *Abrus precatorius*. Besides, *in-vitro* study was performed to find out the cytotoxic effect of the ethanol extract of *Abrus precatorius* on Raw 264.7 as well as SK-N-SH cell lines using MTT assay.

1.1 Raw 264.7 Cell line

Macrophages are the one of the specialized cells that response quickly to various particles to initiate and propagate inflammatory reactions [14,15]. It plays an important role in the regulation of inflammatory process by releasing cytokines, prostaglandins and leukotrienes [16]. In addition, they are involved in several other immune processes such as lymphocyte activation, proliferation, differentiation, angiogenesis, and apoptosis [17,18].

1.2 SK-N-SH Cell line

SK-N-SH cell line was derived from human metastatic neuroblastoma tissue and used as target cell for cytotoxicity assays. It constitutes morphologically contrasting cell types which includes a small spiny cell and a large epithelioid cell [19,20]. It also exhibits a neuronal phenotype, and have multiple neurochemical markers [21]. Besides it was characterized by high dopamine β-hydroxylase activity, an enzyme distributed only in sympathetic nervous tissue [22].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Absolute ethanol (S.D. Fine Chemicals Ltd, Mumbai, India) of AR grade was used for extraction. Vanquish Quaternary HPLC coupled with Q-Exactive high resolution orbitrap mass spectrometer (Thermo Scientific Pvt Ltd) was used. The solvents such as formic acid, methanol (MeOH) and acetonitrile (ACN) were of HPLC grade used for the LC-MS experimentation.

2.2 Collection and extraction of plant material

The leaves of *Abrus precatorius Linn*. were collected near Theni, Tamil Nadu, India. The plant was identified and authenticated by Dr.L.Mullainathan, Professor, Department of Botany, Annamalai University, Annamalai Nagar, Tamilnadu, India and the voucher specimen was deposited in the Department of Botany, Annamalai University. The leaves of *Abrus precatorius* were washed with fresh water and air

dried for 15 days. Then the dried material was made into coarse powder using mechanical grinder. The powdered material was packed and the extraction was carried out using Soxhlet apparatus.

2.2.1 Research laboratory

Soxhlet extraction and phytochemical study was conducted at the Department of Pharmacy, Annamalai University. The cytotoxicity study was performed at our tissue culture lab, Department of Pharmacy, Annamalai University, and Greensmed labs, Chennai.

2.3 LC-MS Analysis

LC-MS study was carried out using Vanquish UHPLC coupled with Q-Exactive Plus mass spectrometer (Sophisticated Analytical Instrument Facility, IIT-Bombay). Mobile phases used were solvent A, is a mixture of 0.1% formic acid in water (FA in water), solvent B is methanol (MeOH) and solvent C is acetonitrile (ACN). Addition of formic acid to the mobile phase enabled protonation. C18 column was used for the separation of the analytes at 40°C. The analysis was carried out with the following conditions such as: t = 0 min, 5% methanol; t = 2 min, 5% methanol; t = 20 min, 95% methanol; t = 25 min, 95% methanol; t = 26 min, 5% methanol; and t = 30 min, 5% methanol at a flow rate of 0.3 ml/min and total run time for LC was 30 minutes. Hamilton syringe was used to inject the sample at a flow rate of 3 µl/min and the MS data was collected in both positive and negative ionization mode with the scan range upto 2000 m/z.

2.4 Cell Culture

2.4.1 RAW 264.7 cell line

The murine macrophage cell line RAW 264.7 was purchased from NCCS, Pune, India and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4 mM L-glutamine, 100 IU/ml of penicillin, 100 µg/ml of streptomycin and 10% fetal bovine serum. The cells were incubated in an atmosphere of 5% CO_2 at 37°C and sub-cultured every 3 days.

2.4.2 SK-N-SH cell line

SK-N-SH, a human neuroblastoma cell line was procured from NCCS, Pune, India. Cells were cultured in Minimum Essential Medium (Eagle's) supplemented with 10% inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37^{o}C until the cells become confluent.

