IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 7 Issue 1, January 2020

ISSN (Online) 2348 – 7968 | Impact Factor (2019) – 6.248

www.ijiset.com

Bioactive Peptides: Its Production and Potential Role on Health

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Abstract

Organic substances containing short sequence of amino acids are gaining importance for their potential health benefits. They are obtained from several sources including plants and animal origin. While some of the peptides are synthesized by the organisms freely, many others are encrypted within proteins and require enzymatic processing for its release. They are generally obtained by enzymatic hydrolysis of their source proteins and through microbial fermentation process. Many other peptides are being chemically synthesized with the required amino acid sequences as well. Several studies have proved the importance of bioactive peptides on human health because of its potential role on many diseases including diabetes, inflammation, cardiovascular disorders, gastrointestinal ailments, nervous disorders and many others. This review article provides a comprehensive description of the various methods of obtaining such bioactive peptides and their major pharmacological potential.

Key words: Bioactive peptides, enzyme hydrolysis, encrypted proteins, milk proteins

1. Introduction

The human body is susceptible to various types of infections that impair normal life with different mechanisms to weaken the immune system. These infections spread due to the release of toxins in the body, which in turn cause different physiological aberrations. The neutralisation of these toxins is carried out by physiological homeostasis. But in case of acute and chronic conditions, supplementing different health-supporting agents can aid the restoration of immune system **[1]**. The recent advancements in food science reveal that functional foods and nutraceuticals can act as effective replacements for conventional medications for physiological aberrations **[2, 3]**. This property is attributed to reduced side effects and ease of availability. This has created an enormous focus of the food industry on food-derived products which can help in the balanced maintenance of general health.

This review is focussed on the protein-derived products, which can effectively reach target to perform certain physiological functions needed. Proteins contain different sequences known as peptides having the amino acid sequences which are basically responsible for the specific physiological action. These amino acid chains with specific positive impacts on health are known as bioactive peptides **[4]**, which are generally obtained by the proteolytic cleavage of a larger protein molecule. These peptides are generated by microbial fermentation or during food processing and hydrolysis of complex proteins followed by post-hydrolysis processing. They are different from naturally occurring peptides in terms their screening, purification and further processing. The specific composition of amino acids after release from their encrypted protein determines the activity of bioactive peptides. They are known for their profound physiological functionalities like anti-microbial, anti-diabetic, anti-hypersensitive, hypocholesterolemic, immunomodulatory etc. **[5]**.

Bioactive peptides can be obtained from a variety of sources including microbes, plants and animals, as they play a major role in metabolism. Despite the main source being food products, some of them are derived from plants **[6]** and animals **[7]**. This study focusses on the bioactive peptides that are derived from milk and other dairy products considering the fact that milk remains the reservoir of the greatest number of nutrients. We also focus on the production and purification methods and different physiological functionalities of bioactive peptides.

2.1. A Brief History of Bioactive Peptides

The story rolls back to the year 1902 when the substance named secretin was discovered. Found in the intestinal lining, this substance was reported to stimulate the secretion of pancreatic digestive enzymes **[8]**. Further, several peptides were reported using similar purification and sequencing mechanisms. This led to the birth of a novel research concept. Phosphorylated casein peptides were discovered in rachitic infants with vitamin D deficiency in the year 1950. This is regarded as the first discovery of bioactive peptides from a food source **[9]**. Significant amount of research has been carried out on the isolation of bioactive peptides from various sources.

2.2. Different Sources of Bioactive Peptides

2.2.1. Food Sources: Bioactive peptides are encrypted predominantly in complex food proteins **[10]**. To date, bovine milk **[11]**, cheese **[12]** and other dairy products **[13]** are regarded as the chief sources of bioactive peptides. Further they can also be extracted from protein complexes present in animal sources like bovine blood, gelatin, meat, eggs, and several fishes like tuna, herring, salmon and sardines. These peptides can also be obtained by some crop plants like maize, soy, wheat, rice, pumpkin, mushrooms, sorghum and amaranth. During food processing, bioactive peptides are released by the microbial enzymes. They are also found abundantly in fermented foods **[4]**.

