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Lunasin: A Medicinally Important Chemo-Preventive Natural Dietary Peptide

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Abstract:- Lunasin is a natural peptide of plant origin possessing potential activity against chronic disorders specially cancer along with associated cardiovascular diseases. The bioavailability and confirmed effectiveness against breast, skin and colorectal cancer has declared lunasin as a promising chemo-preventive agent. Lunasin, a 43 amino acids long peptide possess anti-inflammatory and anti-oxidative activity. Modern biotechnological techniques viz. genomics, proteomics along with other biochemical tools are continuously elucidating the molecular mechanisms of lunacin action. Lunasin enriched natural product-based healthcare remedies for the prevention of life style induced cancer is suggested as an alternative to the conventional chemo, immuno and hormonal therapy.

Keywords:- *Lunasin, Chemo-Preventive, Soybean Peptide, Cancer, Anti-Inflammatory, Anti-Oxidant, Nutraceutical, Proliferation, Differentiation.*

I. INTRODUCTION

Initially identified in soybean seed cotyledon (Galvez et al., 1997), Lunasin belongs to the category of most studied plant peptides due to its potential beneficial effects against chronic diseases (Hsieh et al., 2018). Biological effects of lunasin have been tested by several research laboratories and more than 20 years of research has presented Lunasin as anti-cancer soyabean derived peptide that possesses chemo-preventive activity not only for mouse and human melanoma cells but also show effectiveness towards endometrial (Goodman et al. 1997), prostate (Lee et al. 2003) and breast cancer (Yamamoto et al., 2003) too. Additionally, adequate soybean dietary consumption (as a source of lunasin) has been found to lower the risk of osteoporosis and cardiac diseases too (McCue and Shetty, 2004).

II. LUNASIN AS NATURAL PLANT PRODUCT

Lunasin was earliest isolated at Niigata University School of Medicine (Japan) in 1987, amid examining the protease inhibitors from soybean seeds and subsequently recognised as a hopeful anticancer candidate (Liu et al., 2014). From soybean seeds lunasin was isolated at concentrations ranging from 0.5 to 8.1 (mg/g seed) (De Mejia et al., 2004) Since then, the soybean is recognized as a principal lunasin source (Liu et al., 2014). Western blot and ELISA assays have quantified lunasin in a variety of dietary foods (Cavazos et al., 2012) as given in Table 1. Out of these barley, wheat, rye and cereal grains reported to contain variable amounts (Fig 1). It is also reported that species based differential expression of lunasin contents is attributed to their variable genotypes (Gonzalez de Mejia et al., 2004). A more diligent and logical research on lunasin and its homologues in different plant seeds is presently being carried out in order to demonstrate a relation between the presence of this very natural peptide and the taxonomic positions of various plant families (Hsieh, et al., 2011a). In addition to soybean, lunasin is reported to be present among whole of the *Solanaceae* family, except the *Leptochola chinensis* (Jeong et al., 2007a).

Table 1 Lunasin Content in Certain Plants

Plant	***Lunasin (mg/g protein)***
Soybean (*Glycine max*)	70.5
Sunberry (*Solanum nigrum L.*)	36.4
Hyyodori-jogo (*Solanum lyratum*)	22.3
Bladder-cherry (*Physalis alkekengi var. francheti*)	17.0
Amaranth (*Amaranthus hypochondriacus*)	12.1
Jimson weed (*Datura starmonium*)	10.3
Barley (*Hordeum vugare L*)	8.7

III. LUNASIN COMPOSITION AND STRUCTURE

Lunasin is a 43 amino acid long unique peptide of 5.5 kDa (Balasubramanyamet al., 2003). Negatively charged aliphatic amino acid ie. aspartic acid is the major component of this peptide and the positively charged amino acids viz. Glutamine and Lysine ranks second along with Glycine (Figure 2). Circular dichroism analysis revealed lunasin structure as 28% β-strands and 29% α-helix that exhibits three α-helical regions interspersed between H^5-C^{10}, C^{22}-I^{30} and

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D^{35}-D^{41} (Dia et al., 2013). C-terminal α-helix comprised of aspartic acid (D) residues (Table 2) plays important role in the recognition and binding with chromatin material, thereby, making lunasin as potent inhibitor of positively charged (basic) H3 and H4 histone proteins acetylation (Balasubramanyamet al., 2003).

