Chapter 1 INTRODUCTION

1.1 Human Nutrition

Human nutrition is the branch of science which deals with the information about the essential nutrients which supports the human life and health. From several years, importance of nutrition for supporting better human life is mentioned everywhere for most populations as malnutrition or malnourishment is the most common cause of various kinds of disease and death in the world.

There are two types of nutrients for the human beings, one is macronutrients which required relatively in greater quantity for the proper functioning of human health and another one is micronutrients, required in smaller quantities to sustain the human viability. Carbohydrates, proteins, fats, fiber and water came under the class of macronutrients whereas minerals and vitamins came under the class of micronutrients. A balance of all the macronutrients and micronutrients is essential for maintaining the healthy human life.

Carbohydrates are the most basic food group and instant energy provider for the human body. It is also termed as saccharide as this group has made up from the units of sugar eg. sugars, starch and cellulose. It is divided in mainly four groups: monosaccharides, disaccharides, oligosaccharides and polysaccharides (Westman, 2002). Proteins are also the energy sources and involved in wide range of bodily metabolic reactions mainly in the body growth, building and repairing of the damaged tissues. These are made up of the one or more long chains of amino acids residues which are arranged in different structure and folding to make a particular protein. Fats are the third class of macronutrients for the energy production for the human body (Passmore, 1974). Fats are the organic compounds made up of long chains of fatty acids, responsible for the both structural and metabolic functions. Fat is mainly storage energy for the future use. Chemical Structure of all kinds of fats is almost similar having the same base of glycerol and fatty acids. Fats are mainly important for enhancing healthy cell functioning, maintaining body temperature and provide insulation to body against shock (Gibney *et* *al.*, 2002). Fiber is also a kind of carbohydrates which can also produce the energy but amount of energy is less than the carbohydrates due to limited absorption and digestibility. Fiber is mainly important for the digestive health of humans.

Minerals and vitamins come under the class of the micronutrients. Dietary minerals are the chemical elements required by the living cells. Almost all living things are composed of the basic elements i.e. Carbon, hydrogen, nitrogen and oxygen. Dietary minerals like sodium, magnesium, phosphorus, potassium, copper etc. are having wide range of functions in the human body such as phosphorus is the component of bones, sodium and potassium are the ions for the electrolytes balance in the body, and magnesium requires for the ATP processing. Vitamins are the organic compounds essentially required in the diet in limited quantity for the good human health. There are two kinds of vitamins, water soluble and fat soluble vitamins. Vitamins A, D, E and K are the fat soluble vitamins however vitamin B complex and C are the water soluble vitamin. It cannot be synthesized by human body in sufficient quantities except the vitamin D which can be synthesized in the skin in presence of UV radiation so the supplementation or consumption via diet is essential. Vitamins have diversity in their biochemical functions some vitamins function as antioxidants, some involved in cell and tissue growth and differentiation and some are having hormone like functions (Huskisson *et al.*, 2007). Largest group of vitamin is B complex vitamin as it contains Vitamin B_1 (Thiamin), B_2 (Riboflavin), B_3 (Niacin), B_5 (Pentothenic acid), B_6 (Pyridoxine), B_7 (Biotin), B_9 (Folic acid) and B_{12} (Cyanocobalamin).

1.2 Importance of vitamin to human health

Vitamins are vital nutrients essential for the healthy maintenance of cells, to consume energy efficiently derived from foods and to process the carbohydrates, proteins and fats ingested through the diet. The importance of consuming vitamins rich foods came into the existence much before the individual vitamins were identified as the ancient Egyptians knew that night blindness could cure by consuming the liver which is supposed to occur due to the vitamin A deficiency (Challem, 1997). Term “vitamin” is coined by polish biochemist Kazimierz Funk in 1912, came from *vital* and *amine* means amines of life (Semba, 2012).

