International Journal of Scientific Research in Biological Sciences **Research Paper**
Vol.6, Issue.2, pp.74-80, April (2019) **E-ISSN:** 2347-7520
DOI: https://doi.org/10.26438/ijsrbs/v6i2.7480

In-vitro ACE inhibitor, Anti-inflammatory and Cytotoxicity study of *Salvia* (Chia) and *Ocimum* (Basil) Seeds

Nidhi Patel[1], Anjali Soni[2]*, Preeti Sharma[3]

[1,2,3]Dept. of Biotechnology, Veer Narmad South Gujarat University, Udhna Magdalla Road, Surat, Gujarat, India

**Corresponding Author: anjalisoni@vnsgu.ac.in*

Available online at: www.isroset.org

Received: 29/Mar/2019, Accepted: 11/Apr/2019, Online: 30/Apr/2019

Abstract: Chia seeds from *Salvia hispanica* and Basil seeds from *Ocimum basilicum* are increasingly being categorized as a novel food and have received scientific attention due to presence of high amounts of nutraceutical compounds. Recent studies demonstrate that both seeds are used as a medicine for the treatment of various kinds of diseases. Present study aims to determine their antihypertensive potential, anti-inflammatory action, and cytotoxicity. Hypertension is a major risk factor and Angiotensin Converting Enzyme (ACE) is one of the major regulators of blood pressure. Inflammation is response of body to an injury or infection. Drugs which are used as ACE inhibitors and anti-inflammatory possess undesirable side effects, while natural inhibitors have no side effects and are potential nutraceutical. *In vitro* assays were used to determine ACE inhibition and anti-inflammatory activity. The cytotoxicity assay for crude extract of defatted chia and basil seeds was done on human histiocytic lymphoma cell line (U937) by MTS assay. ACE inhibition activity assay showed that ethanolic and hexane extracts of both seeds have distinct ACE inhibitory activity. Both seed extracts had shown higher activity at 150 and 50 µg/mL concentrations respectively. Results of anti-inflammatory assay showed maximum protection of HBRC (Human Red Blood Cell Membrane Stabilization Method) at 50 and 75 µg/mL concentrations for both seeds in ethanolic and methanolic extracts. MTS assay showed 73.20% and 70.85% cytotoxicity of Chia and Basil seeds at 12.50 and 50µg/mL concentrations respectively. IC_{50} values of Chia and Basil seeds were found to be 14.09µg/mL and 23.46µg/mL. This study provides direction for future investigation concerning detailed assessment of therapeutic potential of Chia and Basil seeds.

Key-Words - *Antihypertension, Anti-inflammation, Cytotoxicity, Human Red Blood Cell Membrane, U-937 cell line.*

I. INTRODUCTION

With increasing health awareness world-wide, demand for functional food with multiple health benefits has also increased. There is an interest to introduce new food to prevent various disorders (1). A large number of drugs have been developed in medicinal practice from natural products. Screening of various bioactive compounds from plants has led to the discovery of new medicinal drug which have efficient protection and treatment roles against diseases (2). Most members of Lamiaceae family possess broad range of biological and pharmacological activities. Chia seeds from *Salvia hispanica* and Basil seeds from *Ocimum basilicum* both belonging to this family, contain high amounts of nutraceutical compounds with outstanding human health benefits and great commercial potential (3).

Cardiovascular diseases (CVD) are chronic, lifelong diseases caused by interaction among health behaviours, genetic predisposition, and environment currently the leading cause of mortality and disease burden world-wide. Obesity, especially visceral obesity, is the most important risk factor for the development of cardiovascular diseases and hypertension (4). The Global Burden of Disease study estimate the age-standardized CVD death rate of 272 per 100, 000 population in India is higher than the global average of 235 per 100, 000 population (5). The most important risk factor of CVDs is hypertension, a condition where the blood vessels have persistently raised pressure. It is a significant problem in India, with 2, 61,694 deaths in 2013; this is an increase of 138% in comparison with the number of deaths in 1990. Angiotensin converting enzyme (ACE) is generally known to play a pathogenic role in the onset of hypertension through its enzymatic activity to catalyse the conversion of angiotensin-I to angiotensin-II, which is an active peptide inducing the elevation of blood pressure, and hence the inhibition of this enzyme is considered to reduce the blood pressure, thereby improving the physical conditions of hypertensive patients. Ang I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) is a prohormone, which is converted to active hormone-Ang II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) by the cleavage of terminal dipeptide (His-Leu). This reaction is catalysed by a zinc dependent metalloenzyme known as 'angiotensin converting enzyme' (ACE). ACE is also