IV. THERMOSTABILITY

Lunasin remains thermostable within 25-100°C temperature range (Dia et al., 2013). Circular dichroism analysis by Dia et al., (2009), observed no apparent changes in its secondary conformations within this temperature zone; revealing its high thermostable characteristic. Lunasin is a immensely heat stable peptide that is able to even retain its activity despite 10 min. of boiling in the water (de Lumen, 2005).

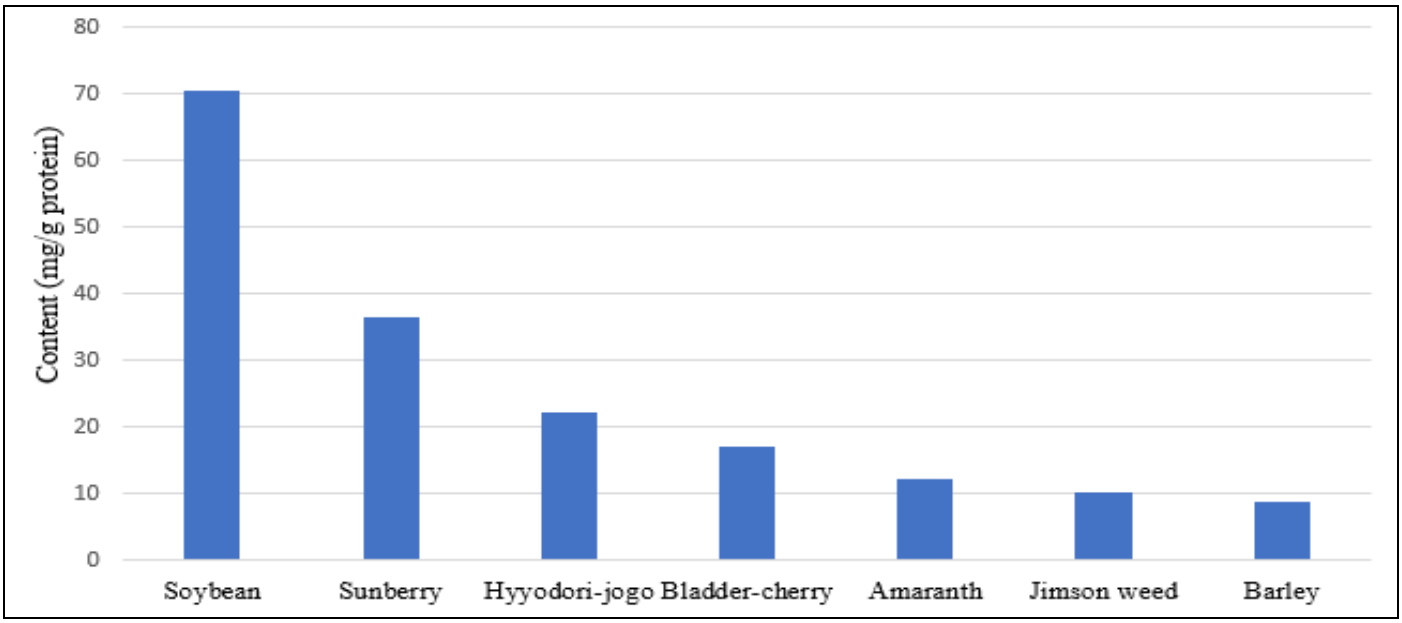

Fig 1 Clustered Column Chart Comparing Lunasin Contents

V. DIGESTIBILITY

In vitro digestibility studies using pure non-natural synthetic lunasin reported that the peptide is digested enzymes sourced from pancreas (Galvez et al., 2001). However, in soy and wheat the naturally present protease inhibitors viz. Kunitz Trypsin inhibitor (KTI) and Bowman-Birk inhibitor (BBI) function to protect the lunasin from digestive action of g.i.t. in humans and other animals.