2.2.2. Animal Sources: Bioactive peptides from animal origin show different health benefits **[14]**. Blood acts as one of the chief reservoirs of proteins. Serum albumin is predominantly present in the blood along with other proteins. Enzymatic hydrolysis of serum albumin results in regulation of glucose activity (DPP-IV inhibition), anti-oxidation and reducing the blood pressure (ACE inhibition). Apart from blood, meat also possesses some bioactive peptides. These peptides were also reported to show anti-hypertensive effects. The hydrolysates obtained from chicken breast, chicken leg bones and beef showed significant ACE inhibitory activity. In addition to this, a study reported antioxidant activity of beef hydrolysates against lipid oxidation **[15]**.

2.2.3. Plant Sources: A variety of plants and plant products produce bioactive peptides with an array of physiological benefits **[16]**. Both soybean seeds and soy milk are reported to possess the bioactive peptides with anti-microbial activity. Further, soy-fermented foods like natto also consist of bioactive peptides with ACE inhibitory activity **[17]**. Cereal grains including barley, millet, sorghum, corn, rice and wheat are reported to possess greatest number of bioactive peptides, in which barley and wheat showed profound ACE inhibitory activity. It is known that whole grains show significant effect against non-communicable diseases like cardiovascular diseases, diabetes and cancer **[16]**. In addition to this, cyclic dipeptides known as 2, 5-Diketopiperazines (DKP's) formed from N-terminal amino acid residues of a protein chain are reported to exhibit physiological activity similar to bioactive peptides. These DKP's can be found in various foods including cocoa, roasted malt, beer and aged sake **[18]**.

2.3. Milk as a source of Bioactive Peptides

Milk is regarded as an important source of energy and possesses an array of essential biomolecules including lactose, lipids, vitamins, minerals and proteins, the source of bioactive peptides. It is generally known as a 'complete food' due the availability of variety of the nutrients **[19]**. Despite claiming the adverse cardiovascular effects of milk fats **[20]**, milk remains the undoubted rich source of nutritional benefits. This study focusses on milk and other dairy sources as important sources of bioactive peptides.

Being the chief sources of bioactive peptides, milk proteins and their fractions are considered as potential components to produce health-promoting functional foods **[4, 5]**. Apart from the nutritional activity, immunoglobulins present in milk play a significant role in maintenance of immune health. These milk proteins yield variety of bioactive peptides upon enzymatic hydrolysis or food processing. Apart from this, fermentation of milk proteins using lactic acid bacterial also yields considerable number of bioactive peptides **[21].**

Peptides screened from cow milk exhibit opioid, ACE inhibitory, immunomodulatory, mineral-binding, anti-cancerous, ant-bacterial, anti-thrombotic and cytotoxic activities. In addition to cow milk, using different methods, the isolation and characterization of bioactive peptides from various milk sources including camel, buffalo, goat, sheep and yak are also reported.

Consumption of fermented milk with rich bioactive peptides resulted in the lowering of blood pressure. This showed the anti-hypertensive activity of the peptides. The opioid peptides released as a result of enzymatic hydrolysis showed similar pharmacological properties to that of morphine on CNS. Lactoferrin, an iron binding glycoprotein is associated with antimicrobial and immunomodulating effects. Lactoferrin and its derivatives are also reported to affect inflammatory and immune processes by affecting the cytokine production in the body. Peptides obtained from casein were reported to possess ACE inhibitory, opioid and anti-microbial activity. In a similar study done using donkey milk, peptides obtained showed antioxidant activity along with ACE inhibition **[21]**.

These activities prove that milk is a reservoir of greatest number of bioactive peptides with an array of positive effects on physiological health. Milk also poses as economical, abundant and healthiest source of bioactive peptides, compared to other sources like animals and plants. Along with the milk, dairy products which are originated from milk also possess the bioactive peptides. Figure 1 depicts the different physiological benefits shown by the bioactive peptides obtained from milk.

2.4. Dairy Products as Sources of Bioactive Peptides

Apart from milk, other dairy products also possess considerable number of bioactive peptides encrypted within their proteins. Though they are derived from milk, proteins from other sources exhibit characteristic physiological functions. For example, colostrum is enriched with immunoglobulins that play an important role in the growth and development of an organism. In addition to this, it also contains high concentration of antimicrobial proteins.