responsible for the elevation of BP by cleaving the terminal dipeptide (Phe–Arg) of vasodilator hormone bradykinin to its inactive form. Inhibition of ACE activity leads to a decrease in the concentration of angiotensin II, which decreases the tensionin blood vessels and consequently reduces blood pressure. ACE inhibitor alters the activity and expression of ACE *in vivo* and *in vitro*. The direct effect of ACE inhibitor treatment is a decreased serum ACE activity (6). ACE-I inhibitors derived from food proteins have attracted particular attention for their ability to prevent hypertension. Certain compounds derived from plant extracts like hydrolysable tannins, phenylpropanes, proanthocyanidins, flavonoids, terpenoids and peptide amino acids have been reported for their in vitro ACE inhibitory activity. Plant seeds, especially cereals are one of the most important sources of protein worldwide and these proteins have been found to be potential precursors of antihypertensive peptides. So Chia and Basil seeds can have an ability to block the activity of ACE and reducing capacity of blood pressure in hypertensive individuals. These findings can open up the possibility of finding newer plant derived compounds which mimic synthetic ACE inhibitors and provide health benefits without any adverse side effects (7).

During recent decades, it has been demonstrated worldwide increase in allergies and intolerances to certain foods, which is associated with nutrition, lifestyle, economic growth and urbanization (8). Inflammation is a body response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Result may be caused: mechanically (e.g., by pressure or foreign body), chemically (e.g., by toxins, acidity, and alkalinity), physically (e.g., by temperature, by internal processes), by microorganisms (e.g., bacteria, virus, and parasites) (9). Inflammation is triggered by the release of chemical mediators from injured tissue and migrating cells. It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration (9). It is associated with infiltration of the mononuclear macrophages, monocytes, neutrophils, fibroblast activation, proliferation and fibrosis (10). Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response in amplifying the disease. Pharmacognostical study of the leaves of the *Salvia hispanica* and *Ocimum basilicum* plants were reported, but there is no scientific evidence that Chia and Basil seeds may act as an anti-inflammatory agent (2).

Cancer is a significant worldwide health problem associated with abnormal uncontrolled cell growth. It is a major health threat in India (11). Cancer greatly contributes to human mortality and is considered as a major threat to humankind and morbidity globally. Over the last 30 years, scientists have worked hard to identify carcinogenic substances in the home, work place and general environment that cause cancer. Evidence for cancer causing substances and their risk comes from three sources namely human, animal and laboratory experiment studies with human cells. Evidences from each of these sources are important in helping public health officials decide whether exposure to certain carcinogenic substances needs to be reduced or eliminated (12). Most cancer treatments such as chemotherapy and radiation damage the nervous system and have common side effects such as peripheral neuropathy, headaches, and chronic pain. However, many chemotherapeutic drugs are presently placed in a predicament of reduced therapeutic effect due to the problem of drug-resistance. For these reasons, research and development of new classes of anticancer agents which exhibit efficient and selective toxicity in tumour cells is enticing increased attention. A large number of drugs have been developed in medicinal practice from natural products. In this study, we aimed to find a new source of low or non-toxic, natural anticancer agent from medicinal plant seeds. Most members of Lamiaceae family possess broad range of therapeutic activities that may protect tissues against genotoxic effects of environmental toxicants and therefore, lower the risk of human chronic disease. Anti-mutagenic effects of essential oils may be confined due to their ability to inhibit penetration of mutagens inside the cells, free radical scavenging activity and activation of antioxidant enzymes. Chia as well as Basil seeds are good source of dietary fiber, protein and antioxidants. The consumption of dietary fiber improves fecal bolus formation and proper evacuation of stool, which helps prevent obesity and cancer (11). The present study aims to preliminary analyse the antihypertensive, anti-inflammatory and cytotoxic potential of Chia and basil seeds using *in vitro* assays.