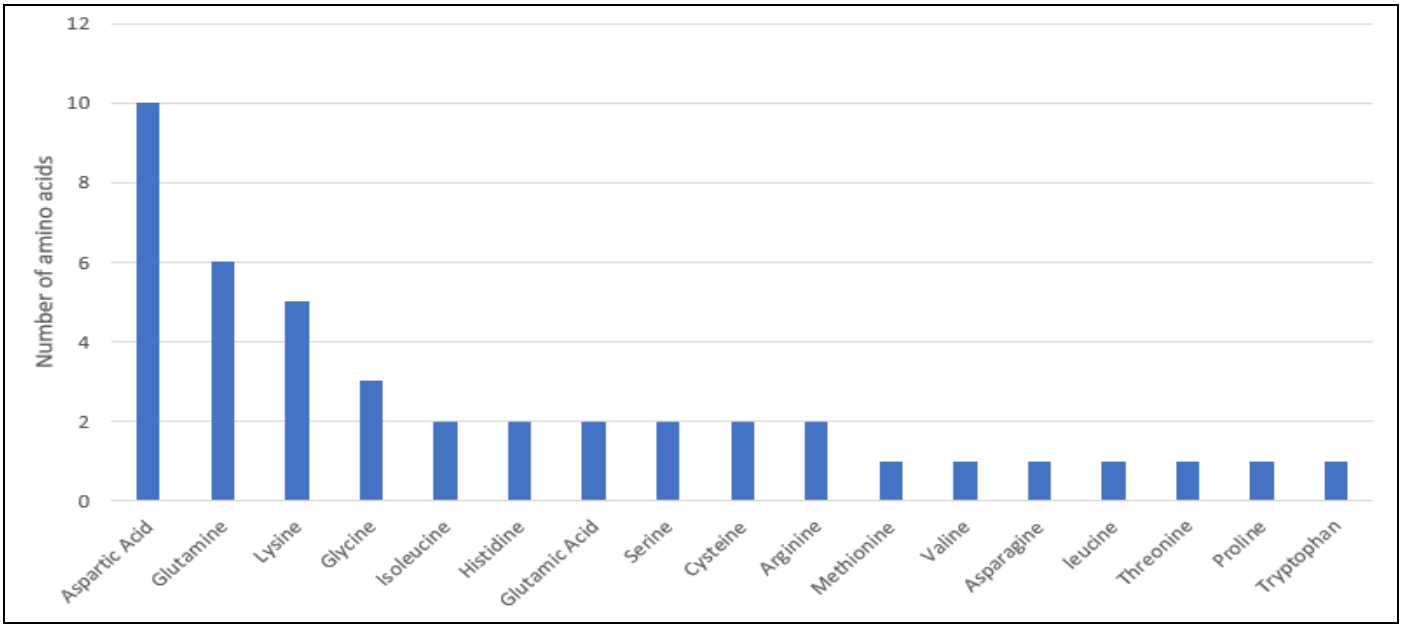

Fig 2 Amino Acid Composition of Lunasin Peptide

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VI. BIOAVAILABILITY

Lunacin is found to resistant towards the action of gastrointestinal as well as serum peptidases and remain actively available.

➢ *Rat and Mice Models:*

Lunasin-enriched soybean protein dietary studies in rat and mice models by Jeong et al., (2007b), regarding its bioavailability reported the 35% distribution of ingested lunasin in the aimed tissues as well as organs in its functionally flawless form.