As vitamins act as regulator in various metabolic functions, its deficiency can result in potential severe diseases. So, Dietary Reference intake (DRI) is given for every nutrient by the Food and Nutrition board of the Institute of Medicine of the U.S. National Academy of Sciences in 1997, before that Recommended dietary allowance (RDA) was used by the U.S. and Canada which introduced earlier during World War II. Constant deficiency of vitamins in body can impact the whole metabolic functions of the body which ultimately leads to disease associated with particular vitamin deficiency such as rickets occur due to the deficiency of the vitamin A in the human diet and scurvy due to the vitamin C deficiency which is involved in the collagen synthesis in humans. A list of the vitamins, their daily recommended intake, sources, their functions in human body and associated disease is given below in the table 1.1.

Table 1.1 Importance of different vitamins to humans

Name of Vitamin	**Daily Recomme-nded intake**	**Sources**	**Functions**	**Associated disease**
Vitamin A Retinol	900 µg in men 700 µg in women	Liver, orange and yellow fruits, leafy vegetables, carrots, pumpkin, squash, spinach, fish, soy milk, milk	Mainly in the maintenance of good vision, skin, hair and immune system.	Night-blindness, hyperkeratosis
Vitamin D Calciferol	5 µg	Fortified Milk, sunlight, eggs, fish, mushrooms and butter	Bone and tooth formation, heart and nervous system functioning	Rickets, osteomalacia
Vitamin E Tocopherol	15 mg	Nuts and oils, multigrain cereals and wheat germ	Protects blood cells, tissue and essential fatty acids from destruction, antioxidant	Sterility in males and females, sometimes hemolytic anemia
Vitamin K Phylloquinone	120 µg in men 90 µg in women	Green leafy vegetables, dairy products and fruits	Mainly for the blood clotting	hemophilia
Vitamin C Ascorbic	90 mg in men	Citrus fruits and berries, vegetables	Maintenance of bone, tissue and cartilage	Scurvy

acid	75 mg in women	mainly in peppers	structure, essential for iron absorption	
Vitamin B_1 Thiamine	1.1 mg in women 1.2 mg in men	Liver, eggs, potato, vegetables and brown rice	Coenzyme in the sugar and amino acid metabolism, acetylcholine and GABA synthesis	Beri-Beri disease
Vitamin B_2 Riboflavin	1.1 mg in women 1.3 mg in men	Milk and dairy products, cheese, , bananas, green beans	wide variety of cellular processes as metabolism of fats, ketone bodies, carbohydrates, and proteins and central component of the cofactors FAD and FMN,	Glossitis, Angular stomatitis
Vitamin B_3 Niacin	14 mg in women 16 mg in men	Meat, eggs, mushrooms, fish and many vegetables,	precursors of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP)	Pellagra
Vitamin B_5 Pentothenic acid	5 mg	broccoli, Meat, avocados	Mainly required to synthesize coenzyme-A (CoA)	Paresthesia
Vitamin B_6 Pyridoxine	1.3 mg	Meat, vegetables, tree nuts, bananas	cofactor in many reactions of amino acid metabolism, including transamination, deamination, and decarboxylation	Anemia, peripheral neuropathy
Vitamin B_7 Biotin	30 µg	Raw egg yolk and liver, peanuts, leafy green vegetables	coenzyme for carboxylase enzymes, involved in gluconeogenesis, fatty acid and amino acid synthesis	Dermatitis

Vitamin B_9 Folic acid	400 µg- 800 µg for pregnant women or women of periconceptional period	Leafy vegetables, pasta, bread, cereal, liver	Rapid cell division and growth in infancy and pregnancy, red blood cells formation, essential to synthesize, repair and methylate DNA	Megaloblastic anemia, neural tube defects in newborns, cardiovascular diseases
Vitamin B_{12} Cyanocobalamin	2.4 µg	Meat and other animal products	Key role in brain and nervous system functioning and blood cell formation	Megaloblastic anemia

All the vitamins are mostly absorbed in the small intestine. Fat soluble vitamins (A, D, E and K) are generally absorbed in the duodenum and stored in the body mainly in the liver, fat and muscle tissues and usually not excreted easily as the water soluble vitamins so higher intake may be toxic for the body. Water soluble vitamins (B complex and C) are absorbed in the duodenum and jejunum and generally freely movable and absorbable in the body with the blood stream and usually excreted from the body constantly. So the regular consumption of water soluble vitamin in adequate amount is very much essential for the healthy maintenance of the body and cells.