II. MATERIALS AND METHODS

Collection of Seeds

The dried seeds of *Salvia* (Chia) and *Ocimum* (Basil) were purchased from the Hakeem Chichi, Ayurvedic pharmacy at Rani Talao, Surat, Gujarat, India. Seeds were grounded well into fine powder and stored in air tight container for further use.

Preparation of Defatted Seed Powder by Soxhlet extraction

Dried seed powder (10g) was packed in a thimble separately and defatted in Soxhlet extractor using 200 mL of petroleum-ether (50ºC) till 4-5 cycles' runs. At the end of the process, the defatted powder present in the thimble was air dried and stored in a container at room temperature.

Angiotensin Converting Enzyme Inhibition Assay

ACE inhibitory activity was done according to method described by Belovic et al. 2013. This assay is based on the hydrolysis of Hippuryl L histidyl L leucine (HHL) (substrate) by ACE. The amount of hippuric acid (product) formed in reaction is determined by measuring the absorbance at 228 nm (absorption maximum of hippuric acid). The difference between absorbance in the absence and presence of inhibitor is proportional to the inhibitory activity of tested sample. Defatted powder of Chia and Basil seeds (5 g) was extracted by maceration. The solvents from both extracts were removed by evaporation in vacuum at 37 °C, using rotary evaporator. Dried extracts were resuspended in HEPES buffer (50 mM HEPES sodium salt and 300mM NaCl, pH adjusted to 8.3 using 1 M HCl) to obtain a concentration of 1 mg/mL. These solutions were used for ACE inhibitory assay. 50 µL of ACE solution (100 mU/mL) was incubated with 50 µL of test samples, at different concentration (50, 100, 150 µg/mL) dissolved in HEPES buffer as well as with standard drug Losar-50 (1 mg/mL) at 37 °C for 10 min. After the addition of 150 µl of Hippuryl L histidyl L leucine (HHL as substrate) 8.3 mM in HEPES buffer, the reaction mix was incubated for 45 min at 37 °C. The reaction was terminated by the addition of 250 µL of 1 M HCl. The resulting hippuric acid was extracted with 3 x 500 µL of ethyl acetate and centrifuged at 800 g for 15 min. 750 µL of the upper layer was transferred into test tube and evaporated under air flow at 37 °C (13). The hippuric acid was dissolved in 1 mL of distilled water, and the absorbance was measured at 228 nm using UV/VIS spectrophotometer (Shimazdu UV1800). Blank samples were prepared without the addition of enzyme and control samples were prepared without the addition of extracts. In order to eliminate the interferences in the analysis, ACE inhibition was calculated according to equation;

$$\% I_{ACE} = \frac{100\ [(A-B)-(C-D)]}{(A-B)}$$

Where, A represents absorbance in the presence of ACE,

B - Absorbance of the reaction blank,

C - Absorbance in the presence of ACE and inhibitor,

D - Absorbance of the sample blank.

Anti-Inflammatory Assay [Human Red Blood Cell Membrane Stabilization Method]

Erythrocytes have been used as a model system by a number of scientists to investigate interaction of drugs with membranes. HRBC (Human red blood cell) membranes are similar to lysosomal membrane components and inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a measure of potency of anti-inflammatory activity of extract. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to red cell membrane will render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. Membrane stabilization leads to prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators (14). Defatted powder of Chia and Basil seeds (5 g) was extracted with (ⅰ) 10 % ethanol by Soxhlet extraction method and with (ⅱ) methanol and n-hexane (50 mL) by maceration. The solvents from both extracts were removed by evaporation in vacuum at 37 °C, using rotary evaporator. Dried extracts were resuspended again in 10% ethanol, methanol, and n-hexane respectively to obtain 1 µg/mL concentration.

