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Pharmaceutical Sciences **Review Article……!!!**

‘‘A REVIEW ON FORMULATION OF MULTIENZYME COMPLEX”

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ABSTRACT

Digestive enzymes are the essential part of any chemical reactions that take place in our body. Thus, various biological reactions, such as digesting food, activities of the brain, cellular energy, and repair process of tissues, etc. are mainly done by various enzymes and related compounds called co-enzymes. The gastrointestinal tract begins with the mouth, and digestion starts with chewing, which breaks up large pieces of food into smaller particles that can be swallowed. The saliva contains the digestive enzyme amylase, which breaks complex sugars, into smaller sugar units. The food is then moved to the stomach where it is subjected to enzymatic action enteric digestive enzymes viz., protein-digesting enzyme (pepsin), and lipid digesting enzyme (gastric lipase). The final stage of digestion and most of the absorption occurs in the small intestine. Here, partially digested carbohydrates, fats, and proteins are broken down by respective digestive enzymes into monosaccharides, fatty acids, and amino acids, respectively. Improper digestion, caused by lack or inefficiency of one or more digestive enzymes, leads to various digestive disorders viz., Insufficient/inefficient lactase leads to lactose intolerance, undigested nutrients leads celiac disease, and studies have shown that colon health is dictated by the proper digestive process. Also, improper digestion in the elderly population leads to flatulence, bloating, and diarrhea. For example, dairy protein-based products contain milk protein and lactose (milk sugar) which is hard for some to digest and may cause bloating. To overcome from this problem, proper functioning of the digestive enzymes should be takes place.

INTRODUCTION:

Enzyme, a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process. The biological processes that occur within all living organisms are chemical reactions, and most are regulated by enzymes. Without enzymes, many of these reactions would not take place at a perceptible rate. Enzymes catalyse all aspects of cell metabolism. This includes the digestion of food, in which large nutrient molecules (such as proteins, carbohydrates, and fats are broken down into smaller molecules; the conservation and transformation of chemical energy and the construction of cellular macromolecules from smaller precursors. Many inherited human diseases, such as albinism and phenylketonuria, result from a deficiency of a particular enzyme.

In general, state of the digestive health determines overall wellness of an individual, because taking control of this intricate system may help improve one's health, wellbeing, and happiness. One may experience a lot of health problems, such as poor nutrient absorption, weight issues, nausea, bloating, constipation, stomach pain, and diarrhea to severe conditions like acid reflux, irritable bowel syndrome, if digestive system is not functioning the way it is supposed to be. Several factors are known to influence digestive health, viz. poor lifestyle and eating habits, stress, age, genetics, food additives and chemicals, and physical inactivity. In today's fast-paced world, changing lifestyle and diet preferences are leading to a number of discomforts with the digestive system is the most affected, causing disturbed activity of digestive enzymes or enzyme insufficiency.[1]

Enzymes can be described as simple proteins found within each living cell, where they act as 'biological catalysts' to regulate various biological reactions, including digestion. Several digestive enzymes are involved in breaking down protein, carbohydrates, and fats and other complex molecules present in food. Through this action, they facilitate the digestive process and thus help better nutrient absorption by the body.[2]

- Amylase breaks down starches and carbohydrates into sugars
- Protease breaks down proteins into amino acids.
- Lipase breaks down lipids, which are fats and oils, into glycerol and fatty acids.

However, in several individuals, due to aging or otherwise, body's natural ability to produce certain pancreatic enzymes like lipase, amylase as well as other extra pancreatic enzymes that play a crucial role in macronutrient digestion starts declining as they age. Hence, lack of optimal digestive function associated with enzyme inadequacy may lead to malabsorption and a host of related health issues, if enzyme insufficiency is left unaddressed. Fortunately, one may replenish enzyme losses/deficiencies via enzyme supplementation, which is critical in today's world.[3]

ENZYME SUPPLEMENTS

Enzyme supplements are digestive enzyme preparations derived from animal, plant, microbial, and others. Pancreatin enzymes are common in the market as digestive enzymes supplements which are bovine or porcine origin. Hence the demand for the plant-based digestive enzymes are increased in the market. These digestive enzymes are of bacterial or fungal origin and are produced through fermentation method. Nowadays, they are being used as a major source of enzymes for supplements, thus representing about 90% of all enzymes produced commercially. Some of the best-known sources of microbial enzymes used in supplements include species like *Aspergillus, Rhizopus* (fungal), *Bacillus* (bacterial), and *Saccharomyces* (yeast).[4]