1.3 Importance of Folic acid

Folic acid is one of the most important water soluble B vitamins also known as folate, folinic acid, B_9 and pteroyl L-glutamic acid. Its structure is composed of basically three subunits: pteridine, p-aminobenzoic acid and glutamic acid. Folate is the generic term used for all the derivatives derived from the parent structure of folic acid. Its name is derived from *"folium"* a latin word, which means leaf as it is found abundant in the green leafy vegetables specially in spinach. It is essential for various metabolic functions in humans such as one carbon transfer reactions in which it acts as a one carbon donar in amino acid inter conversions and nucleotide synthesis (Ames, 1999; Sarma & Duttagupta, 1995). The humans need folic acid to synthesize, repair, and methylate DNA as well as a cofactor in certain biological reactions. However humans cannot synthesize folic acid *de novo* so it is essential to consume it through the diet in adequate amount to meet the daily requirements (LeBlanc *et al.*, 2007). Folic acid deficiency causes several diseases such as megaloblastic anemia, certain forms of cancer such as colorectal cancer and breast cancer and neural tube defects in newborns as folic acid is essential for the proper cell growth and development of embryo (LeBlanc *et al.,* 2011). Thus folic acid prevents the disease occurrence in the human body which is quite beneficial than the cure in terms of both physical health and financial aspects. Some of the important metabolic functions of folic acid in the human body are briefly discussed here (Figure 1.1) to understand the importance of folic acid in humans and disease occurrence due to its deficiency.

