CHAPTER 2

LITERATURE REVIEW

2.1 Prevalence of anaemia and IDA

In 2019, there were 1.8 billion prevalent cases of anaemia across the world, increased from 1.4 billion cases in 1990. WHO reported that the global prevalence of anaemia among children aged 6-59 months and women aged 15-49 is 39.8% and 29.9%, respectively. This is equivalent to 269 million children and over half billion women. The prevalence was higher among pregnant women than non-pregnant with 36.5% and 29.6%, respectively. South Asia, East Asia and Southeast Asian experienced the largest number of prevalent cases in 1990, while in 2019, the highest were found in South Asia and Africa (Safiri *et al*., 2021). In Malaysia, Malaysia's National Health and Morbidity Survey (NHMS) 2019 found an overall anaemia prevalence of 21.3% among individuals over the age of 15, with mild, moderate, and severe anaemia accounting for 13.5%, 6.8%, and 1.0%, respectively. When comparing prevalence by gender, women had a greater rate (30.4%) than men (12.6%). Systematic review by Abd Rahman *et al*., (2022) reported overall anaemia prevalence of 19.3 to 57.4% among pregnant women, while the prevalence of iron deficiency was 31.6 to 34.6%. Among preschool children, Shanita *et al.,* (2018) reported that the prevalence of anaemia and iron deficiency was 4.0% and 5.2% respectively, with the anaemia prevalence significantly higher among children of indigenous bumiputra and children living in a rural area. Meanwhile, in rural school children in Kudat, Sabah, Roslie *et al*., (2019) reported the anaemia prevalence of 31.4% with prevalence of IDA of 13.8%.

2.2 Symptoms of IDA

Symptoms of IDA are generally vague and non-specific thus often go undiagnosed. As the condition progress, it can become more severe. Clinical presentation of IDA depends on the anaemia severity, age, comorbidities and chronicity and onset speed. Fatigue, pallor of the skin and mucous membrane, breathlessness, headaches, mouth soreness and ulcers, dry and damaged nail, skin, and hair are among symptoms very frequently associated with IDA. Paleness is associated with IDA due to less haemoglobin in the blood and is easily recognised on the face, nails, inner mouth and lining of the eyes. Headaches, shortness of breath and poor concentration, are also among common symptoms very frequently associated with IDA as a result of low oxygen delivery to the body tissue and declined activity of iron-containing enzymes (Camaschella, 2015). Headache is often coupled with light-headedness or dizziness due to low oxygen concentration reaching the brain resulting in the swellnesss of brain's blood vessels causing pressure and headaches (Emel & Mehmet, 2015). Swelling and soreness of the tongue and mouth can also be an indication of IDA, with IDA patients having significantly higher frequencies of oral manifestations such as a burning sensation of the oral mucosa, lingual varicosity, dry mouth, oral lichen planus and atrophic glossitis when compared to healthy individuals (Wu *et al*., 2014). IDA also negatively affect epithelial cells, resulting in skin dryness and roughness, as well as dry and damaged hair. Women with iron deficiency are at risk of telogen hair loss (Moeinvaziri *et al.*, 2009). Abnormalities in the colour, shape and texture of nails can also be related to nutritional deficiency, and IDA is often associated with brittle nails that easily chip and crack and nails that have a rounded appearance like a spoon-shaped known as koilonychia (Cashman & Sloan, 2010).

2.3 Causes of IDA

Referring to Figure 1, IDA may arise as a result of increased iron demand, decreased iron intake, decreased iron absorption, increased iron loss, inflammation and genetic factor. Increased iron demand is common in infants, preschool children, adolescent growth spurts and during pregnancy particularly during second and third trimesters. The growth of the foetus and placenta, as well as the expansion of maternal red cell mass, significantly increases iron requirement during pregnancy (Fisher & Nemeth, 2017). Decreased iron intake can be a direct result of malnutrition and poverty especially in underdeveloped countries or attributed to iron- deficient vegan or vegetarian diet. The consumption of inhibitor such as calcium, phytate and tannin in diet and surgical procedure like gastrectomy, duodenal bypass and bariatric surgery decreased iron absorption (Samtiya *et al.*, 2020; Steenackers *et al.*, 2018: Jun *et al.*, 2016). Medical condition like *H. pylori* infection, coeliac disease, atrophic gastritis and the consumption of proton-pump inhibitors also contribute to decreased iron absorption. Chronic blood loss due to gastrointestinal, genitourinary and systemic bleeding increase iron losses which eventually lead to IDA (Camaschella, 2017). Heavy menstruation and frequent blood donation also cause IDA. Non-steroidal anti-inflammatory drugs (NSAID) also result in drug-induced blood loss as it can increase bleeding risk and irritate the gastric mucosa (McEvoy *et al.*, 2021). Prolonged immune activation in diseases such as cancers, chronic kidney disease and congestive heart failure cause inflammation that hinder iron absorption which eventually led to IDA (Weiss *et al.*, 2019). Genetic factor also contributes to IDA, for instances, a malfunction in the TMPRSS6 gene, which encodes for Matriptase-2, a protein involved in regulation of hepcidin, a key regulator of iron haemostasis causes iron-refractory iron deficiency anaemia (IRIDA) (Thangavelu *et al.*, 2019).

