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INDUCTION OF APOPTOSIS BY CASPASES IN THE LEAVES OF *SANSEVIERIA ROXBURGHIANA* SCHULT. AND SCHULT. F.

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ABSTRACT

Cancer is one of the most life-affecting disease with many different types. Since most of the chemotherapeutic agents sold for the treatment of cancer are highly expensive, mutagenic, carcinogenic and side effects limit their applications, the compounds derived from plants have a special role in anti-cancer treatment. Apoptosis is a highly organized physiological process to eliminate damaged cells and is involved in maintaining homeostasis. Therefore induction of apoptosis in cells has been considered as a methodology for the identification of anticancer drugs. Apoptosis are triggered by activation of extrinsic and intrinsic pathways, which results from activation of caspases. *Sansevieria roxburghiana* is an herb that is used for various ailments by traditional healers. The medicinal uses of *S. roxburghiana* include treatment for abdominal pains, earache, diarrhoea and hemorrhoids. In treating ear aches and hemorrhoids, the leaf of this plant is heated and the warm juice is squeezed onto the affected area. In the present study, Effect of methanol extract of leaves of *S. roxburghiana* on the activities of caspase-3, caspase-8, and caspase-9 were studied in HepG2 cancer cell line using caspase colorimetric assay which showed caspase-3 and caspase-9 activity whereas caspase-8 showed no activity suggesting, extrinsic apoptotic pathway is probably not involved in this process.

KEYWORDS: life-affecting disease, *Sansevieria roxburghiana*, HepG2 cancer.

INTRODUCTION

Recent studies on tumor inhibitory compounds of plant origin have yielded wide spectrum of chemical structures. Epidemiological studies suggest that consumption of diets containing fruits and vegetables, which are major sources of phytochemicals and micronutrients, may reduce the risk of developing cancer. Since many of the antitumor agents sold are non affordable, the search for an effective anti-cancer drug which would be non toxic, highly efficacious against multiple cancer, cost effective, palatable and accepted by human population is an active research field.[1]

Certain products from plants are known to induce apoptosis in cells.[2,3,4] Apoptosis or programmed cell death is a highly organized physiological process to eliminate damaged or abnormal cells and is involved in maintaining homeostasis in multicellular organisms.[5] It also plays a major role in embryogenesis where apparently normal cells undergo apoptosis. It has become increasingly evident that apoptosis is an important mode of action for many anti-tumour agents, including ionizing radiation alkylating agents such as cisplatin and camptothecin.[6,7,8] Apoptosis are triggered by activation of the death receptor (extrinsic) and mitochondrial (intrinsic) pathways, results from activation of members of cysteine protease family called caspases.[9,10] Mitochondria are involved in a variety of key events, including release of caspase activators, changes in electron transport etc.[11,12] Alterations in mitochondrial structure and function have been shown to play a vital role in caspase-9-dependent apoptosis.[13] by releasing apoptotic factors from mitochondria including cytochrome C. In this manner, released cytochrome C interacts with Apaf-1 and pro-caspase-9 to form the apoptosome. Then caspase-9 cleaves and activates caspase-3, the executioner caspase, which cleaves poly (ADP-ribose) polymerase (PARP) and activates endonucleases leading to DNA fragmentation. [14] The caspase colorimetric provides a colorimetric substrate and a cell-permeable inhibitor that allows quantitative measurement of caspase (DEVD, IETD and LEHD ase), protease activity, which is an early regulatory event in the apoptotic cell death process. The colorimetric substrate is labeled with the chromophore p-nitroaniline (pNA) which is released from the substrate upon cleavage by the respective protease. Free pNA produces a yellow colour that is monitored by a spectrophotometer at 405 nm. The amount of yellow colour produced upon cleavage is proportional to the amount of protease activity in the sample.[13]

MATERIALS AND METHODS

Preparation of solvent extracts: The fresh leaves of *Sansevieria roxburghiana* were washed, air dried, cut into small pieces and pulverized in a mechanical blender. Powdered plant material was used for the preparation of solvent extracts.

The pulverized leaves was mixed with sufficient quantity of solvent methanol. It was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The dried extract thus obtained was weighed and preserved at 4ºC for future use.[14]

Caspase activation was measured using caspase colorimetric assay kits (Millipore) following the manufacturer's protocol. The HepG2 liver cancer cells were treated with 0 (control), 100, 200, 300, 400 and 500 µg/ml of methanol extract and 2 µg/ml of camptothecin as positive control. Cells were collected and cell lysates were prepared by incubating 2×10^6 cells/ml lysis buffer for 10 min on ice. Lysates were centrifuged at 10,000×*g* for 1 min. The supernatants (cytosolic extract) were collected and protein concentration was measured using Bradford protein assay method using Bovine Serum Albumin (BSA) as a standard. Protein extract (100 µg), was diluted in 50 µl cell lysis buffer for each assay. Cellular extracts were then incubated in 96-well microtiter plates with 5 µl of the 4mM p-nitroanilide (pNA) substrates, (DEVD-pNA for caspase -3, IETD-pNA for caspase -8, and LEHD-pNA for caspase -9), and incubated at 37°C for 2 h in the dark. Caspase activities were assessed in parallel by cleavage of the above substrates to free pNA. Free pNA (cleaved substrates) was measured by absorbance at 405 nm in a microtiter plate reader. Relative caspase activity was calculated as a ratio of the absorbance of treated cells to untreated cells.[15]