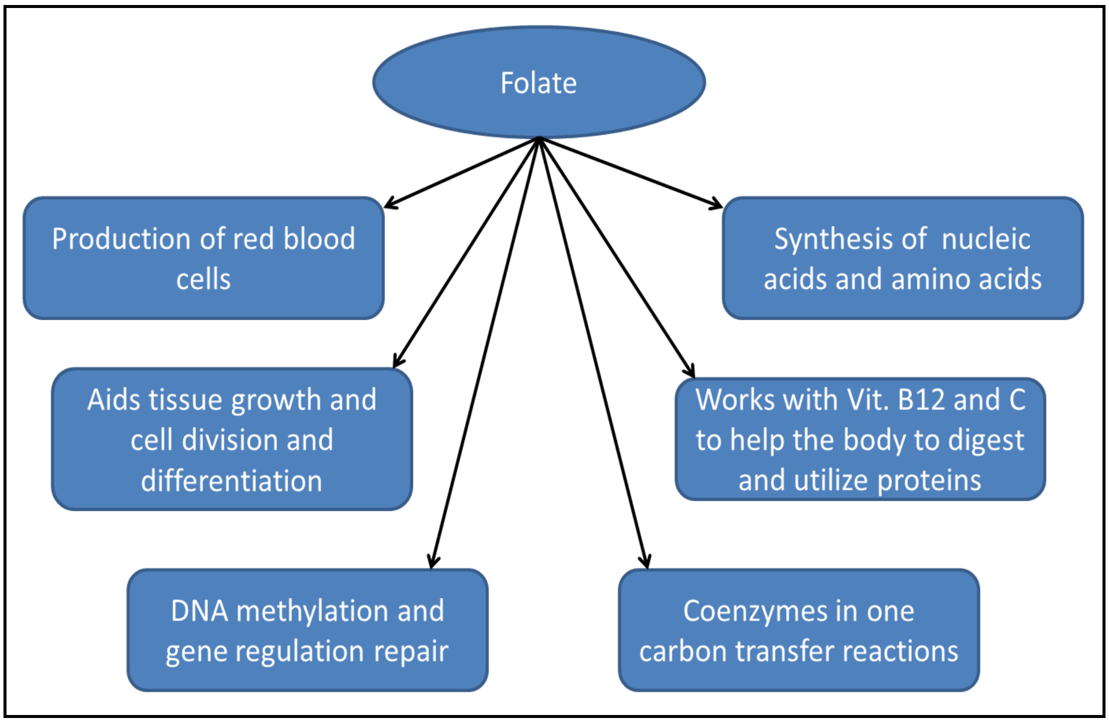


Fig 1.1: Folate and its role in various metabolic functions of Human body

1.4 Sources of folic acid

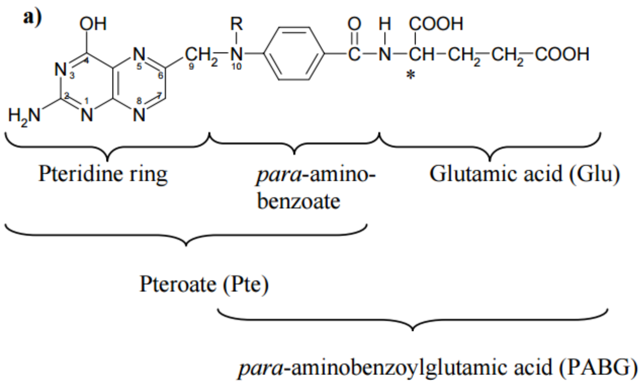
Mainly green leafy vegetables (e.g., spinach, broccoli, asparagus, and romaine lettuce), have abundant folic acid (Dietary Supplement fact sheet: Folate). Certain fruits and fruit juices eg. orange juice, and other citrus fruits, kiwis and strawberries are also good sources of folate. Beans, lentils, grains are also contained folic acid. But fresh fruits and vegetables could provide only the good amount to the adolescents because folate is destroyed by the heat used in cooking and canning and other storage procedures as it is quite sensitive to heat and light. The body can absorb almost 100% of chemically synthesized folic acid (the form used in vitamin supplements and fortified cereals). However, the amount of folate (natural form occurs in food sources) absorbed varies from individual to individual even to individual foods. Overall the absorption from the person diet is approximately 50% of the total folate intake of the food. Dietary folate equivalent (DFE) system was established due to the difference in bioavailability of natural and supplemental folic acid. 1 DFE is defined as 1 μg of dietary folate, or 0.6 μg of folic acid supplement. This is reduced to 0.5 μg of folic acid if the supplement is taken on an empty stomach (Suitor & Bailey, 2000). So, it is very important to consume a variety of folate-rich foods every day. Supplements could also be a choice to a person needing higher doses specially women of childbearing age and pregnant women to obtain the recommended amount to prevent neural tube defects. Almost all the multivitamins contain 400 micrograms (0.4 mg) of folic acid, the daily recommended intake for the pregnant women for the prevention of neural tube birth defects. However the supplement cannot be a substitute of diet because benefits of fibres and minerals are missing in the supplements.

Several microorganisms such as *Lactobacillus*, *Bifidobacterium* sp. and *Streptococcus* sp. also reported from the decades which can produce the folic acid in complex media but requirement of downstream processing for the purification of folic acid from the complex fermentation media enhanced the cost of the final product. Another option could be the fortified breakfast cereals and other fortified food products that could contain 100% of the recommended daily amount of folic acid in one serving to provide 400 micrograms (0.4 mg) of folic acid (Dietrich *et al.*, 2005). Fortification can be done by various ways but today scientist are focusing on the microbial fortification as commercial and industrial fortification involves the usage of chemically synthesized folic acid supplements. However microbial fortification is focused on the usage of probiotics to enhance the natural folate content of the food products which could provide the double advantages of folate supplementation along with the probiotics beneficial effects. According to the definition given by FAO/WHO, (2002) "probiotics are live beneficial microorganisms which when administered in adequate amounts confer a health benefit on the host". Species belonging to mainly *Lactobacillus* and *Bifidobacterium* are considered as probiotics.