(a) Preparation of Red Blood Cells (RBCs) Suspension

Fresh whole human blood (10 mL) was collected from healthy volunteer and transferred to the heparinised centrifuged tubes. The blood sample was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride). The tubes were centrifuged at 3000 rpm for 10 min and pellet was washed three times with equal volume of normal saline and 10% v/v suspension (HBRC suspension) was made with normal saline.

(b) Membrane Stabilizing Activity (Heat Induced Haemolysis)

Different concentrations of extracts were prepared (25, 50, 75, 100, 125, 175 and 200 µg/mL) using distilled water and to each concentration 1 mL of phosphate buffer, 2 mL hyposaline and 0.5 mL of HRBC suspension was added. It was incubated at 37ºC for 30 min and centrifuged at 3,000 rpm for 20 min. Haemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560 nm (15). ECOSPRIN®-150 (25to200µg/mL) was used as reference standard and a control was prepared without extract. The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

% Protection = 100- O.D. of drug treated sample/ O.D. of control X100

Cytotoxicity Assay

Cell Line Acquisition and Maintenance

Human histiocyte lymphoma cell line (U937) was procured from National Centre for Cell Sciences (NCCS), Pune, Maharashtra, India. Cell line was maintained with regular subculturing and in RPMI 1640 complete medium supplemented with 10% Fetal Bovine Serum (FBS), 20

µg/mL of penicillin-streptomycin solution at 37°C with an atmosphere of 5% CO_2.

Preparation of crude extracts

Defatted powder of Chia and Basil seeds (5 g) was extracted with 10 % ethanol by Soxhlet extraction method. The solvents from extracts were removed by evaporation in vacuum at 37°C, using rotary evaporator. Dried extracts were resuspended again in 10% ethanol. Stock solutions having final concentration 1 mg/mL of Chia and Basil seed extracts were prepared in serum-free RPMI 1640 medium. The samples were syringe filtered using 0.22 µM membrane filters to remove contaminants. Range of dilutions (6.25, 12.50, 25.00 and 50.00 µg/mL) of extracts was prepared in sterile conditions in laminar flow hood by adding calculated amounts of RPMI 1640 to stock solution.

MTS Assay

MTS assay was done by using the EZcount^{TM} MTS Cell Assay kit purchased from HiMedia, India (16). Briefly, the cells were harvested and the cell density was adjusted to 1×10^5 cells/mL. Cell suspension (100 µL) was seeded in a 96-well microtiter plate at the required cell density of 1×10^4 cells/well. 100 µL of extracts having concentrations 6.25, 12.5, 25 µg/mL of Chia and Basil seed extracts were added. The plate was incubated at 37ºC in a 5% CO_2 atmosphere for 24 hours. After incubation period, the plate was removed from incubator and 20 µl activated MTS reagent was added to each well. The plate was wrapped with aluminium foil to avoid exposure to light. The plate was incubated for 2 to 4 hours again. The absorbance was taken on ELISA reader at 490 nm. The average values from triplicate readings were determined at 490 nm and subtract from this value the average value for blank (i.e. Medium control). The IC_{50} values were calculated by plotting O.D. readings versus the drug concentrations.

$$\text{Specific absorbance} = \text{Absorbance (490 nm)} - \text{Blank (490 nm)}$$

The cell cytotoxicity was calculated.

$$\% \text{ Cell viability} = \frac{\text{Specific absorbance}}{\text{Average of control}} \times 100$$