➢ *Humans:*

Similar lunasin bioavailability study involving adult human males reported the presence of the 4.5% active content of ingested soybean lunasin in the plasma. Above studies revealed the high resistance of the lunasin in the course of its translocation from the alimentary and digestive tract (g.i.t) to other organs as well as the blood. Lunasin's capability to reach the target tissues in the bioactive form even after oral administration makes lunasin a potent anti-cancer natural agent (Hernández-Ledesma & De Lumen, 2008).

VII. LUNASIN AS ANTI-INFLAMMATORY AND ANTI-OXIDATIVE AGENT

Lunasin reported to inhibit the biosynthesis of pro-inflammatory mediators viz. Tumor necrosis factor-α (TNF-α), Interleuquine-6 (IL-6) and Prostaglandin-E2 (PGE-2) (de Mejia & Dia, 2009). Lunasin reduces the induced generation of reactive oxygen species (ROS) by macrophages, thereby, function as free radical scavenger as well as potent antioxidant (Hernández-Ledesma et al., 2009). So to this wise, it suppresses the hydroxyl radical generation and protects the DNA strands from the oxidative damages (Jeong et al., 2010). Therefore, both the antioxidant as well as the anti-inflammatory effects of lunasin peptide contribute towards the chemoprevention.

➢ *Lunasin in Chronic Disorders*

High mortality rates due to prevailing chronic disorders is the mojor medical challenge in the 21st century. Dietary and lifestyle modulations are proposed as the best preventional strategies for these chronic diseases. Multiple dietary compounds have already been described for their nutritional as well as health promoting effects. One of these compounds is the soybean peptide lunasin which has already been extensively studied in most recent decades (Hsieh, 2018). For Example, In the cardiovascular disorders, being the most immensely serious worldwide prevailing public health issues in the modern times, the average daily dietary intake of 25 g of protein from soy source is already approved by the Food and Drug Administration (FDA) to minimize the risk of cardiovascular disorders (FDA, 1999).

➢ *Lunasin as Chemo-Preventive Phytochemical*

Cancer prevails worldwide as a leading cause of death. In 2020, about 19.3 million cancer cases leading to 10 million mortalities have been reported by International Agency for Research on Cancer (IARC). The prevalence of disease has been expected to raise (47% increase) ie. approx. 28.4 million by the 2040 (Sung et al.,2021). American Cancer Society (ASC) reported about 7.6 million mortalities and 12.3 million newer cancer cases globally in the year 2007 (Garcia et al., 2007). Manson (2003), estimated that 35% of cancer cases among these are related to the imbalance of the dietary factors. Phytochemicals from various plant foods are reported to interact with the carcinogenesis by way of regulating multiple key proteins related to the complex processes of signal transductions involved in the series of step wise mechanism involving cellular proliferation, differentiation, metastasis, angiogenesis and apoptosis, thereby, possessing potential health promoting and disease controlling properties (Fimongnari et al., 2008; van Breda et al., 2008). Béliveau & Gingras (2007) reported that the generation of micro-tumours in the human body can be restricted by taking diet rich in anticancer phytochemicals.

Table 2 Amino Acid Sequence and Regional Functionality of Lunacin

Sequencing Order	Amino Acid	Regional Functionality
1	S (Serine)	--
2	K (Lysine)	
3	W (Tryptophan)	
4	Q (Glutamine)	
5	H (Histidine)	
6	Q (Glutamine)	
7	Q (Glutamine)	
8	D (Aspartic Acid)	
9	S (Serine)	
10	C (Cysteine)	
11	R (Arginine)	
12	K (Lysine)	
13	Q (Glutamine)	
14	K (Lysine)	
15	Q (Glutamine)	
16	G (Glycine)	
17	V (Valine)	