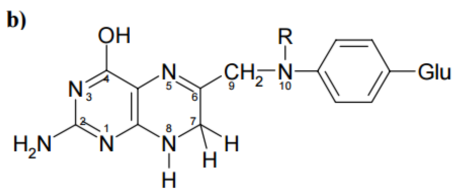
1.5 Chemical Structure and Nomenclature of folic acid and related compounds

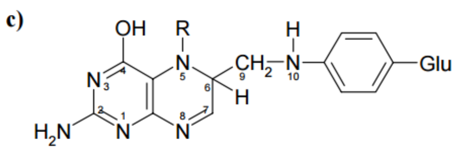
Nomenclature of folic acid and related compounds is given by the IUPAC-IUB Joint Commission on Biochemical Nomenclature (Blakley, 1988). This nomenclature of folate is very much complicated due to the various existing different carbon substituted forms. All folates are built up of a basic unit of pteridine ring, p-aminobenzoic acid and glutamate. Combination of pteridine ring and para-aminbenzoate also termed as “pteroate” which is made up of (Eitenmiller & Landen, 1999). Some structural differences existed in the different form of folates which are as follows:

Different forms exist on the basis of reduction of the pteridine ring. Folates with fully reduced pteridine rings are termed as tetrahydrofolates, partially reduced are called as dihydrofolates, and fully oxidised are known as folic acid. Different substituents can be placed in the N-5 and N-10 position of the pteridine ring such as hydrogen (H), methyl (CH_3), formyl (HCO), formimino (NHCH), methenyl (CH+) and methylene (CH_2). Nomenclature was done according to the nature of the substituent and reduction level of pteridine ring (Figure 1.2 a-d). L-glutamate can be conjugated via γ-peptide linkage of the carboxyl group in repetitive units which is known as diglutamate, triglutamate and polyglutamate on the basis of number of residues. In general, term folate polyglutamates is used for all folates having more than one glutamate residue regardless of substituents. The term folates may be used in general meaning any derivatives of folate or mixture of them regardless of reduction of the pteridine ring or one-carbon substituents and number of glutamate chains (Patring, 2007). Tetrahydrofolate or dihydrofolate loses its hydrogen to form folic acid (Scott *et al.*, 2000).

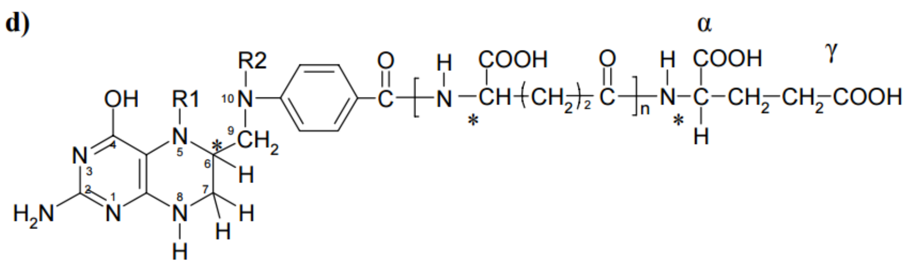


a) Fully oxidized pteridine ring	
Substituent (R)	Folate derivative
-H	Folic acid
-HCO	10-formyl-folic acid (10-HCO-folic acid)





b & c) Partially oxidized pteridine ring	
b) Substituent (R)	Folate derivative
-H	Dihydrofolate (H_2folate)
-HCO	10-formyldihydrofolate (10-HCO-H_2folate)
c) Substituent (R)	
-CH_3	5-methyldihydrofolate (5-CH_3-H_2folate)
-HCO	5-formyldihydrofolate (5-HCO-H_2folate)



* = tetrahedral stereocentres

d) Fully reduced pteridine ring		
Substituent (R1)	Substituent (R2)	Folate derivative
-H	-H	Tetrahydrofolate (H_4folate)
-CH_3	-H	5-methyltetrahydrofolate (5-CH_3-H_4folate)
-CH=NH	-H	5-formiminotetrahydrofolate (10-NHCH-H_4folate)
-HCO	-H	5-formyltetrahydrofolate (5-HCO-H_4folate)
-H	-HCO	10-formyltetrahydrofolate (10-HCO-H_4folate)
Bridge R1-R2		
-CH_2-		5,10-methylenetetrahydrofolate (5,10-CH_2-H_4folate)
-CH^+=		5,10-methenyltetrahydrofolate (5,10-CH^+=H_4folate)

Figure 1.2: a-d) Structure and name of some folate derivatives with fully oxidised, partially oxidised and fully reduced pteridine ring and nature of substituents (adapted from Patring, 2007).