$$\% \text{ Cytotoxicity} = 100 - \% \text{ Cell viability}$$

III. RESULTS AND DISCUSSION

Angiotensin Converting Enzyme Inhibitory Activity

Enzyme inhibition refers to a decrease in enzyme activity. In this assay, HHL was used as substrate for ACE enzyme. In absence of enzyme inhibitor, ACE and HHL react to form white precipitate in the form of hippuric acid under favourable condition and were observed at the wall of eppendrof tube. In the presence of extracted samples, white precipitate either not formed or observed in very less amount in comparison to control which indicated that enzyme-substrate reaction was not occurred properly. The difference between absorbance in the absence and presence of inhibitor is proportional to the inhibitory activity of tested sample (13). The influence of ACE on blood pressure has made it an ideal target for studying hypertension. The inhibition of ACE activity is generally recognized to contribute to the reduction of blood pressure in hypertension. Role of inhibitors is being considered to be one of the effective measures to improve the physical conditions of patients. Various synthetic medications such as captopril, enalapril, and lisinopril that inhibit angiotensin converting enzyme (ACE) are widely prescribed in the treatment and prevention of cardiovascular disease. However, these drugs are often accompanied by undesirable side effects, including a persistent dry cough that has been reported in up to 44% of patients, as well as less common but more serious side effects such as allergic reactions triggering anaphylaxis, retention of potassium and difficulty swallowing or breathing due to angioedema. In contrast to the synthetic ACE inhibitor drugs, no such side effects have been observed for ACE inhibitors derived from natural ACE inhibitor containing food. In our study two plant seeds *Salvia* (Chia) and *Ocimum* (Basil) regarded now a days as functional food with multi-bioactivities were selected. Organic extracts of powdered seeds made in three different solvents ethanol, methanol and hexane were used to perform this assay. Three concentrations 50, 100, 150 µg/mL of the extracts were tested. Maximium percentage inhibition (52.8) was shown by ethanolic extract of Basil seed while lowest (20) by hexane extracted Chia seed sample at 150 µg/mL. The IC_{50} value of Chia and Basil seed extracts were 223.68 µg/mL and 136.42 µg/mL respectively which is quite high in comparison to standard, Losar, was found to be 31.95 µg/mL (Table 1). The extracts inhibited ACE in a concentration dependent manner only in ethanolic extracts of both seeds (Figure1).

Similar studies were conducted by other research groups such as (Muthuswamy et al., 2012) (17) found that the seeds of *Apium graveolens* possess ACE inhibitory activity at a concentration of 800µg/ml, showing 50% inhibition. In this respect, the search for diet-related preventive agents for hypertension is of interest as functional foods (4).

Table 1. Results of standard drug (Losar) and Chia and Basil seeds extracts for ACE inhibition

Concentration (µg/mL)	% I_{ACE} of Standard Drug	% I_{ACE} of Chia seed extract		% I_{ACE} of Basil seed extract	
		Ethanolic	Hexane	Ethanolic	Hexane
10	24	-	-	-	-
25	56	-	-	-	-
50	60.8	25.6	43.2	31.2	45.6
100	-	27.2	21.6	42.4	38.4
150	-	40.8	20	52.8	37.6

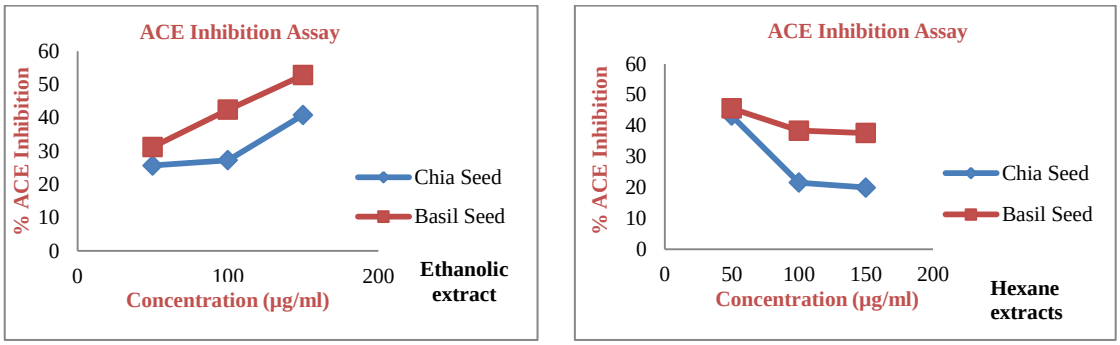


Figure 1. *Angiotensin converting enzyme %inhibition vs extract concentration curve of Chia and Basil seeds (a) Ethanolic extract (b) Hexane extract*