2.4.3 MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay

The cells were cultured in 96 well plates at a density of 5x10^4 cells/100 µl of culture medium and incubated for 24 h. Test samples of various concentrations such as 3.125, 6.25, 12.5, 25, 50 and 100µg were prepared in dimethyl sulfoxide (DMSO) and added to the 96 well plates. The cells were incubated for 24 h and then 20 µl of MTT reagent was added to each well and incubated for 4 h. The quantity of formazan crystals formed was measured at 570 nm using a micro plate reader (Robonik, India).

3. RESULTS AND DISCUSSION

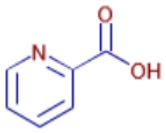
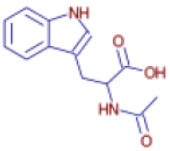
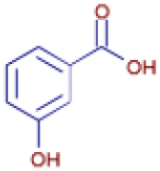
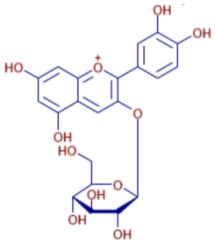
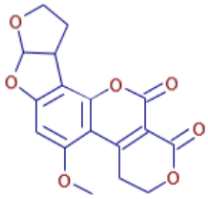
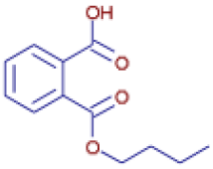
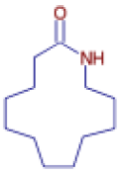
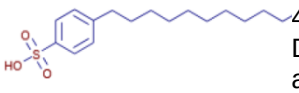
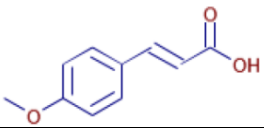
3.1 LC-MS Analysis

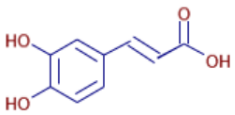
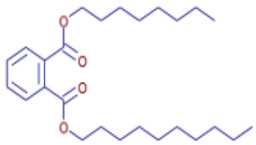
Liquid chromatography-Mass spectrometry (LC-MS) analysis was performed to identify the secondary metabolites present in the ethanol extract of leaves of *Abrus precatorius* Linn and the study exhibited the presence of secondary metabolites such as anthocyanin, carboxylic acid, amino acid and monoesters (Table 1 and Fig. 1). The compound kuromanin, an anthocyanin is used as neuroprotective [23]. The phenolic compound used as carcinogenic inhibitor, and also known for its antioxidant, antibacterial activity as well as anti-atherosclerotic agent [24-27]. 4-Methoxy cinnamic acid possessed antiproliferative activity [28]. It is also used as anti-browning preservative in post harvesting mushroom [29].

3.2 Cytotoxicity Studies

In the present study, the cytotoxicity of ethanol extract of *Abrus precatorius* (APET) was tested on two different cell lines such as Raw 264.7 and SK-N-SH cells using MTT assay. From the study, it was observed that the extract has no adverse effect on Raw 264.7 cells upto the concentration of 100 µg/ml when compared to DMSO control (Table 2, Fig 2 and 3). Interestingly, the viability of the neuroblastoma cell lines was decreased as a function of increasing concentration of ethanol extract. Cisplatin was used as control for neuroblastoma cell line SK-N-SH and the IC_{50} values were calculated for the each of the two cell lines separately. The percentage viability of SK-N-SH cell line was 11.79 % when treated with Cisplatin at the concentration of 100 µg/ml (Table 3A as well as B, Fig 4 and 5).

Table 1. LC-MS derived compounds with their structure, empirical formula, molecular weight and retention time

Sl. No	Chemical structure	Compound	Molecular formula	Molecular weight	RT(min)
1		Picolinic acid	$C_6H_5NO_2$	123.11	1.04
2		N-Acetyl-DL-tryptophan	$C_{13}H_{14}N_2O_3$	246.10044	10.7
3		3-Hydroxybenzoic acid	$C_7H_6O_3$	138.03169	10.9
4		Kuromanin	$C_{21}H_{21}O_{11}$	448.10056	13.48
5		Aflatoxin G2	$C_{17}H_{14}O_7$	330.29	14.30
6		Monobutyl Phthalate	$C_{12}H_{14}O_4$	222.08921	15.81
7		Laurolactam	$C_{12}H_{23}NO$	197.1779	18.7
8		4-Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S$	326.19157	25.7
9		4-Methoxycinnamic acid	$C_{10}H_{10}O_3$	178.06299	21.86