2.4 Impacts of IDA

IDA generally reduced the overall physical ability, productivity, and tolerance to exercise due to diminished oxygen transport associated with anaemia and decreased cellular oxidative capacity associated with iron deficiency. Iron is essential to oxidative metabolism for energy generation in skeletal muscle, which involved in oxygen storage in myoglobin and mitochondria. Both components rely substantially on oxygen-carrying ability, which reflects adequate oxygen supply provided by haemoglobin (Stugiewicz *et al*., 2016). IDA impairs individual physical capacity and work performance, resulting in productivity losses of roughly US$2.32 per capita or 0.57% of GDP in both low- and middle-income nations. Thus, IDA not only decreases individuals' and populations' work capacity but also puts a serious strain on economic and national development. Global Burden of Disease Study in 2016 reported that, iron deficiency anaemia was one of the leading causes affecting women's quality of life globally (Vos *et al.*, 2017).

Iron demand is fundamentally increased in pregnancy to balance the physiological requirement of increased haematocrit, fetoplacental development and losses during delivery and lactation, with an approximately 1,000mg of total iron is lost during pregnancy and lactation (Mcdonagh *et al*., 2015). Anaemia is frequently observed in pregnant women as a result of a prominent expansion of plasma volume by 30-40% in contrast with a 20-25% rise in Hb mass and erythrocyte volume. IDA in pregnant women has been considered harmful for maternal and foetus well-being and is often linked to increased morbidity and foetus death rate. Severe IDA in pregnancy significantly increased the incidence of premature delivery due to prolong chronic hypoxia caused by anaemia, resulting in elevated corticotropin-releasing hormone (CRH) production, which is recognised as a major risk factor in premature births (Di

Renzo *et al.*, 2015). Additionally, the risk of premature delivery may further increase due to oxidative damage to both erythrocytes and the fetoplacental unit. Besides, maternal IDA is frequently linked to an increased risk of caesarean section delivery, perinatal haemorrhage, preeclampsia, abruptio placentae, poor maternal thyroid status and cardiac failure (Gupta, 2018; Tangeda *et al.*, 2016; Erez Azulay *et al*., 2015; Drukker *et al*., 2015; Arnold *et al*., 2009; Zimmermann *et al*., 2007).

Fetal iron deficiency causes persistent impairment in postnatal outcomes for infants. Published animal studies demonstrated that when the iron is critically restricted in the neonate, the available iron is redirected to red blood cells, at the expense of the brain, heart and muscle (Zamora *et al*., 2016; Tran *et al.*, 2015,2009). Iron deficiency impairs brain and hippocampal development, myelination and dopaminergic neurotransmission, causing adverse effects on motor performances, mental development, cognitive and behavioural function, marked in infancy and early childhood where the brain growth and development are heightened (Larson *et al.*, 2017; Doom & Georgieff, 2014). Qin *et al*., (2019) showed a significant longitudinal association between anaemia and cognitive decline in middle age and older Chinese population especially the episodic memory decline regardless of gender. El Shemy *et al.*, (2019) demonstrated that IDA children with spastic cerebral palsy showed significantly lower motor function and decreased muscle strength and endurance compared to non-anaemic children. A longitudinal study of Costa Rican children found that iron deficiency had long-lasting negative effects on children's motor development over time, with children who suffered from chronic iron deficiency in infancy having a lower motor trajectory with both fine and gross motor skills equally affected when compared to the control group (Shafir *et al*., 2006). The same cohort population also showed a poorer executive functioning and recognition memory as young adults and

exhibited more behaviour problems compared to children with normal iron status (Corapci *et al*., 2010). IDA also adversely influenced the immune system. A study by Hassan *et al.,* (2016) conclude that IgG, IL-6, phagocytic activity and oxidative burst were significantly lower in IDA children compared to normal control. Described next in figure 1 is the conceptual framework demonstrating the common cause of IDA and the negative effects of IDA illustrated and supported from the various literature as described before.