1.6 Folate Biosynthesis Pathway

Folate biosynthesis pathway (Figure 1.3) is almost similar in plants and bacteria (Quinlivan *et al.,* 2006; Hanson *et al.,* 2002; Green *et.al.,* 1996). Folate biosynthetic pathway is lacking in mammals so humans cannot synthesize folic acid itself. In Bacteria, whole folate biosynthesis process is completed in cytosol. However in plants, it is divided into three subcellular compartments plastids, in which p-Amino benzoic acid moiety is formed from chorismate, cytosol in which pteridine moiety is formed from GTP and mitochondria where pteridine and p-ABA moieties coupled together to form Tetrahydrofolate. Then, short chains of γ-linked glutamate can be added further to form the polyglutamate form (Bekaert *et al.,* 2007). Folate biosynthesis pathway in microorganism can be divided in several parts. The pteridine portion of folate is made from GTP that is synthesized in purine biosynthesis pathway. p-Aminobenzoic acid originates from chorismate and can be synthesized *via* the same biosynthesis pathways required for aromatic amino acids involving glycolysis, pentose phosphate pathway and shikimate pathway. Third component of the folate is glutamate that is normally taken up from the medium. Different parts of folate synthesis are described here as follows:

1.6.1 Synthesis of Pterin

Guanosine triphosphate converted into the dihydroneopterin triphosphate, which undergo a two-step dephosphorylation in presence of dihydroneopterin triphosphate pyrophosphatase to form the dihydroneopterin (DHN). Aldol cleavage of trihydroxypropyl side chain of DHN by the DHN aldolase enzyme results in the formation of 6-hydroxymethyldihydropterin (HMDHP). Finally the HMDHP is pyrophosphorylated in presence of enzyme hydroxymethyldihydropetrin pyrophosphokinase before its condensation with p-aminobenzoate (pABA) (Quinlivan *et al.,* 2006; Basset *et al.,* 2002; Klaus *et al.,* 2005; Rebeille & Douce, 1999; Iwai & Kobashi, 1975)

1.6.2 Synthesis of p-Aminobenzoate

First of all chorismate, obtained from shikimate pathway, undergo for the replacement of hydroxyl group with an amino group which form 4-amino-4-deoxychorismate (ADC). This whole reaction is catalyzed by two enzymes; PabA acts as a glutamine amido-transferase, supplying an amino group to PabB, which carries out the amination reaction in bacteria. However, single fused enzyme catalyzes both the process in plants. The final step is the elimination of pyruvate and aromatization of the ADC ring to form pABA which is catalyzed by ADC lyase (PabC) (Viswanathan *et al.*, 1995; Basset *et al.*, 2004a; Green *et al.*, 1992; Basset *et al.*, 2004b; Quinlivan *et al.*, 2006).

1.6.3 Synthesis of Dihydropteroate and dihydrofolate

Finally the pABA is condensed with the pyrophosphorylated HMDHP to form the dihydropteroate catalyzed by 7, 8-dihydropteroate synthase. The phosphorylated dihydropteroate undergo the glutamate addition and phosphate release to form the dihydrofolate (DHF), which is finally reduced to tetrahydrofolate (THF) which is mainly used in the one carbon transfer reactions. Dihydrofolate synthase enzyme catalyzes the both final steps of folate synthesis i.e. phosphorylation and glutamylation (Ferone &Webb, 1975; Griffin & Brown, 1964; Brown *et al.*, 1961; Quinlivan *et al.*, 2006).