Sl. No	Chemical structure	Compound	Molecular formula	Molecular weight	RT(min)
10		Caffeic acid	$C^9H^8O^4$	180.16	23.6
11		Octyl decyl phthalate	$C_{26}H_{42}O_4$	418.30831	24.3

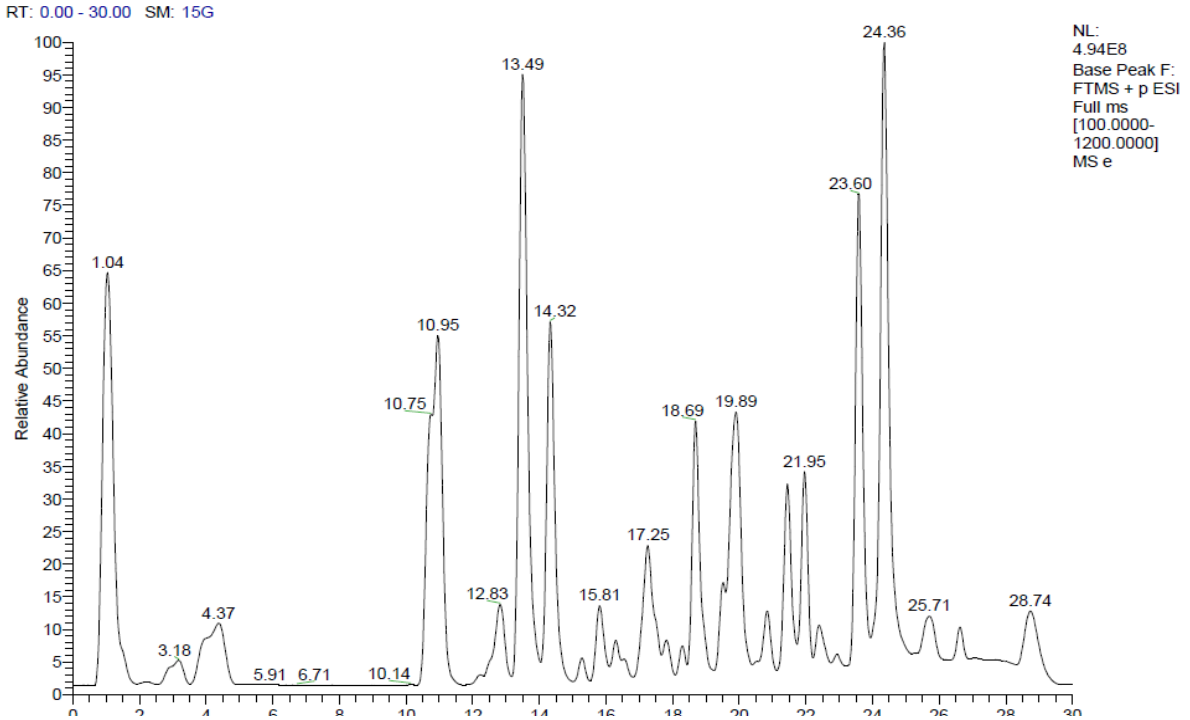


Fig. 1. The chromatographic profile of the compounds derived from ethanol extract of *Abrus precatorius* leaves using LC-MS coupled with ESI interface in both positive and negative ionisation mode

Table 2. Cytotoxicity of ethanol extract of *Abrus precatorius* on Raw 264.7 cell lines

S. No	Concentration of test sample (µg/ml)	Percentage of cell viability (in triplicates)			Mean value (%)
1.	Control	100	100	100	100
2.	3.125	99.36	99.04	99.2	99.2
3.	6.25	96.88	97.44	96.8	97.04
4.	12.5	94.32	94.08	94.56	94.32
5.	25	87.52	88	87.36	87.62
6.	50	83.28	82.56	82.88	82.90
7.	100	79.84	80.4	80.72	80.32