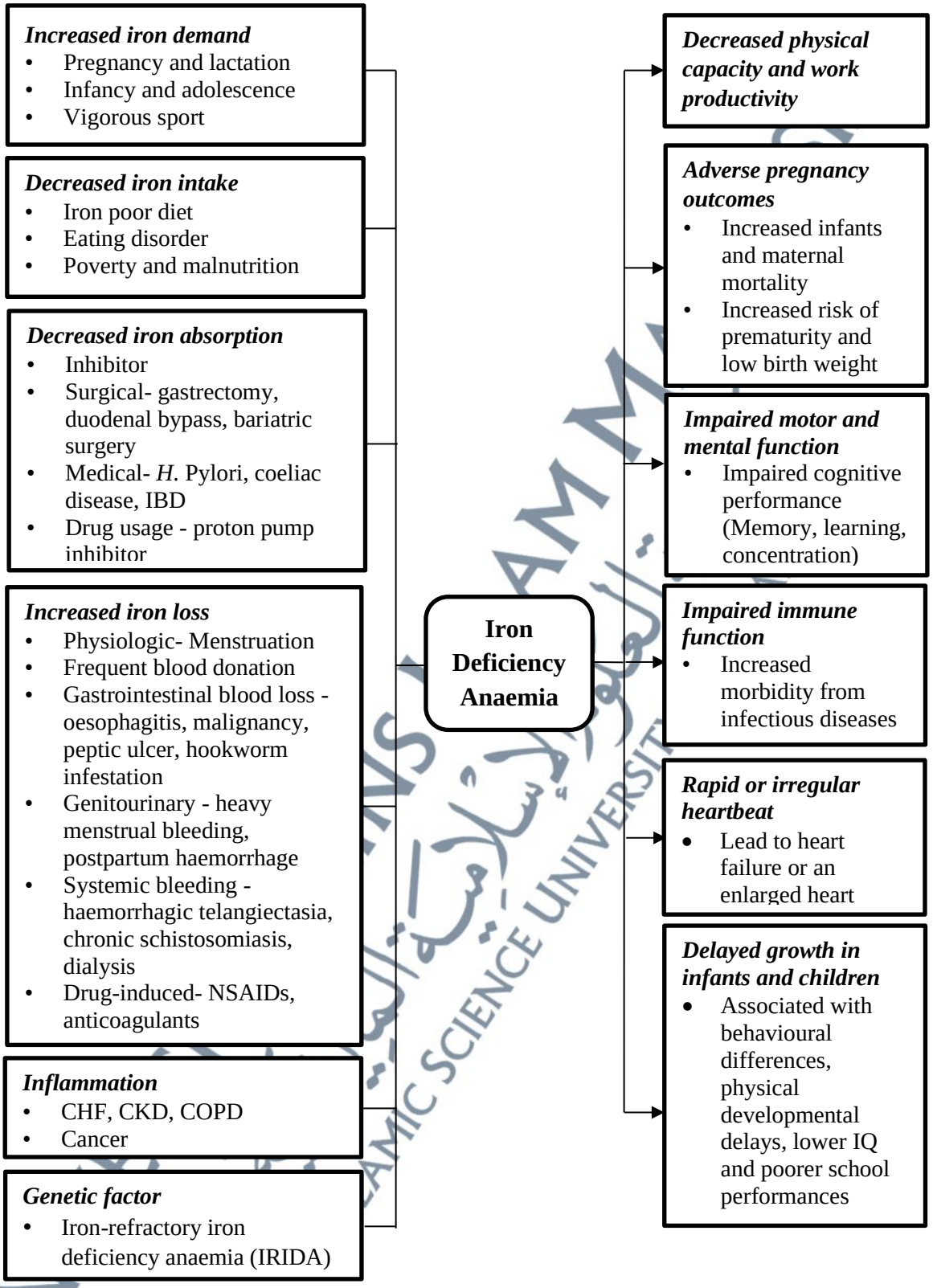

Figure 1: Theoretical framework of the common cause of IDA and the negative impact on the human body. CHF: congestive heart failure; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease

2.5 Current treatments for IDA

IDA treatment aims to provide adequate iron to normalise Hb level and restore iron stores, hence, improving quality of life, symptoms and the prognosis of chronic disorder related to iron deficiency. Food-based approaches that promote the access and consumption of iron-rich food are highly recommended by WHO. People at risk are encouraged to consume meat, fish, poultry, legumes, fruits, and leafy vegetables. The bioavailability of iron can also be improved by consuming food that is rich in ascorbic acid and amino acids such as cysteine and histidine. Inhibitor that interferes with iron absorption such as calcium and phytate that are found in cereal and polyphenol and tannins which are present in tea and coffee should be avoided. Food fortification is one of the main strategies imposed by WHO and the United Nations Food and Agriculture Organisation (FAO) for improving dietary intake worldwide. Fortification of widely consumed staple food such as rice, maize and wheat has effectively improved dietary diversity and decreased micronutrient deficiencies. Iron fortification commonly used includes sodium iron ethylenediaminetetraacetate (NaFeEDTA), ferrous sulfate, ferrous bis-glycinate, ferrous fumarate and electrolytic iron. According to a meta-analysis by Keats *et al.*, (2019) there was a 34% reduction in anaemia prevalence following large scale food fortification with the greatest impact was noticed for women of reproductive age followed by school-age children.

Oral iron supplementation is commonly prescribed when an individual is experiencing symptoms of IDA to restore normal iron stores and to replenish haemoglobin deficit. Oral iron supplements contain different amounts of easily absorbed elemental iron and are available in the form of pills, capsules, drops and extended-release tablets. The most commonly available oral iron is ferrous sulfate, ferrous gluconate and ferrous fumarate. In adults, therapeutic doses ranging from 100

to 200mg of elemental iron daily has been recommended (Stoffel *et al.,* 2020). Elemental oral iron has been recommended as the first line defense against IDA and has been used as a standard prenatal care and as a prophylactic measure to alleviate anaemia in pregnant women and children (Division of Family Health Development, 2020). Parenteral therapy involves the administration of iron intravenously, available in various form that includes iron dextran, ferric carboxymaltose, iron sucrose, iron isomaltoside-1000, ferric gluconate and ferumoxytol. This method circumvents the problem of iron intolerance, lack of response to oral iron or in the case of a clinical need for rapid and efficient treatment of anaemia.