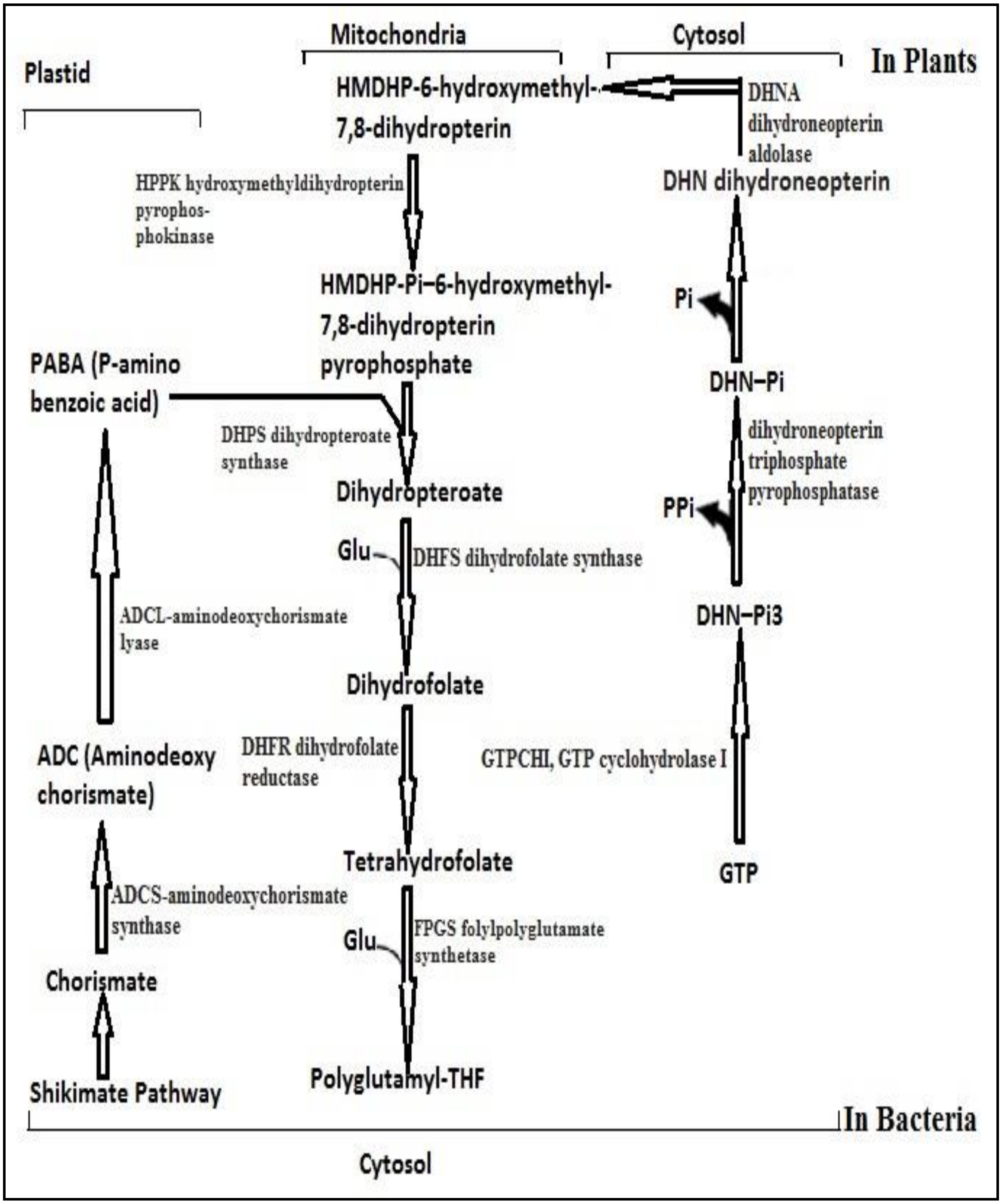


Figure 1.3 Whole folate biosynthesis pathway and enzymes involved in the biosynthesis pathway