Anti-Inflammatory Activity

The investigation of anti-inflammatory action is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as well as substitutes for chemical therapeutics (2). Plant-based drugs used in the practice of traditional treatment of many diseases including inflammation have become the objective of current research. Chia and Basil seed extracts exhibited membrane stabilization effect by inhibiting hypotonic induced lysis of erythrocyte membrane. Ethanolic extract was prepared by Soxhlet method while in other method seeds were dissolved in methanol and hexane and kept for 24 hr shaking condition at 60 rpm, 25^0C. Almost equal maximum membrane stabilization activity was observed with ethanolic extracts of Chia and Basil seeds i.e. 88.73 and 88.28 % at 75µg/m respectively. Compared to above results methanolic extracts of Chia and Basil seeds showed maximum membrane stabilization activity of 88.28 and 86.48 % at lower concentration of 50µg/mL respectively. ECOSPRIN®-150 is used as standard drug which showed maximum membrane stabilization activity of 91.81 % at 50µg/mL. Hexane extracted samples of both seeds did not resulted in any positive effects.

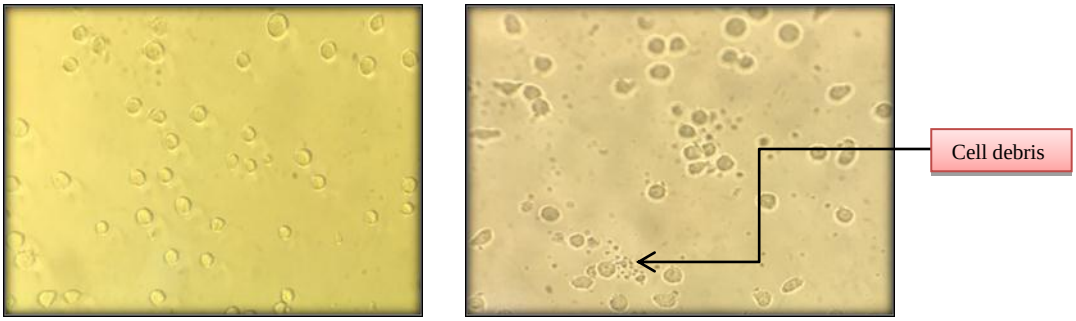
Table 2. Results of *in vitro* anti-inflammatory activity of Standard drug and different extracts of Chia and Basil seeds

Concentration (µg/mL)	% Protection of Standard drug [ECOSPRIN®-150]	% Protection by Chia seed extracts		% Protection by Basil seed extracts	
		Ethanolic	Methanolic	Ethanolic	Methanolic
25	81.98	79.72	78.82	77.47	80.18
50	91.81	86.03	88.28	84.23	86.48
75	89.18	88.73	88.28	88.28	85.58
100	88.73	83.33	85.13	70.72	81.98
125	80.18	64.41	82.43	50.90	77.47
150	78.82	77.47	77.47	46.84	74.77
175	74.77	73.87	76.12	23.42	72.97
200	74.77	54.95	76.12	23.42	59.45

MTS assay

Research into food- derived bioactive components for cancer prevention as well as cancer therapy is a growing area of research due to the relatively low or no detectable toxicity and better bioavailability (18). Due to limitations of chemotherapeutic agents commonly used to treat cancer, the needs to search new anticancer agents which are plant based have been increased (11). Therefore, the present study aims to perform cytotoxicity assay of these plant seed extracts on Human histiocyte lymphoma cell line (U937) using MTS assay for 24 hrs. Results of this assay had revealed that