Consumption of oral iron in long run is accompanied by several side effects including epigastric discomfort, heartburn, constipation, diarrhea, nausea, black stool and vomiting (Girelli *et al.*, 2018). A meta-analysis by Tolkien *et al.*, (2015) demonstrated that supplementation of ferrous sulfate resulted in significant gastrointestinal side effects in adults. Iron supplements have also been linked to an increase incidence of diarrhoea in infants and children. The introduction of iron supplementation results in excess unabsorbed iron entering the colon. Sharp increases in colonic iron caused a declination in beneficial barrier commensal gut bacteria and increases the abundance of bad enteropathogenic bacteria. Beneficial gut bacteria from *Bifidobacteria* and *Lactobacilli* genera require little to no iron and are often growth-limiting nutrients. Contrarily, iron is essential for the virulence and colonisation of enteric gram-negative bacteria like *E. coli* and *S. shigella*. Excess non-absorbed iron from iron supplementation will increase colonic iron that eventually modify the microbiota equilibrium in favouring the pathogenic strains over the healthy barrier strain, thus, increasing the intestinal pathogen concentration (Jaeggi *et al*., 2015; Paganini & Zimmermann, 2017). Even though intravenous iron replenishes iron stores

more effectively, this therapy is limited by several factors such as the possibility of anaphylactic reactions and a high cost. (Onken *et al*., 2014). Intravenous iron preparation exacerbates oxidative stress resulting in upregulation of various transcription factors that trigger the release of TNF-α and other cytokines that eventually lead to progressive tissue damage. According to a meta-analysis by Litton *et al*., (2013), intravenous iron promotes bacterial growth thus increasing the risk of infection. Excess iron has been associated to a variety of disorder, notably in age related macular degeneration, age-related Alzheimer and Parkinson, and cancer (Kenkhuis *et al*., 2021; Mochizuki *et al*., 2020; Song *et al*., 2019; Jung *et al*., 2019; Liu *et al*., 2018). Iron's ability to be oxidised and reduced allows it to participate in free radical generating reaction such as the Fenton reaction, which produce hydroxyl radical, a powerful reactive oxygen species (ROS). As the results, iron is potentially mutagenic by inducing DNA strand breakage, which causes cellular transformation that eventually resulted in cancer progression (Jung *et al*., 2019). Excess iron also adversely affects cellular immunity by decreasing polymorphonuclear leucocytes (PMNs), thereby impairing the body's host defence mechanism (Maynor & Brophy, 2007).

Poor patient compliance also put a challenge in reducing IDA prevalence. In Malaysia, several studies reported on the poor compliance with oral iron supplementation (Abd Rahman *et al*., 2022; Kadir *et al*., 2021; Daud *et al.*, 2020). Half of pregnant women in Malaysia do not consume the iron supplements due to forgetfulness and intolerance to iron tablet's side effects (Thirukkanesh & Zahara, 2010). While iron fortification is a more cost-effective strategy for IDA prevention in risk populations, the cost may be prohibitively expensive for some individual. Several technological challenges also need to be thoroughly handled, particularly with regards to adequate levels of nutrients, stability of fortificant, nutrient interaction, physical

qualities, high cost and customer acceptance. Iron is the most challenging micronutrient to be incorporate into fortified food as it is easily interact with food constituents, producing unpleasant organoleptic side effect especially after prolonged storage (Prentice *et al.,* 2017). Furthermore, in the case of multiple fortifications, free iron that is produced from iron compound degradation can oxidise some of the vitamins supplied in the same fortificant mixture (Allen *et al.*, 2006).

2.6 Regulation of iron metabolism

Dietary iron can be classified into two categories according to its chemical form. Heme iron is derived from haemoglobin and myoglobin abundantly found in meat, poultry, seafood, and fish. Non-heme iron, by contrast, exists in plant-based food, like cereal, vegetables, fruits, nuts, and seeds. It can also be found in animal products such as eggs and dairy. Referring to figure 2, many enzymes involved in essential cellular and organismal activities require iron as a cofactor in order to function normally. Iron is indispensable for the synthesis of haemoglobin, a protein present in red blood cells that transports oxygen throughout the body. Most iron is utilised for erythropoiesis with bone marrow erythroblasts acquire more than 70% iron. Iron is also a component of myoglobin – an oxygen binding protein that is found in the cardiac and skeletal muscle tissues that provide oxygen to the working muscle. Iron is also essential for enzymes and proteins involved in mitochondrial respiratory chain, synthesis of haemoproteins and iron-sulfur clusters, DNA and RNA repairs, microRNA biogenesis and cell cycle control and proliferation. Of note, iron is particularly important for nervous system, involved in neurotransmitter synthesis, myelin formation and synaptic development. Iron also involved in the production of thyroids hormones and immune cell proliferation (Alnuwaysir *et al*., 2022; Zohora *et al*., 2018).