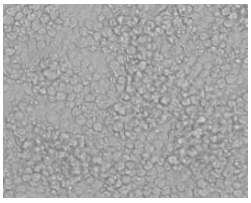
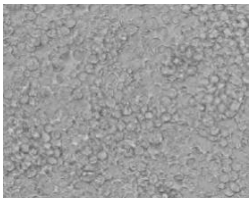
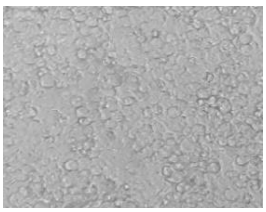
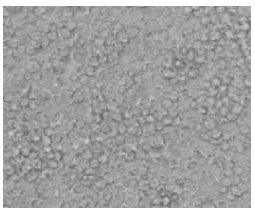
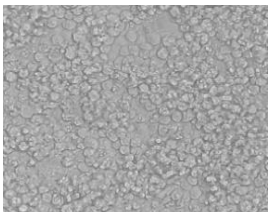
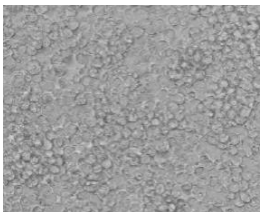
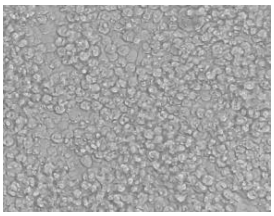
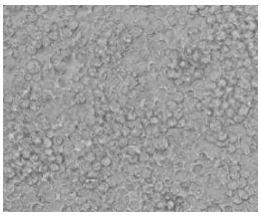
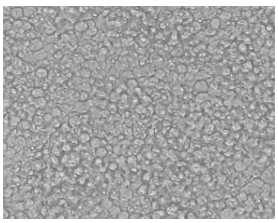
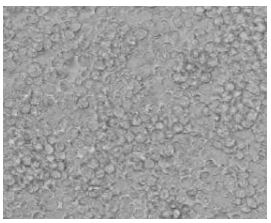
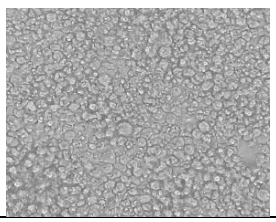
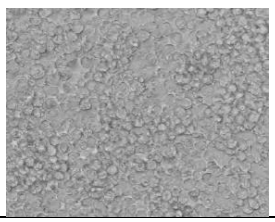
Concentration	APET	Control
3.125 µg/ml		
6.25 µg/ml		
12.5 µg/ml		
25 µg/ml		
50 µg/ml		
100 µg/ml		

Fig. 2. The cytotoxic effect of various concentrations ethanol extract of *Abrus precatorius* (APET) on the viability of Raw 264.7 cell line compared to normal control, DMSO