ENZYMES	SOURCES
Alpha amylase	*Aspergillus oryzae*
Neutral Protease	*Bacillus subtilis*
Lactase	*Aspergillus oryzae*
Lipase	*Rhizopus oryzae*
Cellulase	*Trichoderma longibrachiatum*

The individual enzymes are Non-GMO (not derived from genetically modified organisms), suitable for Vegans, gluten-free, and TSE (Transmissible Spongiform Encephalopathy) and BSE (Bovine Spongiform Encephalopathy) free. The table shows the component enzymes of this blend and their respective sources. The protease and lactase enzymes in this blend not only help to digest milk proteins but also lactose, thereby help control bloating caused by lactose

indigestion. Plant based proteins (soya, pea, almond, rice, potato proteins etc.) contain protein and phytonutrients, antioxidants, vitamins, minerals, and fibre.This blend can digest the plant components more efficiently since it contains cellulase and protease, the digestive enzymes that can digest cellulosic material as well as plant proteins, respectively. So, preparation is an ideal multi-enzyme blend for both dairy and plant protein-based products.[5]

UNIQUE FEATURES:

- Vegan: Derived from non-animal sources
- Each digestive enzyme in the blend is food-grade, Non-GMO and gluten-free
- Adheres to the most stringent international standards and regulatory norms
- Resistant to the action of gastric juices, while retaining optimal activity
- Safe, traceable origins, validated quality & science-backed efficacy

Understanding of enzyme supplementation's benefits has gone beyond digestive aid with recent research into new and different areas drawing more attention to the category. In addition to playing a role in managing a range of conditions related to gastrointestinal tract, research indicates **digestive enzymes** may be helpful to immune function, joint and mood support, and weight loss. This is an opportunity for the category to expand further and reach a broader audience.[6]

Digestive juices and enzyme	Substance digested	Product formed
Saliva Amylase	Starch	Maltose
Gastric Juice Protease (Pepsin) and HCl	Proteins	Partly digested Proteins
Pancreatic Juice Protease (Trypsin) Lipase Amylase	Proteins Fat emulsified by bile Starch	Peptides and amino acids Fatty acids and glycerol Maltose
Intestinal enzymes **Peptidases** **Sucrase** **Lactase** **Maltase**	Peptides Sucrose (sugar) Lactose (milk sugar) Maltose	Amino acids Glucose and fructose Glucose and galactose Glucose
Bile from the liver Bile salts	Fats globules	Fat droplets

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Several factors are known to influence digestive health, viz. poor lifestyle and eating habits, stress, age, genetics, food additives and chemicals, and physical inactivity. In today's fast-paced world, changing lifestyle and diet preferences are leading to a number of discomforts with the digestive system is the most affected, causing disturbed activity of digestive enzymes or enzyme insufficiency.[6]

Pancreatic enzymes, also known as pancreases or pancrelipase and pancreatin, are commercial mixtures of amylase, lipase, and protease. They are used to treat malabsorption syndrome due to certain pancreatic problems. These pancreatic problems may be due to cystic fibrosis, surgical removal of the pancreas, long term pancreatitis, pancreatic cancer, or MODY 5, among others. The preparation is taken by mouth. Common side effects include vomiting, abdominal pain, constipation, and diarrhoea. Other side effects include perianal irritation and high blood uric acid. The enzymes are from pigs. Use is believed to be safe during pregnancy. The components are digestive enzymes similar to those normally produced by the human pancreas. They help the person digest fats, starches, and proteins.

Pancreatic enzymes have been used as medications since at least the 1800s. They are on the World Health Organization's List of Essential Medicines. In 2020, it was the 262nd most commonly prescribed medication in the United States, with more than 1 million prescriptions.[7]

MULTI-ENZYME COMPLEX

This proprietary preparation is a unique blend of specific digestive enzymes that offers much more than just aiding proper digestion and better absorption of nutrients.

α-Amylase - Starch hydrolyzing enzyme

Protease - Protein hydrolyzing enzyme

Lipase - Fat hydrolyzing enzyme

Cellulase - Cellulose hydrolyzing enzyme

Lactase - Lactose hydrolyzing enzyme

This off-white to creamy-white powder consists of following digestive enzymes[8]

This Formulation has been focusing on the importance of rebalancing, maintaining adequate levels and the optimal activity of digestive enzymes not only in easing the gastrointestinal symptoms, but also to support digestive system associated disease management.[9]