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18	N (Asparagine)	
19	L (leucine)	
20	T (Threonine)	
21	P (Proline)	
22	C (Cysteine)	
23	E (Glutamic Acid)	Putative helical region enhancing histone binding for chromatin targeting (homologous to chromatin-binding protein conserved region)
24	K (Lysine)	
25	H (Histidine)	
26	I (Isoleucine)	
27	M (Methionine)	
28	E (Glutamic Acid)	
29	K (Lysine)	
30	I (Isoleucine)	
31	Q (Glutamine)	
32	G (Glycine)	
33	R Arginine	Cell adhesion Arg-Gly-Asp motif for attachment to extracellular matrix and lunacin internalization
34	G (Glycine)	
35	D (Aspartate)	
36	D (Aspartate)	Histone protein (positively charged) binding aspartate tail (negatively charged) for acetylation inhibition
37	D (Aspartate)	
38	D (Aspartate)	
39	D (Aspartate)	
40	D (Aspartate)	
41	D (Aspartate)	
42	D (Aspartate)	
43	D (Aspartate)	

➢ *Lunasin as Mitosis Inhibitor*

Lunasin has been found to suppress the cell proliferation by obstructing the cell cycle during S- phase and down-regulating the mRNA levels of cell signaling genes. Transfection of the lunasin gene segment into the mammalian cells viz. murine hepatome, breast cancer cells (BRC) and murine fibroblasts and its constitutive expression reported to hinder the mitosis as well as apoptosis (Galvez & de Lumen, 1999). Lunasin mediated mitosis disruption in mammalian cells involves combining of the negatively charged (anionic) lunasin peptide to the highly positively charged (basic) histones in the nucleosome structures of the condensed chromosomes, more specifically to the hypoacetylated chromatin material in the telomeres as well as centromeres. The resulting displacement of the kinetochore proteins that normally connect to the centromere then leads to the collapse of the spindle fibre.

➢ *Lunasin as Breast Cancer Inhibitor*

Lunasin's role defensive as chemopreventive deterrent against breast cancer has been revealed using mouse models. Hsieh et al., (2010a) reported the lunasin induced reduction in the incidences of tumor formation in a xenograft mouse by using MDA-MB-231 cell line (human breast cancer cells). In another study using DMBA-induced SENCAR mice, Hsieh et al., (2010b) reported inhibitory effect of lunasin rich diet on the tumor development in the mammary glands compared with the control group. Also, lunasin/aspirin in combination finds to obstruct the foci formation as well as cell proliferation in the chemical carcinogens (DMBA and MCA) induced-NIH/3T3 cells (Hsieh et al., 2011b). Dia & De Mejia (2011), demonstrated the lunasin induced cytotoxicity in the highly metastatic KM12L4 human colon cancer cell lines.

➢ *Lunasin as Nutraceutical*

In recent decades, food proteins demonstrating disease preventive functional activities like cancer initiation, progression, are included in the nutraceuticals category (de Mejia & Dia, 2010); Now a days, the use of these nutraceuticals, due to the advantages like low toxicity, high affinity and specificity for targets, are described as a promising anticancer strategical alternative to chemotherapy in cancer treatment (Bhutia & Maiti, 2008).

➢ *Lunasin in Commercial Perspectives*

Seber et al. (2012), developed one commercial method to isolate large-scale highly purified lunasin protein from the defatted soy flour employing certain biochemical techniques of separation viz. ultrafiltration anion-exchange and reversed-phase liquid chromatography (RP-LC) achieving 99% purity and yielding 442 mg/kg of defatted soy flour; now regarded as a robust method for industrial scale purification of lunasin. The bulk availability due to this large-scale purification of lunasin from natural dietary plant source may greatly facilitate the research in developing lunasin as a potential anticancer novel nutraceutical for therapeutic use (Figure 3) and help ailing humanity.