RESULTS AND DISCUSSION

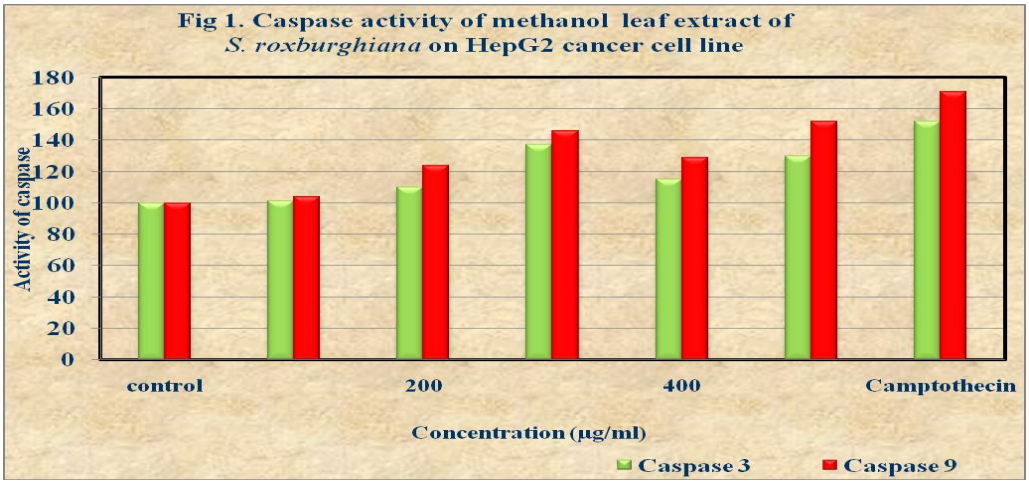
Caspase activity

Effect of methanol extract of *S. roxburghiana* leaves on the activities of caspase-3, caspase-8, and caspase-9 were examined in HepG2 cells (Table 1). The results showed a significant increase in caspase activity in the HepG2 cell line by the proteolytic cleavage of caspase-3 and caspase-9 only. Activation of Caspase- 8 was not observed in the assay. In a dose dependent proteolytic cleavage of caspase activity, 500 µg/ml of methanol extract showed an increase in the fold of caspase-3 and caspase-9 activity of 150 and 165 respectively which were comparable to the standard Camptothecin that showed a fold of caspase activity of 152 and 171.

Table 1: Caspase activity of methanol leaf extract of *S. roxburghiana* on HepG2 cancer cell line

S. No.	Concentration of extract (µg/ml)	Caspase-3 activity		Caspase-9 activity	
		OD	Fold of caspase 3	OD	Fold of caspase 9
1	0	0.58	100	0.548	100
2	100	0.591	101.90	0.573	104.56
3	200	0.637	109.83	0.681	124.27
4	300	0.775	133.62	0.796	145.26
5	400	0.662	114.14	0.714	130.29
6	500	0.749	129.14	0.834	152.19
7	Camptothecin	0.884	152.41	0.942	171.90

Apoptosis is characterized by chromatin condensation and is mediated by caspases.[16,17] The family of caspases regulates apoptosis. Caspases are normally present in the cell as proenzymes that require limited proteolysis to activate enzymatic activity.[18] Once activated, caspases cleave a variety of intracellular polypeptides, including major structural elements of the cytoplasm and nucleus, components of the DNA repair machinery, and a number of protein kinases.



Collectively, these divisions disrupt the survival pathways and disturb the important architectural components of the cell, which contribute to the stereotypic morphological and biochemical changes that characterize apoptosis. Among the caspases, caspase-3 is most commonly activated in the apoptotic process.[19] Caspase-3 is a key executioner of apoptosis, whose activation is mediated by the initiator caspases such as caspase-9 that cleave a number of substrates which act in response to DNA strand breaks leading to apoptosis.[20] This biochemical and morphological changes in apoptotic cells are cell shrinkage, chromatin condensation, DNA fragmentation, and plasma membrane blebbing.[21,22]

The anticancer activity of methanol leaf extract of *S. roxburghiana* is already established.[23] Therefore elevation in activities of caspase-3 and caspase-9 (Fig 1) suggests that methanol extract of leaves of *S. roxburghiana* can probably induce apoptosis through activation of these proteases, particularly caspase-3 and caspase-9. [15,24]

CONCLUSION

From the present study, we can conclude that the methanol extract of *S. roxburghiana* showed cytotoxicity and induced apoptosis in HepG2 cells by caspase-3 and caspase-9 activity only whereas caspase-8 showed no activity suggesting, extrinsic apoptotic pathway is probably not involved in this process.

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