AMYLASE

Amylase, any member of a class of enzymes that catalyze the hydrolysis (splitting of a compound by addition of a water molecule) of starch into smaller carbohydrate molecules such

as maltose (a molecule composed of two glucose molecules). Three categories of amylases, denoted alpha, beta, and gamma, differ in the way they attack the bonds of the starch molecules. Amylase is widespread among living organisms. In the digestive systems of humans and many other mammals, an alpha-amylase called ptyalin is produced by the salivary glands, whereas pancreatic amylase is secreted by the pancreas into the small intestine. The optimum pH of alpha-amylase is 6.7–7.0. Ptyalin is mixed with food in the mouth, where it acts upon starches. Although the food remains in the mouth for only a short time, the action of ptyalin continues for up to several hours in the stomach until the food is mixed with the stomach secretions, the high acidity of which inactivates ptyalin. Ptyalin's digestive action depends upon how much acid is in the stomach, how rapidly the stomach contents empty, and how thoroughly the food has mixed with the acid. Under optimal conditions as much as 30 to 40 percent of ingested starches can be broken down to maltose by ptyalin during digestion in the stomach.[10]

When food passes to the small intestine, the remainder of the starch molecules are catalysed mainly to maltose by pancreatic amylase. This step in starch digestion occurs in the first section of the small intestine (the duodenum), the region into which the pancreatic juices empty. The by-products of amylase hydrolysis are ultimately broken down by other enzymes into molecules of glucose, which are rapidly absorbed through the intestinal wall.[11]

PROTEASE

Protease is an enzyme that catalyses the hydrolysis of peptide bonds present in proteins**.** In most of the living organisms, protease enzymes are essential for digestion and absorption of proteins. Proteases or proteolytic enzymes are found in all living organisms, e.g. bacteria, algae, plants and animals and in some of the viruses too. They are involved in the catabolism and digestion of proteins There are many different types of proteases, that take part in various biochemical processes. On the basis of the site of the peptide bond cleavage, proteolytic enzymes are divided into two broad groups:

Exopeptidase: They catalyse the cleavage on terminal peptide bond, e.g. aminopeptidases, carboxypeptidases, etc.

Endopeptidase: They facilitate the cleavage of internal peptide bonds of proteins, e.g. pepsin, trypsin, chymotrypsin, elastase, etc.

Oligopeptidase refers to enzymes, that act on a specific peptide bond.

Different types of protease enzymes remain active in the different pH range, e.g. acid proteases, alkaline or basic proteases and neutral proteases.

Protease enzymes are essential for many biological processes. They are required for the regulation of various metabolic and cellular processes.

- They are proteolytic, they help in digestion and catabolism of proteins. They catalyse the hydrolysis of peptide bonds and convert them to amino acids, which is then absorbed and utilised by cells.
- They are required for the blood coagulation process.
- Protease enzymes are involved in the cell division, growth, apoptosis and migration.
- Protein recycling and transport across membranes.
- They are involved in the activation of precursor proteins and zymogens.
- Proteases provide immune support and regulate the process of tumour growth, metastasis, inflammation, etc.
- They may help in wound healing and muscle soreness.[12]

LIPASE

Lipases are a family of enzymes that break down triglycerides into free fatty acids and glycerol. There are expressed and active in multiple tissues; for example, hepatic lipases are in the liver, hormone-sensitive lipases are in adipocytes, lipoprotein lipase is in the vascular endothelial surface, and pancreatic lipase is in the small intestine. Lipases in pancreatic secretions are responsible for digestion and hydrolysis of fat and absorption of fat-soluble vitamins. Understanding the lipase function is crucial for the pathophysiology of fat necrosis and acute and chronic pancreatitis

Lipases are present in pancreatic secretions and participate in fat digestion and metabolism. They play an essential role in lipid transport and serve individual functions in several tissues, including hepatic lipase in the liver, hormone-sensitive lipases in the adipocytes, lipoprotein lipase in the endothelial cells, and pancreatic lipase in the small intestine. Hepatic lipase in the liver degrades the triglycerides that remain in intermediate-density lipoprotein (IDL). Hormone-sensitive lipase is found within fat tissue and is responsible for hydrolysing the triglycerides stored within adipocytes. Lipoprotein lipase is found in the vascular endothelial cells and is responsible for degrading triglycerides that circulate from chylomicrons and very low-density lipoproteins (VLDLs). Pancreatic lipase is found within the small intestine and is involved in degrading dietary triglycerides.