Bioactive peptides can be obtained using digestive enzymes during the fermentation of milk. Dairy products like milk protein hydrolysates, fermented milk and cheese varieties also acts as chief sources of bioactive peptides. Fermented sour-milk products are reported to possess antihypertensive activities. In these beverages, the *lactobacillus* species were added to bring out fermentation, and the resultant peptides thus obtained showed anti-hypertensive activity.

Further, physiological functionalities highlighted by screened peptides from fermented milks, yoghurt and a variety of cheeses were reported in many studies. The beneficial effects exerted by these peptides include regulation of nervous system, gastrointestinal system and cardiovascular system **[22]**. **Figure 1** depicts the different sources of bioactive peptides.

2.5. Production of Bioactive Peptides

The most common methods that are employed to carry out bioactive peptide production are enzyme hydrolysis of food proteins and microbial fermentation. These methods result in the release of variety of bioactive peptides from where they are screened and purified based on the requirement.

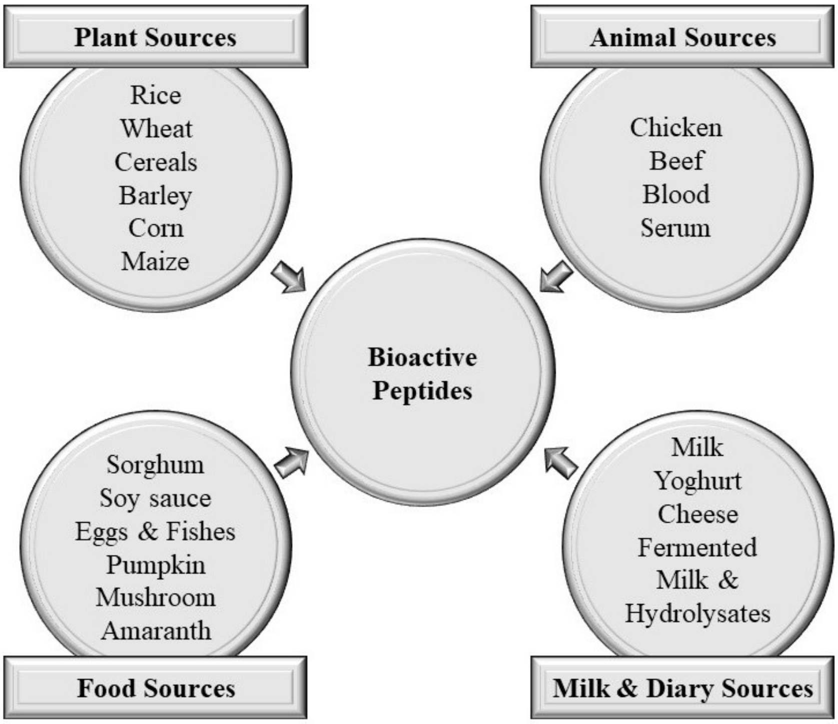

Figure 1: Different Sources of Bioactive Peptides

2.5.1. Enzymatic Hydrolysis: In this method, the complex proteins are subjected to hydrolysis with a set of enzymes, at optimal temperature and pH **[23]**. Enzymatic hydrolysis is preferred over microbial fermentation due to its short reaction time, ease of predictability and scalability. A set of crude or purified enzymes are added either simultaneously or sequentially based on the optimal temperature and pH of the enzymes. These proteolytic enzymes release bioactive peptides of specific molecular weight. However, sustained hydrolysis would result in the production of low molecular weight peptides **[24]**. To obtain an optimum level of hydrolysis, it is important to maintain a balanced enzyme to substrate ratio. Thus, the peptide sequences and their biological functionalities depend on the type, concentration, time of hydrolysis and the ratio of the enzyme used. It does not mean that bioactive peptides with low molecular weight exhibit reduced biological functions. In some studies, low molecular weight peptides (<10 kDa) are reported to have more antioxidant and antihypertensive activities than that of high molecular weight peptides. Thus, it would be noted that low molecular peptides would be helpful in case of commercial production of antihypertensive and antioxidant peptides.

During the production of bioactive peptides, it is advised to separate some non-protein bioactive compounds. For example, phenolic compounds which are known for their antimicrobial, antioxidant, antihypertensive and antidiabetic activities can interfere with biological functions when they are present in hydrolysates. Therefore, it becomes necessary to remove such components during the production of bioactive peptides. These components are usually removed by acetone extraction, pressurized water extraction, ethanol extraction, supercritical carbon dioxide, and ultrasound-assisted extraction before enzymatic hydrolysis.