extracts of Chia and Basil seeds were able to inhibit the proliferation of the cancer cells. U- 937 cell line cells were treated with extracts for 24 hrs. Percentage cell viability was calculated by measuring the absorbance of yellow colour formazan formed from reduction of MTS solution by the presence of mitochondrial dehydrogenase in viable cells. Chia and Basil seeds extracts at 6.25, 12.5, 25, and 50 µg/mL concentrations resulted in a significant increase in the cell death, though it was not in dose dependent manner. Cells observed under inverted microscope shows distinct changes in morphology after treatment with the extracts (Fig.2). Chia seed extract had shown highest cytotoxicity of 73.20% at 12.5 µg/mL concentration while in Basil seeds extracts it was 70.85% at 50µg/mL concentration. IC_{50} value of Chia and Basil seed extracts were 14.05µg/mL and 23.46µg/mL respectively (Fig.3). In present study, the reduction in percent cell viability after 24 h of treatment showed potent cytotoxic effects of Chia and Basil seeds on cancer cell line. Similar studies performed by Hashim et al. (2012) (19), found that curcumin exhibited notable cytotoxic activity towards lymphoblast leukemic cells (U937) with 80.02% in concentration dependent.



(A) (B)

***Figure 2**. Cyotoxic effect of Chia seed ethanolic extracts on U937 cell line (A) Control (Untreated) and (B) Treated cells with Chia seed extract (12.5 µg/mL)*

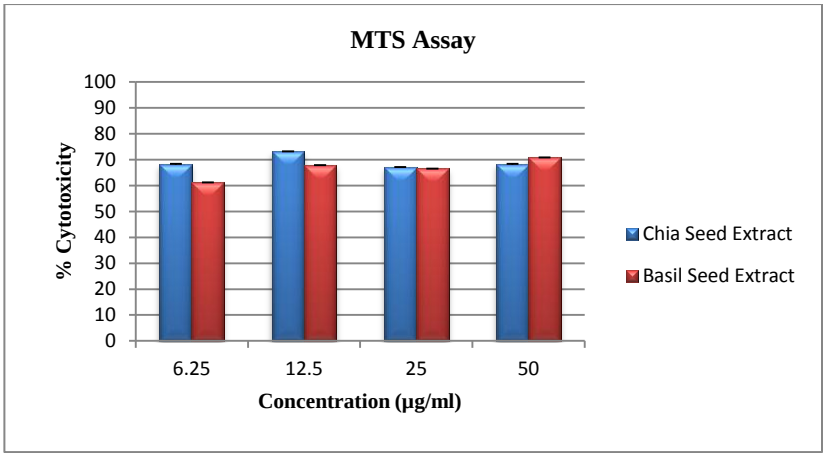


***Figure 3**. Graphical representation of Cytotoxicity of Chia and Basil seeds extracts in U937 cell line.*

IV. CONCLUSION

Neutraceuticals are gaining much importance as important dietary compounds in present days. According to our preliminary studies on Chia and Basil seeds it is observed that both of them possess very significant medicinal properties as antihypertensive, anti-inflammatory and cytotoxicity. Basil seeds had shown distinct ACE inhibitory activity while Chia seeds were found to be better in case of anti-inflammatory action. Low IC_{50} values obtained for Chia seeds in comparison to Basil have implied to their cytotoxic effect on cancer cells. On the basis of above results it can be concluded that both seeds posses potential to be the source of natural antihypertensive, anti-inflammatory and cytotoxic agents which might play an important role in therapeutic control of these disorders.

REFERENCES

[1] N. Mohd Ali, S. Yeap, H. Wan Yong, B. Kee Beh, *"The Promising Future of Chia, Salvia hispanica L"*, Journal of Biomedicine and Biotechnology, Hindawi Publishing Corporation, pp. 1-10, 2012.

[2] K. Pant, K. Agarwal, P. Saini, *"To study in vitro anti-inflammatory activity of Anthracephalus cadamba leaves extract"*, DHR International Journal of Pharmaceutical Sciences, Vol. 3, No. 1, pp. 55-60, 2012.

[3] S. Suri, S. Jain Passi, J. Goyat, "*Chia seed (Salvia Hispanica L.) – A new age functional food" [Conference]*. New Delhi, pp. 752-765, 2016.

[4] M. Shamloo, P. Eck, T. Beta, *"Angiotensin converting enzyme inhibitory peptides derived from cereals"*, Journal of Human Nutrition & Food Science, Vol. 3, No. 1, pp. 1-10, 2015.