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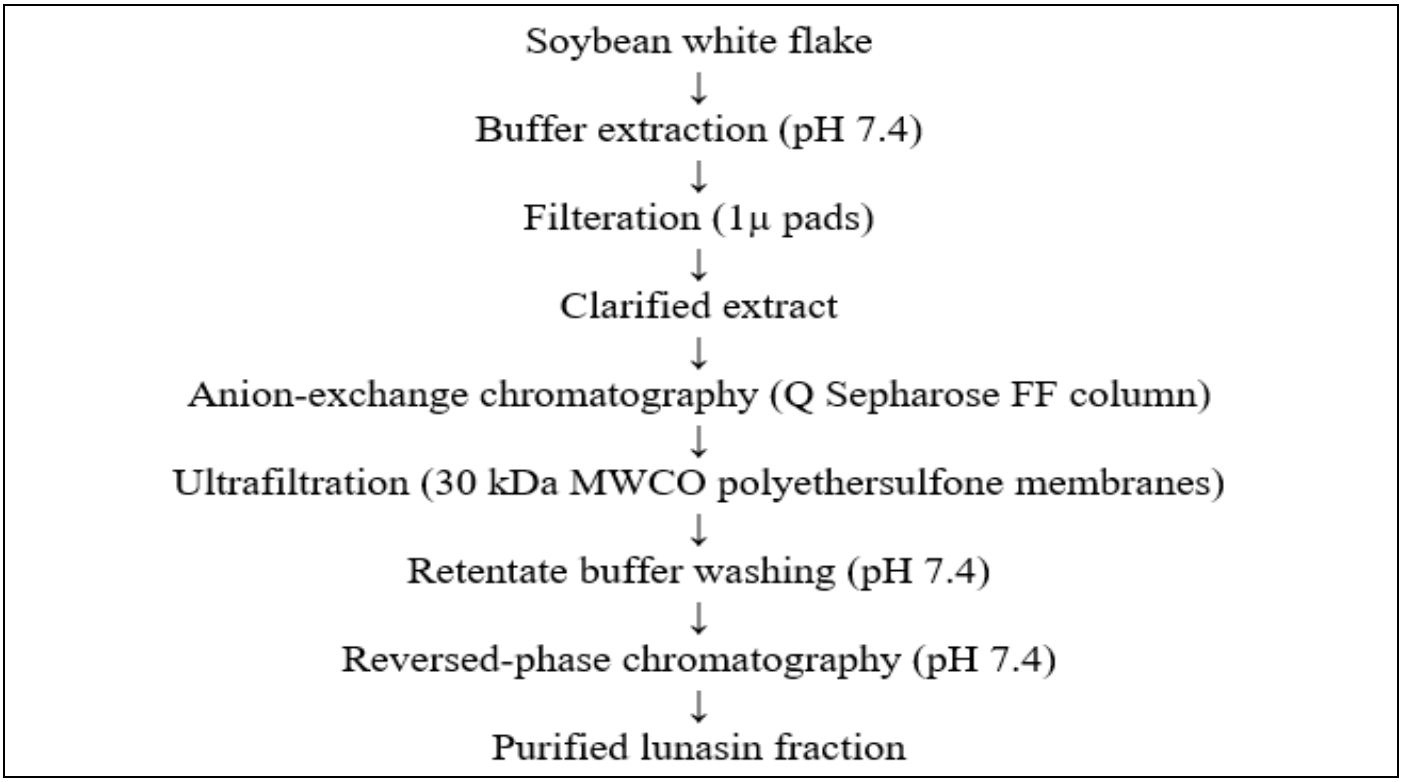

Fig 3 Schematic Extraction of Lunasin from Soyabean

VIII. CONCLUSION

As a major cause of mortality, cancer poses a great medical challenge throughout globe and to block carcinogenesis, prevention by use of natural substances is the most important strategy. Dietary components from plant sources with bioactive phytochemicals in significant amounts can be utilized to control the cancer. In the recent past, various proteins as well as peptides have been described in the nutraceutical category and the most recent lunasin peptide identified in soybean with potential to control the progression of cancer is added as new generation natural chemo-preventive agent. Now, the crucial challenge for the utilization of lunasin in the cancer treatment is its drug design and optimization against various forms of cancer.

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