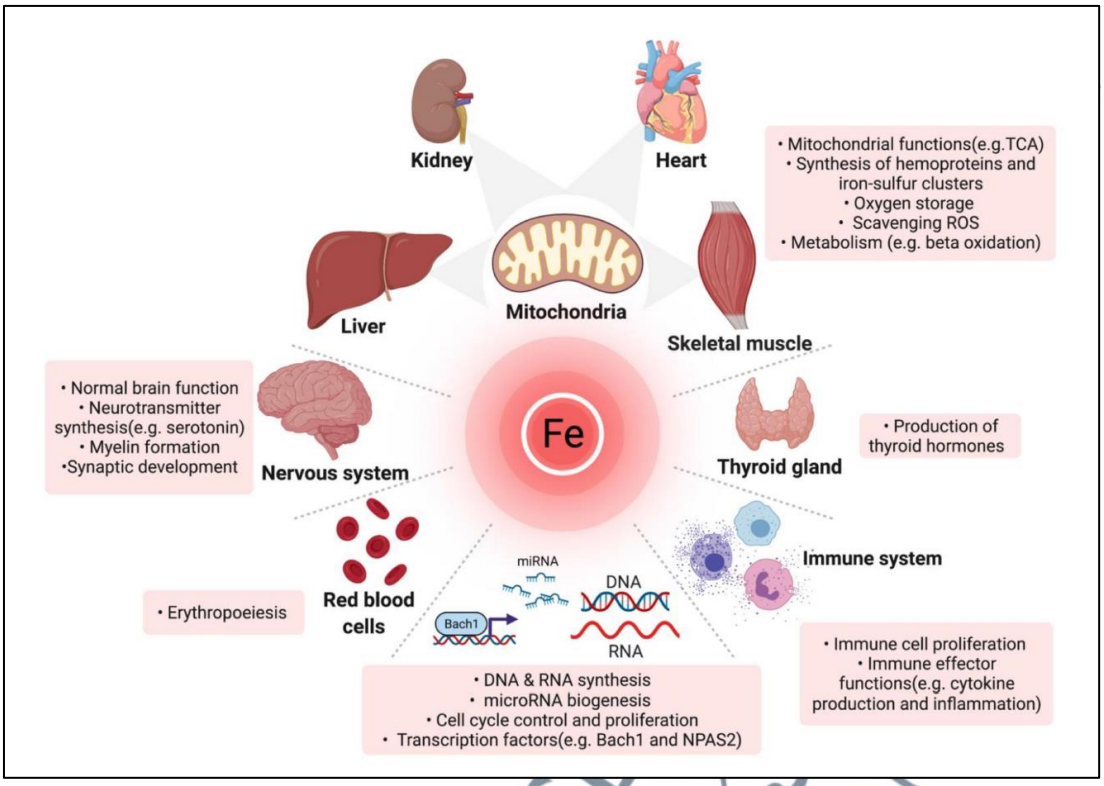

Figure 2: An overview of the iron's function in the body. Most iron is utilised for erythropoiesis for the production of haemoglobin. Iron is essential for enzymes and protein involved in the oxidative metabolic process with significant amount of iron was found in cell with high energy demands such as in skeletal muscle, heart, kidney and liver. Iron also crucial in DNA and RNA metabolism, the function of the thyroid gland, central nervous system and immune system (Alnuwaysir *et al.*, 2022).

The average human body has 3 to 5g of iron, the majority of which is contained as heme in erythroid cells' haemoglobin (more than 2g) or muscle's myoglobin (approximately 300mg). Meanwhile, macrophages in the liver, bone marrow and spleen stored a transient amount of iron (around 600mg). Excess iron is stored within ferritin shells in the parenchyma cells of the liver (approximately 1000mg). Other cellular iron-containing proteins and enzymes are estimated to bind a total of 8 mg of iron. Iron is acquired in the body from duodenal enterocytes, which absorb 1-2 mg of dietary iron daily, and macrophages, which internally recycle 20-25 mg of iron from senescent erythrocytes. As there is no regulated pathway for iron excretion, dietary iron absorption represents a critically controlled process that compensates for obligatory losses of iron (~1-2mg). Iron is lost through sloughing of intestinal epithelial cells, desquamation of

skin and urinary tract epithelial cells, blood loss, and perspiration. The absorption of iron can either be enhanced or suppressed depending on physiological need, for example, iron absorption increases during pregnancy, blood loss or physical development and is suppressed during iron overload (Hentze *et al.*, 2010).

Figure 3 illustrate the mechanism of iron distribution and circulation The mechanism for dietary iron absorption for heme and non-heme iron in the small intestine is different in which heme iron can be transported directly into the enterocyte via heme carrier protein (HRG1). Meanwhile, dietary non-heme iron in the form of ferric ion (Fe^{3+}) is firstly reduced to ferrous ion (Fe^{2+}) by duodenal cytochrome b reductase (Dcytb), a membrane-bound oxidoreductase enzyme, before being transported into the enterocytes by iron transporter, divalent metal transporter 1 (DMT1). Inside the enterocyte, iron can be either used directly for intrinsic cellular metabolic mechanisms, stored in ferritin or transported out across the basolateral membrane for systemic iron delivery. Fe^{2+} is exported into the bloodstream through membrane ferroportin (FPN1), and after oxidation by hephaestin or ceruloplasmin to Fe^{3+}, it binds to transferrin (Tf). Under physiological conditions, almost all iron in circulation is bound to transferrin (Tf). Tf is made up of two high affinity Fe^{3+} binding sites that keeps Fe^{3+} in a redox-inert state, preventing the formation of reactive oxygen species (ROS). It acts as the major transport glycoprotein for Fe^{3+}, responsible in delivering iron to site of iron utilization. The majority of the iron is transported to the bone marrow for erythrogenesis, stored in muscle as myoglobin or utilised by other metabolic pathways. Once the iron-bound Tf interact with transferrin receptor (TfR) expressed on the plasma membrane of cells, the complex is internalised by receptor-mediated endocytosis where iron is released via endosomal acidification. Tf-TfR complex is returned to the cell surface where it is released back into the circulation for re-utilisation. Once inside the