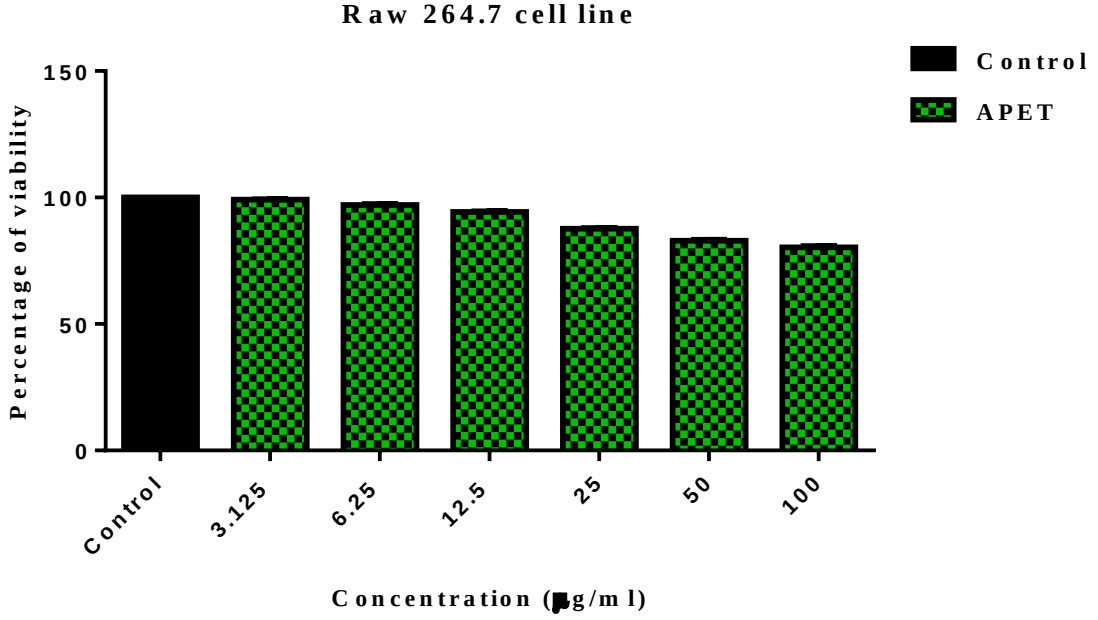


Fig. 3. Cytotoxic effect of ethanol extract of *Abrus precatorius* (APET) on Raw 264.7 cell line. The percentage cell viability was calculated where the data represents the mean of triplicate values. The statistical analysis was performed and the calculations were carried out by means of one-way ANOVA using GraphPad Prism software, version-6.0. P value < 0.0001

Table 3A. Cytotoxicity of ethanol extract of *Abrus precatorius* (APET) on SK-N-SH cell lines

S. No	Concentration of test sample (µg/ml)	Percentage of cell viability (in triplicates)			Mean value (%)
1.	Control	100	100	100	100
2.	3.125	97.19	97.19	95.50	96.62
3.	6.25	95.50	94.10	96.06	95.22
4.	12.50	91.57	92.41	89.88	91.29
5.	25	79.77	80.33	81.17	80.43
6.	50	69.94	72.47	71.06	71.16
7.	100	52.80	51.96	53.37	52.71

Table 3B. Cytotoxic effect of Cisplatin on SK-N-SH cell line

S. No	Concentration of test sample (µg/ml)	Percentage of cell viability (in triplicates)			Mean value (%)
1.	Control	100	100	100	100
2.	3.125	63.48	61.23	62.35	62.35
3.	6.25	38.48	39.32	37.64	38.48
4.	12.5	30.33	28.65	29.49	29.49
5.	25	19.94	20.50	21.06	20.50
6.	50	14.32	16.57	14.60	15.16
7.	100	12.35	11.23	11.79	11.79