Proteolysis usually results in the generation of protons, which fluctuates the pH of the medium and may affect the hydrolytic process. Though addition of acid or alkali can adjust the pH, addition of alkali may result in high salt concentration in hydrolysates. Thus, it is advised perform the proteolysis using a buffer. In addition to this, type of enzyme, temperature and duration of hydrolysis may also affect the generation of peptides. After the enzymatic digestion, the mixture is centrifuged to remove the low molecular weight peptides. These low molecular peptides are usually present in supernatant. The peptides are recovered by desalting, freeze-drying, membrane ultrafiltration, crossflow membrane filtration or column chromatography. Low molecular weight peptides can be effectively separated based on their size using gel filtration.

2.5.2. Microbial Fermentation: This method involves the culturing of microbes (bacteria or yeast) on media enriched with protein substrates. The secretion of proteolytic enzymes by the microbes results in the breakdown of proteins to release bioactive peptides. The exponential growth of desired bacterial species in a medium result in the profound growth of cells. These cells are harvested, washed and suspended in sterile distilled water. Later these are used as starters to inoculate the medium rich in protein substrate. The extent of hydrolysis depends on the strains of microbes used, time of fermentation and concentration of substrate so does the number of peptides generated. For this process, *lactobacillus* species are predominantly used. From this, it becomes clear that, functionality of protein hydrolysates may differ between cultures due to the presence of different proteolytic systems in microorganisms. In addition to this, bacteria of same species also differ in their mechanisms of proteolysis, thus generating peptides with different bioactivities.

Apart from bacteria, yeast and filamentous fungus are also reported to be used in the generation of bioactive peptides. The fermentation can be done even with the combination of yeast and bacteria enhance the proteolysis. The recovered supernatant after the centrifugation usually comprises of peptides, which may be hydrolysed further to get shorter peptide sequences. Further, the low molecular weight peptides in the supernatant can be recovered by different extraction methods listed above. Thus, the peptides are purified, and their amino acid sequencing can be done using mass spectroscopy **[25]**. The production of peptides using different methods has been depicted in **Figure 2.**