[5] D. Prabhakaran, and P. Jeemon, "Cardiovascular diseases in India: Current epidemiology and future directions", [Online] http://circ.ahajournals.org, 2016.

[6] P. Arora, and A. Chauhan, *"Ace Inhibitors: A Comprehensive Review"*, International Journal of Pharmaceutical Science and Research, Vol. 4, No. 2, pp. 532-548, 2013.

[7] D. Orona-Tamayo, M. Valverde, B. NietoRendon, O. Paredes-Lopez, "*Inhibitory Activity of Chia (Salvia hispanica L.) Protein Fractions Against Angiotensin Iconverting enzyme and Antioxidant Capacity"*. Food Science and technology, pp. 236-242, 2015.

[8] K. Kacmarova, B. Lavova, P. Socha, D. Urminska, *"Characterization of protein fractions and antioxidant activity of Chia Seeds (Salvia Hispanica L.)"*. Potravinarstvo® Scientific Journal for Food Industry, Vol. 10, No. 1, pp. 78-82, 2016.

[9] V. Godhandaraman, and S. Ramalingam, "*In vitro anti-inflammatory activity of different parts of Pedalium murex (L.)"*, International Journal of Herbal Medicine, Vol. 4, pp. 31-36, 2016.

[10] C. Anosike, O. Obidoa, L. Ezenyika, *"Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum)"*, DARU Journal of Pharmaceutical Science, Vol. 20, No. 76, pp. 1-7, 2012.

[11] G. Dugganaboyana, C. Palanisamy, K. Kumar, S. Muthusami, K. Velliyur, *"Dietary Evaluation, Antioxidant and Cytotoxic Activity of Crude Extract from Chia Seeds (Salvia hispanica L.) against Human Prostate Cancer Cell Line (PC-3)"*, International Journal of Pharmacognosy and Phytochemical Research, Vol. 8, No. 8, pp. 1358-1362, 2016.

[12] S. Ingole, A. Kakde, P. Bonde, *"A Review on Statistics of Cancer in India"*. IOSR Journal of Environmental Science, Toxicology and Food Technology, : Vol. 10, No. 7, pp. 107-116, 2016.

[13] M. Belovic, N. Ilic, A. Tepic, Z. Sumic, *"Selection of conditions for angiotensin– converting enzyme inhibition assay: influence of sample preparation and buffer"*, Food and Feed Research, Vol. 40, No. 1, pp. 11-15, 2013.

[14] N. Kumar, A. Singh, D. Sharma, K. Kishore, *"In-vitro anti-inflammatory and antioxidant activity of Onosma hispidum (Ratanjot) roots"*, International Journal of Pharmacy and Biological Sciences, Vol. 7, No. 3, pp. 30-35, 2017.

[15] M. Musfiq, and T. Ananthi, *"In vitro antiinflammatory activity of seed extract of Zea mays (L.)"*, Journal of Global Bioscience", Vol. 4, No. 5, pp. 2168-2173, 2015.

[16] EZcount^{TM} MTS Cell Assay Kit (Product Code: CCK053) .

[17] U. Muthuswamy, A. Mathew Puthenpurackal, A. Kuppusamy, S. Thirumalaiswamy, S. Varadharajan, J. Puliyath, *"In vitro angiotensin converting enzyme inhibitory and antioxidant activities of seed extract of Apium graveolens Linn"*, Annals of Biological Research, Vol. 3, No. 3, pp. 1274-1282, 2012.

[18] A. Gajendiran, V. Thangaraman, S. Thangamani, D. Ravi, J. Abraham, *"Antimicrobial, antioxidant and anticancer screening of Ocimum basilicum seeds"*, Bulletin of Pharmaceutical Research, Vol. 6, No. 3, pp. 114-119, 2016.

[19] F. Hashim, M. Shawkat, H. Al-Jewari, *"Cytotoxicity of curcumin against Leukemic Cell Lines via apoptosis activity"*, Current Research Journal of Biological Sciences, Vol. 4, No. 1, 60-64, 2012.