cells, internalized iron becomes part of cytosol's the labile iron pool (LIP) which works as intermediate and can be used for (i) biological activities such as DNA synthesis, repair and cell division, (ii) stored in ferritin as an inert storage form or (iii) transported out via ferroportin. Senescent red blood cell is efficiently recycled by splenic macrophages, providing iron for the synthesis of new red blood cells. Systemic iron regulation is control by the hepcidin-ferroportin regulatory system. Hepcidin is a hormone synthesized in the liver, that upon binding with ferroportin will induces its internalization and degradation, subsequently inhibit iron release from the basolateral membrane of enterocytes into the hepatic portal system and from macrophages into the systemic circulation. By inhibiting the iron efflux into the circulation, it maintains the systemic iron homeostasis, thus critical in regulating iron metabolism during inflammation, hypoxia, iron deficiency and iron overload. Hepcidin expression is controlled by several factors such as the amount of iron, represented by body iron store and Tf saturation; inflammation commonly via the pro-inflammatory cytokine (IL-6) and the iron requirement for erythropoiesis. (Ward *et al*., 2022; Muckenthaler *et al*., 2017; Girelli *et al*., 2016; Lane *et al*., 2015; Pantopoulos *et al*., 2012)

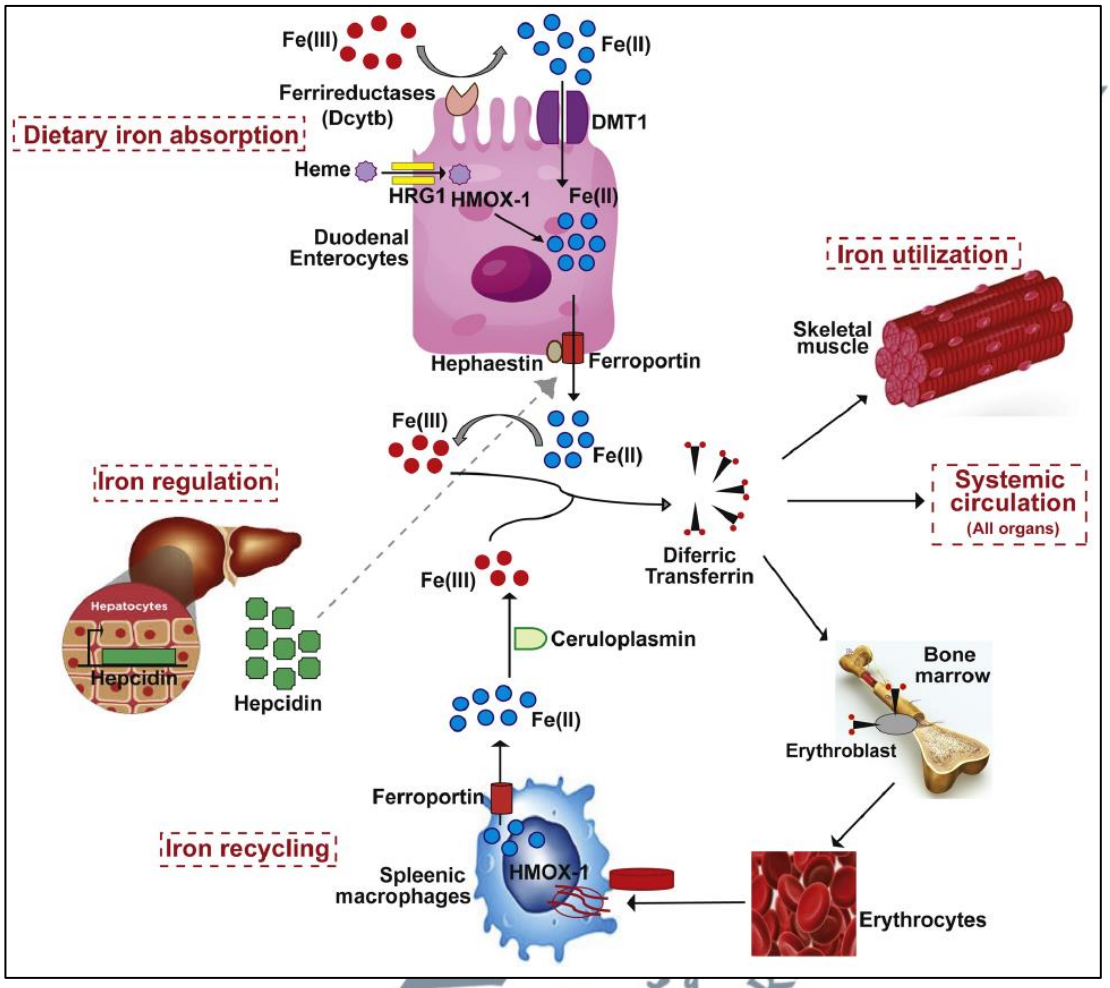

Figure 3: Schematic illustration of cellular iron homeostasis. Dietary non-heme iron is reduced from Fe^{3+} to Fe^{2+} by iron reductase Dcytb before being transported into the enterocytes via DMT1. Fe^{2+} can be either used for biological processes, stored in ferritin, or released into circulation thru ferroportin. Hephaestin then oxidises Fe^{2+} into Fe^{3+} which then is captured by transferrin (Tf), the plasma iron carrier. Iron is transported for various tissue with most iron is utilised for erythrogenesis in bone marrow, as an oxygen storage myoglobin in muscle and other metabolic cellular processes. Tf- iron complex bound to transferrin receptor (TfR) on the cell surface and then internalized by endocytosis. Internalized iron will enter the labile iron pool (LIP) and can be used for storage, utilized for downstream metabolic pathways, or being exported out via ferroportin. Senescent erythrocytes are phagocytosed by macrophages, and iron is recycled back into the circulation. Systemic iron regulation is regulated by hepcidin that bind to ferroportin inducing it internalisation, subsequently control the iron efflux into the circulation (Jayakumar *et al*., 2022)

2.7 Regulation of erythropoiesis