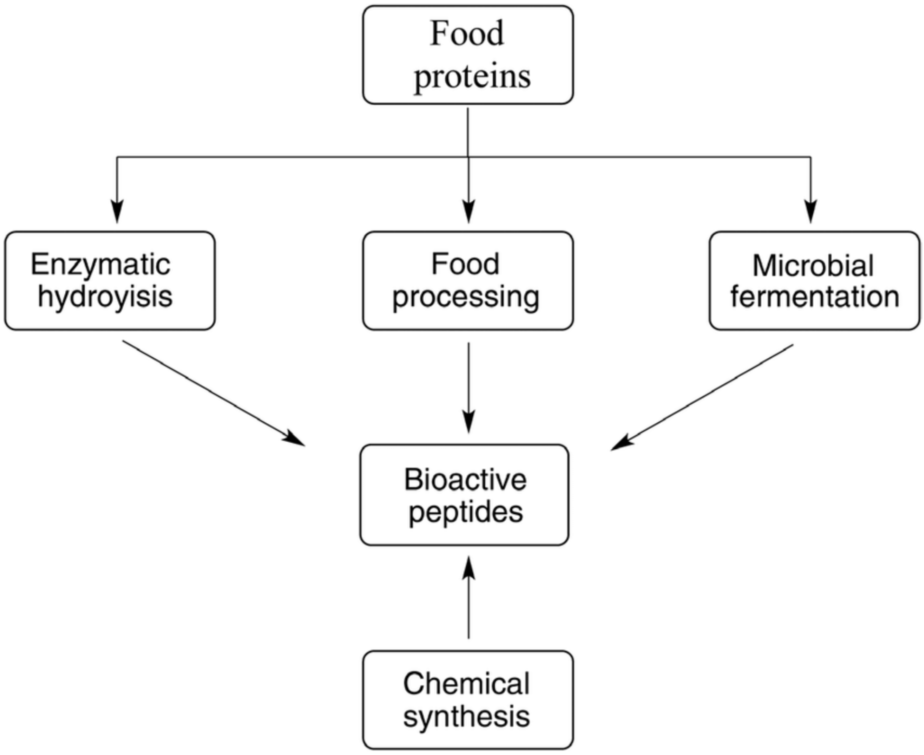

Figure 2: The schematic representation of Bioactive Peptide Production **[4]**

2.5.3. Chemical Synthesis: organic synthesis is a crucial tool to get organic molecules exhibiting particular physicochemical properties. Application of *in silico* protocols, conventional experimental approaches, in solution/solid phase, consent the design & construction of molecules with diverse molecular complications that can be employed for biological studies. The beneficial roles exhibited by bioactive peptides drew the curiosity to acquire them by chemical synthesis to treat most pathological conditions interrelated to oxidation. Amongst antioxidant tripeptide library, the tripeptides *viz.*, Tyr-His-Tyr & Pro-His-His is principally active in stabilizing non-radical oxygen species and radical, as well as lipid peroxide and peroxonitrite. In recent times, there has been a growing interest in de novo design and construction of novel synthetic peptides that mimic protein secondary structures to develop potent peptide analogues and peptidomimetics exhibiting unique pharmaceutical activities. Bioactive peptides are considered the new generation of biologically active regulators not only to avert oxidation and microbial degradation of foods but also to enhance the treatment of diverse disorders and diseases.

2.6. Methods of Purification

The enzymatic hydrolysis and microbial fermentation processes results in the release of an array of bioactive peptides. The protein hydrolysates contain hundreds of bioactive peptides with variable bioactivity. Before subjecting them for commercial production, they need to be purified and their exact nature should to be determined. Thus, purification plays an important role in the development of a completely functional bioactive peptide. Based on the literature, the most common methods that are followed in the purification are membrane-based separation and extraction processes.

2.6.1. Membrane Based Separation: Membrane filtration involves ultrafiltration and nano-filtration **[26]**. The separation of bioactive peptides depends on the pore size, concentration and the time of filtration. The usage of electrical field as a driving force was performed as an addition to the conventional filtration process. This resulted in the selective separation of charged target compounds. However, the selectivity and purity were limited by application of pressure and membrane fouling. To overcome these shortcomings, a new technique called electro dialysis with filtration membrane (EDFM) was developed. This technique efficiently carried out the separation of bioactive peptides with physiological functionalities including anti-hypertensive, anti-cancer, anti-diabetic and anti-microbial activities **[26]**. EDFM requires no pressure applied on the ED cell and it can also simultaneously carry out the hydrolysis and fractionation of generated bioactive peptides using different molecular weight cut-offs.

2.6.2. Extraction process: This type of extraction process is employed based on hydrophobic property of peptides. Studies have reported the selective separation of opioid or antimicrobial peptides through ion-pairing-assisted extraction in aqueous/organic solvent biphasic systems. In addition to this, antioxidant, ACE inhibitory, antihypertensive activities are also reported **[27]**.

2.6.3. Foaming-Draining Processes: Selective separations of bioactive peptides are rarely reported. This method works on surfactant properties of peptides. The first study reported the anti-microbial activity of peptides present in hydrolysate. Along with this, they also considered hydrophilicity, hydrophobicity and molecular size of the peptides. This process also highlighted the influence of parameters to improve the enrichment of the bioactive peptide fractions **[28]**.

2.7. Biological potential

The biological properties of the peptides are determined by their respective amino acid sequence and its length, properties such as charge and hydrophobic or hydrophilic character and the terminal amino acids. Yet, the exact structure-function relationship cannot be determined with the present knowledge **[29]**. In order to be assigned as a bioactive peptide, it should be able to show measurable biological property under physiological conditions, yet remain nontoxic to the organism **[30]**.

Studies have shown that milk peptides possess antioxidant properties. Digestion of casein for instance produces potential peptides that have the ability to exert antioxidant activity through metal ion chelation as well as neutralizing the reactive oxygen species **[31]**. In addition, fish industry is produces a large chunk of waste products which are now being utilized for the production of enzymes. These extracts of protein hydrolysates contain bioactive peptides with known antioxidant properties. Further, studies on the bioactive peptides of Spanish dry-cured ham have demonstrated potential DPPH and superoxide radical scavenging activities up to 92% and 50%, respectively. In addition, in this study remarkable antihypertensive properties were also observed **[32]**. Leu-His-Tyr, Leu-Ala-Arg-Leu, Gly-Gly-Glu, Gly-Ala-His, Gly-Ala-Trp-Ala, Pro-His-Tyr-Leu and Gly-Ala-Leu-Ala-Ala-His were obtained from sardinelle (*Sardinella aurita*), which constitutes a major industrial waste-product obtained from sardine (*Sardina pichardus*) also shows remarkable antioxidant properties **[33]**. Mainly, proteins from soy, egg yolk, casein and potato exhibit proven antioxidant properties **[34].** Enumerable such pepties have been identified with potential antioxidant properties that are associated with many other beneficiary properties.