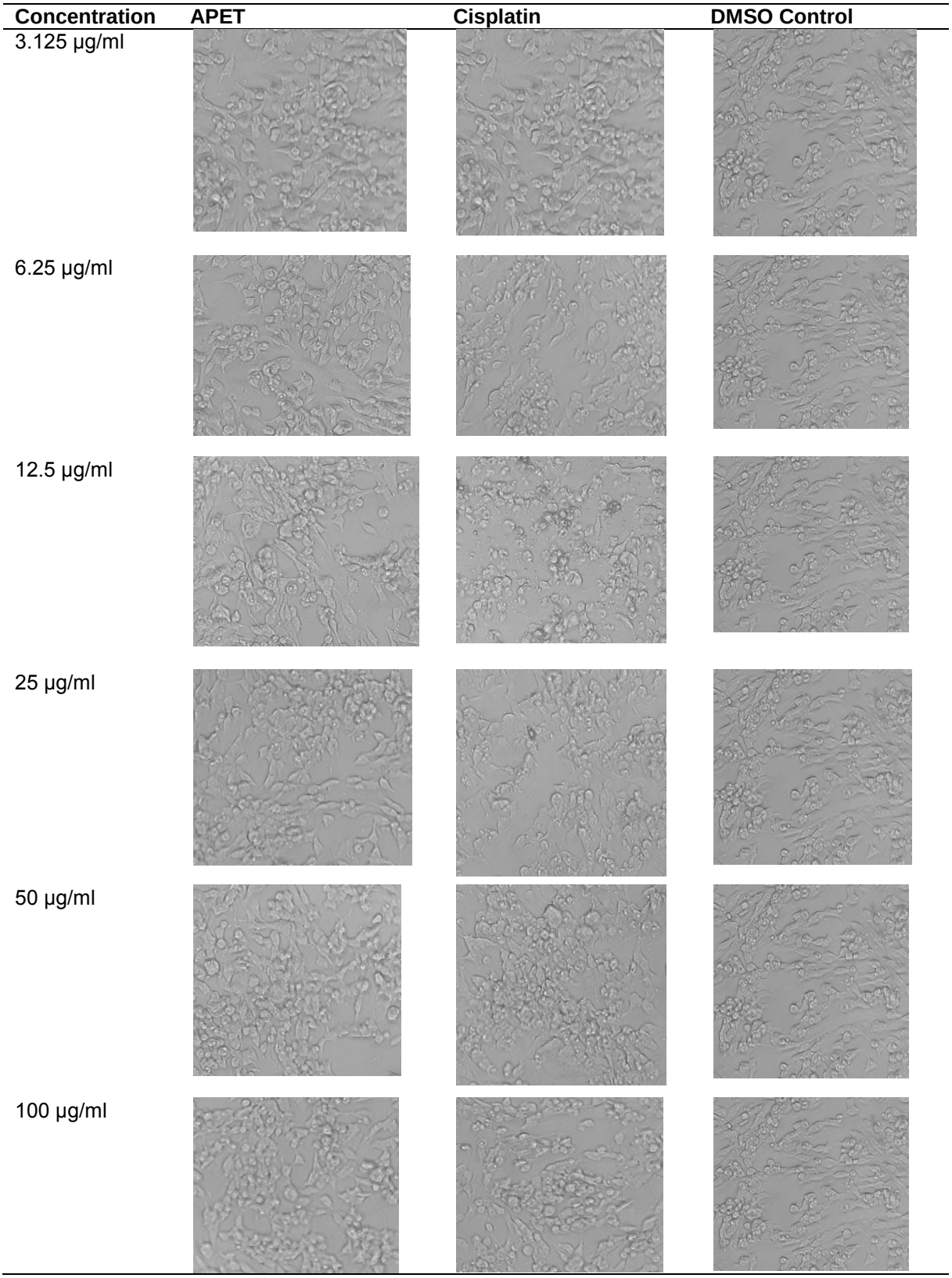


Fig. 4. The The cytotoxic effect of various concentrations of ethanol extract of *Abrus precatorius* (APET) on the viability of SK-N-SH cell lines compared with normal control, DMSO and positive control, Cisplatin