Erythropoiesis is a complex multi-step process wherein hematopoietic stem cells (HSCs) undergo differentiation to become fully matured erythrocytes or red blood cells (RBCs). Erythropoiesis take places in a specialised bone marrow niches known as erythroblastic island (EBIs) that are composed of acentral macrophage surrounded by erythroblast at various stages of differentiation (Borges & Sesti-costa, 2022). Burst-forming unit erythroid (BFU-E) and colony-forming unit erythroid (CFU-E) cells, are the committed erythroid progenitors that are specifically committed to developing into erythrocytes. These cells have progressed beyond the earlier stages of hematopoietic stem cells and multipotent progenitor cells, and have committed to the erythroid lineage. BFU-Es and CFUEs further differentiate into erythroid precursor cells with distinct morphologies. Referring to figure 4, proerythroblast is the earliest morphologically identifiable erythroid precursor, which differentiates sequentially into the basophilic, polychromatophilic and orthochromatic erythroblast before enucleate to form a reticulocyte. The precursors show a gradual reduction in cell and nuclear size, while a robust increase in the accumulation of haemoglobin occurs. Reticulocytes then mature into fully developed erythrocytes, or red blood cells, which are anucleate and contain only haemoglobin (Nandakumar *et al*., 2016).

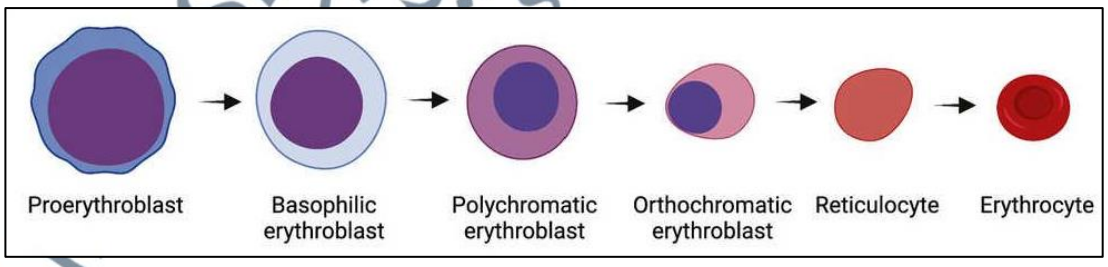

Figure 4: Schematic diagram of the process of erythropoiesis. Proerythroblast undergone several differentiations into the basophilic, polychromatophilic and orthochromatic erythroblast, which then enucleate to form a reticulocyte before fully developed into erythrocytes (Borges & Sesti-costa, 2022)

Erythropoiesis relies heavily on iron, which is necessary for the creation of haem centres in haemoglobin that carry oxygen. Therefore, physiological signals and molecular mechanisms that maintain proper levels of iron can concurrently affect erythropoiesis. At a systemic level, the concentration of iron in the bloodstream is regulated by two key hormones: hepcidin and erythroferrone. Hepcidin limits the amount of iron in the plasma, while erythroferrone enhances it. This regulation ensures that the iron demand necessary for red blood cell production is met. Figure 5 showed the systemic talk between iron regulation and erythropoiesis. In conditions where there is enough iron in the body, hepatocytes can directly sense transferrin-bound iron, which then stimulate the production of hepcidin. Hepcidin will bind to ferroportin, inducing it degradation which subsequently limit the release of iron from enterocytes, macrophages and hepatocytes. On the other hand, when the body requires more iron, differentiating erythroblasts produce erythroferrone, a hormone that suppresses hepcidin production. This result in the accumulation of ferroportin on the iron reservoir cells, leading to increased circulating plasma iron that can be used for the synthesis of haemoglobin in erythroblasts (Liang & Ghaffari, 2016).