The LDL ultimately serves to transport cholesterol from the liver to peripheral tissue. Hepatic lipase plays a crucial role in developing and delivering low-density lipoprotein (LDL). The LDL is formed by modifying the IDL in the peripheral tissue and liver by hepatic lipase. These

LDL particles are taken up, or endocytosed, via receptor-mediated endocytosis by target cell tissue. The LDL ultimately serves to transport cholesterol from the liver to peripheral tissue.[13]

CELLULASE

Cellulases break down the cellulose molecule into monosaccharides ("simple sugars") such as β-glucose, or shorter polysaccharides and oligosaccharides. Cellulose breakdown is of considerable economic importance, because it makes a major constituent of plants available for consumption and use in chemical reactions. The specific reaction involved is the hydrolysis of the 1,4-β-D-glycosidic linkages in cellulose, hemicellulose, lichenin, and cereal β-D-glucans. Because cellulose molecules bind strongly to each other, cellulolysis is relatively difficult compared to the breakdown of other polysaccharides such as starch.

Cellulases are one of the widely used industrial enzymes which are commercially available for more than 30 years. These are inducible enzymes synthesized by a various microorganism including, bacteria and fungi, during their growth on cellulosic materials. Consequently, cellulose is converted to simple sugar, glucose, which can be fermented into cellulosic biofuels.[14]

LACTASE

Lactase enzyme is normally produced by the body to help break down (digest) lactose. This product is an enzyme supplement used to help people who have trouble digesting milk and other dairy products (lactose intolerance). Lactose is a type of sugar found in milk products. However, this can result in a lack of important nutrients and vitamins such as calcium, vitamin D, riboflavin, and protein. Some people use lactose-free milk or milk substitutes such as soy or rice milk. Most people with low lactase enzyme levels can tolerate small amounts of milk products at one time (2-4 ounces) and get symptoms only with larger servings (6 ounces or more). Taking lactase enzyme with milk products as directed can help prevent upset stomach, abdominal bloating/cramping, gas, and diarrhea caused by these products. The FDA has not reviewed this product for safety or effectiveness. Consult your doctor or pharmacist for more details.[15]

ENZYME FORMULATONS

In order to adapt to various needs, and taking into account economic and application effects, enzyme preparations are often supplied in four formulations.

Liquid Enzyme Preparation

The liquid enzyme preparation includes a dilute enzyme solution and a concentrated enzyme solution, and is usually directly prepared or concentrated without removing impurities. Liquid enzyme preparations are economical, but unstable, and complex in composition, and are only suitable for direct application in certain industrial sectors such as the textile industry.[16]

Solid Enzyme Preparation

Some solid enzyme preparations are prepared by directly concentrating the fermentation broth after sterilization, and some are prepared by spray drying the fermentation broth, and some are filled with starch and the like. These preparations are mostly used for softening and depilating of leather, hydrolysis of cellulose and the like. Some of these preparations are also prepared by removing impurities from the fermentation broth, such as enzyme preparations for the production of detergents, pharmaceuticals, and the like. Formulations used to process or produce a product must remove the interfering enzymes that interfere with it. For example, polynucleotide phosphorylase(PNPase) is commonly used to synthesize various polynucleotides (Poly l, Poly c, etc.). If the nuclease or phosphatase is still contained in the enzyme preparation, the synthesis of the polynucleotide chain is disrupted and the quality of the product is affected. The solid crude enzyme preparation is convenient for transportation and short-term storage, and the cost is not high.[17]

Pure Enzyme Preparation

Pure enzyme preparations, including crystallization enzymes, are commonly used as analytical reagents or as medical drugs, requiring high purity. When a pure enzyme preparation is used as an analysis tool enzyme, in addition to the requirement that no hybrid enzyme is present, it is required that the enzyme activity per unit weight of the enzyme preparation reaches a certain amount. The tool enzyme used for genetic engineering requires no non-specific nucleases or no nucleases at all. Enzymes that are the subject of protein structural analysis must be “absolutely” pure, while medical enzymes for injection should seek to remove the heat source.[18]

The heat source is a kind of toxoid secreted by the bacteria after the bacteria is infected, and the preparation with such substances can cause the body temperature to rise after being injected. The heat source material belongs to glycoprotein, and the relative molecular mass is more than 100,000, and there are small differences due to different bacterial sources. The heat source material is quite stable to heat, and often needs to pass a long time high temperature action, for example, after 18°C-22°C, two hours or more of treatment, the heat source material will decompose. The heat source material is also very acid resistant, but it will gradually break down in the base, for example, pH>10, 48h. The heat source material is very sensitive to the oxidant and can be removed in freshly prepared lotion for one hour. In addition to the above methods, in order to solve the heat source problem, the following measures can also be taken:

- For example, an anion exchanger such as DEAE cellulose, calcium phosphate, alumina gel or the like can be used to adsorb and remove such substances, and activated carbon can also be used, but its specificity is not high.
- Affinity separation. For example, a heat source antibody has been isolated and made into an affinity adsorbent to specifically remove the heat source material, and it can also be adsorbed and removed by an affinity column made of lectin.
- The heat source material has the characteristics of an organic solvent, so the product obtained by the organic solvent purification has a higher proportion of the heat source material, and the product purified by the salting out method is less.[19]

Immobilized Enzyme Preparation

The immobilized enzyme preparation is a preparation that is advantageous for preservation and application. Immobilized enzyme is an enzyme preparation prepared by physically or chemically immobilizing an enzyme on a water-insoluble or water-soluble carrier. The earliest immobilized enzymes were reported in 1953, but it was not until the late 1960s that the development of immobilization technology was rapidly developed. In 1969, immobilized amino acid acylase was officially used in industrial production. The emergence of immobilized enzymes has opened up a broad prospect for the application of enzyme preparations in industrial and agricultural production and pharmaceutical practice.[20]

APPLICATION OF ENZYMES

There are approximately 1300 different enzymes found in human cells, which include amylase, pepsin, trypsin, pancreatic lipase, ribonuclease and deoxyribonuclease. All these enzymes are involved in the different chemical processes such as the breakdown of large starch molecules, **proteins**, fats and other nucleic acids.

Enzymes are very useful catalysts in many different industrial processes, which includes:

Food processing industries

Application of enzymes in the process of food processing is a very old practice, which has been used by our ancestors and these include a very simple process.

Enzymes are used by the food industries for processing raw materials for the production of numerous and common products such as bakery products, dairy products, meat products, fruit products, beer and wine.

Enzymes are broadly used in the production of alcoholic beverages and certain food products.

Other than the production, these enzymes are also used in improving the quality of food products, such as improving the quality of the food, taste, flavour, colour, etc. The applications of enzymes include:

1. Addition of α-Amylase and glucoamylase for improving the quality of the food.
2. Addition of peroxidase for the colour and nutritional quality of the food.
3. Addition of lipase, xylanase and glucose oxidase for the improvement of the flavour.

In the modern world through **biotechnology** enzymes used in the food industry are extracted directly from the plants and animal sources and are also obtained by microbial fermentation. [21]

Pharmaceutical industries

With the invasion of new technologies and the unique characteristics of enzymes, the different types of enzymes are used as drugs. These drugs specifically bind to the target and also have an incomparable potential and advantages compared to ordinary small molecular drugs.

Applications of some important therapeutic enzymes, which are widely used in the production of drugs for the treatment of various dreadful diseases and other **infectious diseases**, including allergies, food and cyanide poisoning, gout, heart attack, inflammation, leukaemia, viral and bacterial infections, plague formations, skin ulcers, thrombosis and a lot more.

Textile industries

Enzymes play an important role in removing impurities, providing a stonewashed effect, in ethanol production to break down the starch and cellulose into fermentable sugars.[22]

RECENT ADVANCE DEVELOPMENT IN ENZYMES

This special issue aims to contribute to recent advances in enzyme technology for the biomanufacturing of chemicals, biomaterials, biofuels, and pharmaceuticals. High-quality original research papers and review articles on the development of new approaches to improve catalytic efficiency of enzymes, biocatalytic process intensification, enzyme immobilization, biocatalytic kinetics and optimization, green chemistry and their applications in the biosynthesis of chemicals, biofuels, pilot, and large-scale developments of various bioproducts are welcome.[23]

Chemical catalysis has been widely adopted for industrial applications due to its high throughput rates and short production time. However, this process has several inherent limitations related to high energy consumption and severe environmental impacts, such as wastewater treatment, expensive products and catalysts recovery, etc. The application of biocatalytic technology has gained immense attention in the chemical and bioindustry due to its sustainable mild reaction conditions. These include low reaction temperature conditions, substrate specificity, recyclability, more natural product separation and recovery, and elimination of wastewater treatment costs. In order to reduce enzyme costs, the catalytic effectiveness and chemo-, regio-,

and stereoselectivity of an enzyme can be enhanced and modified using protein engineering. The state-of-the-art includes directed evolution, genetic code expansion, strain improvement, the use of high throughput productive recombinant strains, immobilization, and discovery of new enzymes. The modification of amino acid sequences that are found in nature leads to improved proteins with unique functionalities, such as resistance to the harsh reaction conditions, high activity, and thermostability. Assisted intensification techniques have gained incredible attention in recent years for enzyme reaction enhancement. The application of intensification techniques such as ultrasonication, microwave, and pulsed electric field has shown a significant reduction in enzyme reaction time, with relevance to lowering bioprocessing costs.[24]

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