Antimicrobial activity is another important property shown by bioactive peptides. Antimicrobial peptides (APMs) are generally produced even by tissues and cells of several plants, animals and invertebrates. Mostly below 10 kDa, these peptides are produced from native protein precursors by the enzymatic hydrolysis and id growing as a promising alternative to the presently available therapeutic agents **[4]**. AMPs are classified into helical linear peptides, disulphide bridged cyclic and open chain peptides and peptides with high proline/glycine/histidine residues. These peptides are generally amphipathic having cationic and hydrophobic properties **[35]**. Remarkable antimicrobial properties have been observed in peptides obtained from the skin of *Xenopus laevis* (XLAsp-P1-peptide) against Gram-negative bacteria as well as anti-breast cancer potential. Further, in a study by Przybylski et al., 2016 **[36]**, a 137-141 region of the haemoglobin (Thr-Ser-Lys-Tyr-Arg) demonstrated antimicrobial properties on par with that of BHT.

Peptides have also been identified as immunomodulators via the generation of ROS. Short peptides obtained from the brain-gut region, posterior lobe peptides, growth factors and opiods released from the anterior pituitary are some of the known immunomodulators. Several marine organisms have been reported to release immunomodulatory peptides important in the treatment of some diseases. Ova transferrin is one such immunomodulatory peptide produced from the egg white protein which has demonstrated effects on granulocytes and macrophages **[4].**

Addition to these, several peptides have been obtained from milk, egg, soy and other plant sources with potential anti-inflammatory and antihypertensive properties. Aguilar-Toala et al., 2017 **[37]** demonstrated 55 crude peptide extracts from *L. plantarum* with anti-inflammatory properties better than the standard diclofenac sodium. Rat seminal vesicle protein SV-IV shows potential anti-inflammatory properties. In a study to obtain shortest peptide with anti-inflammatory properties, using Fmoc chemistry several SV-IV peptides were synthesized **[38]**. In addition, several antihypertensive peptides are being produced either by direct synthesis or protein hydrolysis and they are collectively known as ACE inhibitors **[39]**. The main source of these peptides is fish, milk and corn. Several tripeptides are known to possess this activity. Two such tripeptides, namely, Ile-Pro-Pro and Val-Pro-Pro obtained from sour milk fermented with Saccharomyces cerevisiae and L. Helveticus are known to have ACE inhibitor properties along with BP modulating activity **[40]**. The commercially available Food for Specified Health Uses (FOSHU) products containing small peptides: Katsuobushi oligopeptide, sour milk, tryptic hydrolysate of casein, the aqueous extract from *Mycoleptodonoide saitchisonii*, seaweed peptides, sardine peptide and sesame peptides inhibit the ACE enzyme.

Advancement in the research on bioactive peptides has reported many more such potential biological and pharmacological properties. These benefits uphold the need to identify more such peptides from various sources for their potential roles.

3. Conclusion

The bioactive peptides can be obtained from different sources of nature. These peptides exert positive effects on physiological health of human. For this to happen, these peptides should be synthesised to carry out their function specifically. This study focusses on the various sources and the generation of bioactive peptides. In addition to this, it highlights the need of purification of peptides and covers the purification techniques that allow the separation of specific peptides with characteristic properties. During the literature study it became evident that, bioactive peptides can be modified into drugs. We suggest synthesis of lead peptides with enhanced specific action and bioavailability to exert positive effects on human physiological functionalities.

Acknowledgement

All the authors are thankful to His Holiness Jagadguru Sri Shivarathri Deshikendra Mahaswamiji (the 24th pontiff of Sri Suttur Math) and obliged to JSS AHER & JSS Mahavidyapeetha, for giving the opportunity to carry out the present work.

Conflict of interest

The authors declare that there is no conflict of interest.

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