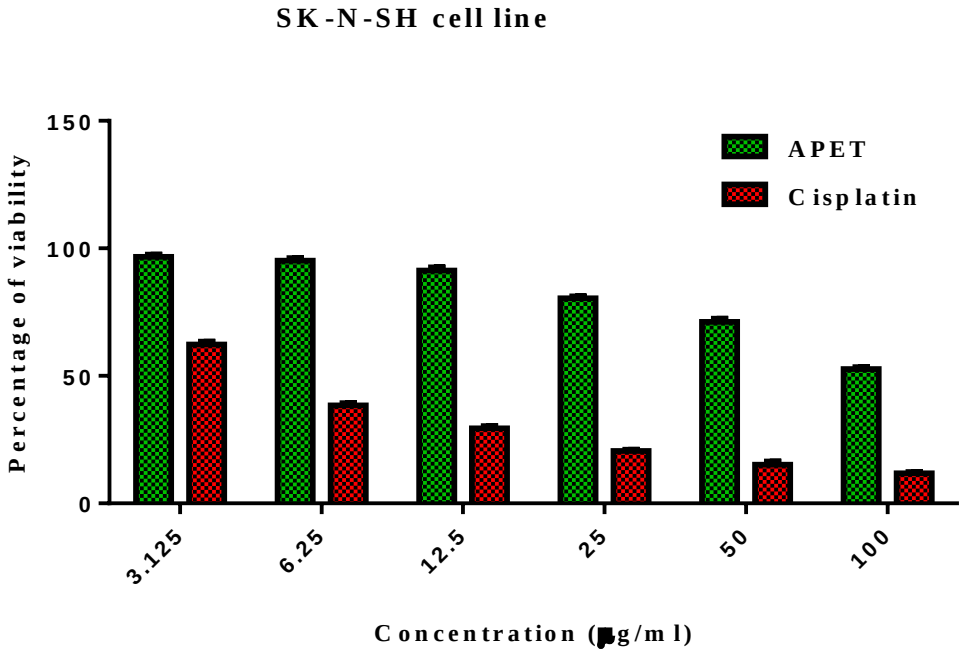


Fig. 5. Cytotoxic effect of ethanol extract of *Abrus precatorius* (APET) on SK-N-SH cell line. The percentage cell viability was calculated where the data represents the mean of triplicate values. The statistical analysis was performed and the calculations were carried out by means of two-way ANOVA using GraphPad Prism software, version-6.0. P value < 0.0001

Green tea extract inhibited the proliferation of SK-N-SH cells by inducing the neuro endopeptidase (NEP) activity [30]. Neuropeptidases involved in the degradation of amyloid beta peptides which plays a main role in the pathogenesis of Alzheimer's disease [31]. The anti-inflammatory effect of chamomile was investigated on IL-1beta stimulated neuroblastoma cell, SK-N-SH. Chamomile extract attenuated the level of MCP-1, IL-6, TNF-α, and IL-8 levels on SK-N-SH cells [32]. It was observed from the study that the SK-N-SH cells have been used for both anti-inflammatory and neuroprotective study. The present study investigated the cytotoxic effect of the extract on murine macrophage Raw 264.7 and human neuroblastoma cell line SK-N-SH.

Raw 264.7 cell lines were treated with ethanol extract of Rubus fruit and Anthocyanin fraction at the concentrations range between 25-200 and 1-50 µg/ml respectively. It was observed that both the ethanol extract and anthocyanin fraction were non-toxic to Raw 264.7 [33]. Red Chinese cabbage possess anthocyanins [34], a major constituent exhibited anti-inflammatory activity without affecting the cell viability of Raw 264.7 cell lines by using MTT assay. Aronia fruits possess polyphenolic compounds [35,36]. Similarly, the mixture of Red Chinese cabbage and Aronia extract was tested on Raw 264.7 cell lines and it was non-toxic to the cells even at high concentration of 1000 µg/ml [34]. From the literature it was observed that the plant derived secondary metabolites anthocyanins or phenolic compounds exerted the pharmacological activity without affecting the viability of Raw 264.7 cell lines. In the present study, we the authors reported that ethanol extract of *Abrus precatorius* consisting anthocyanins and phenolic compounds were non-toxic to the Raw 264.7 cell lines and this result is in agreement with the reported studies.

4. CONCLUSION

LC-MS is a strategy to identify the phytochemicals present in the crude extracts and it is one of the hyphenated techniques used to investigate the metabolites/compounds in a crude extract to avoid unnecessary isolation of previously isolated compound. The study was carried out to findout the cytotoxic effect of ethanol extract of *Abrus precatorius* on murine macrophage as well as human neuroblastoma cell lines. The present study showed that the

ethanol extract of *Abrus precatorius* has no cytocidal effect on growth of murine macrophage Raw 264.7 cells upto 100 µg/ml. But the same extract obtained from *A.precatorius* actively inhibited the viability of neuroblastoma cell line SK-N-SH. This effect may be due to the presence of secondary metabolites such as anthocyanin or phenolic compounds. Hence, further study is warranted to prove the compounds responsible for inhibition of neuroblastoma cell line SK-N-SH.

DISCLAIMER

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

SUPPLEMENTARY MATERIALS

Supplementary material is available in the followinglink:https://www.journaljpri.com/index.php/JPRI/libraryFiles/downloadPublic/24

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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