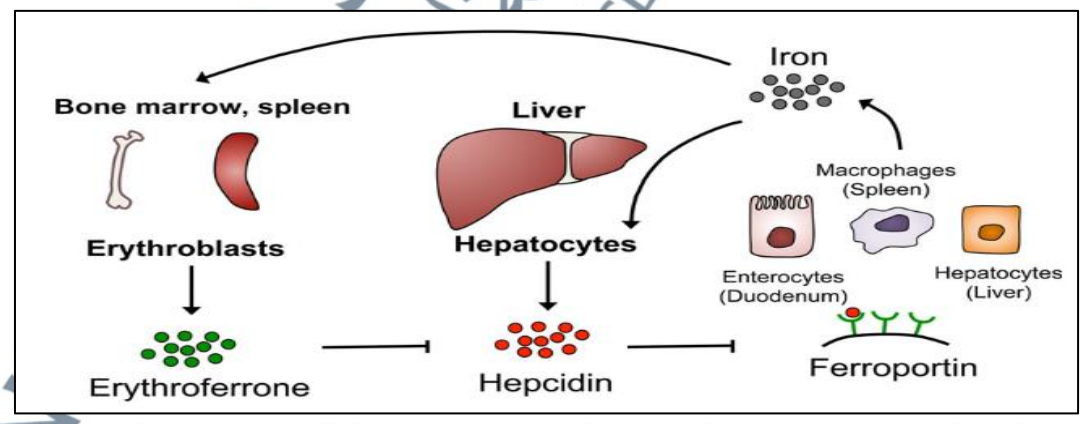

Figure 5: The systemic talk between iron regulation and erythropoiesis. When there is enough iron in the body, more hepcidin is being produced which then bind to ferroportin, inducing it degradation. This limits the release of iron into the circulation. When more iron is required, differentiating erythroblasts produce erythroferrone, that suppresses hepcidin production, increasing circulating iron for erythropoiesis (Liang & Ghaffari, 2016).

2.8 Iron bioavailability

Bioavailability is described as "*the efficiency with which a dietary component is used systemically through normal metabolic pathway*" (Aggett, 2010 page 1434S). The amount of iron ingested is necessary for bioavailable iron since consuming a meal without iron has no effect on iron absorption regardless of how potent an enhancer or inhibitor may be. The type of iron, composition of food and the time between meals can also affect iron absorption thus should be taken into account in understanding the notion of iron bioavailability which can be defined as the proportion of iron ingested that is utilised or stored for bodily function (Blanco-Rojo & Vaquero, 2019).

The absorption mechanism for heme and non-heme iron is different. For non-heme iron, once the non-heme iron complexes are degraded and released from the food component, it presents in the ferric form (Fe^{3+}). The absorption rate of non-heme iron is low, around 2 to 20%, and is susceptible to being affected by dietary components that impede or facilitate its bioavailability (Lesjak & Srai, 2019). Components such as ascorbic acid, cysteine and histidine are capable of converting Fe^{3+} to Fe^{2+} which then readily absorbed into the cell. Additionally, most of the reduction activity is also done by metal reductase enzyme including duodenal cytochrome b reductase (Dcytb) found on the apical membrane of the enterocyte. In contrast, heme iron absorption is essentially unaffected by interactions with other dietary components, resulting in a more consistent and uniform absorption (Hurrell & Egli, 2010). Heme iron is imported into the enterocytes via heme carrier protein -1 (HCP-1) and once internalised, heme oxygenase (HO) will release the heme iron which then will follow the same route as non-heme iron. According to Staron *et al*., (2017), heme iron is exported intact through the basolateral membrane via FLVCR1 to the plasma, where it is bound by haemopexin. The heme-haemopexin complex is then delivered throughout the circulatory system.

Ascorbic acid has been consistently proven to boost intestinal iron absorption and is recognised as one of the most potent iron enhancers. It prevents iron from interacting with other ligands by forming a chelate with iron at low pH. Besides, ascorbic acid also functions as a cofactor for Dcytb enzyme that reduces Fe^{3+} into Fe^{2+} on the enterocyte membrane. Both mechanisms favour iron absorption. According to a meta-analysis comparing 22 studies, the addition of ascorbic acid to test meals significantly increased the percentage of iron absorption (Heffernan *et al*., 2017). This study also found that long term ascorbic acid supplementation significantly boosted Hb level from baseline to follow-up, concluding that ascorbic acid enhances non-heme iron absorption and improves the biomarker of iron status overtime.

Phytic acid, phenolic compounds, oxalic acids, saponins, and tannins are inhibitors, commonly bind to metal, forming insoluble compounds that may impede nutrient absorption. They reduce the bioavailability of nutrient causing micronutrient malnutrition mineral deficiency (Samtiya *et al.*, 2020). A study in Bangladeshi pregnant women showed dietary phytate intake inhibited iron absorption from the diet of all participants and inhibited calcium absorption in 52% of the women (Al Hasan *et al.*, 2016). Calcium is known to interacts with iron for DMT1 transporter, thus high consumption of calcium is associated with lower iron status. Benkhedda *et al.*, (2010) demonstrated that the supplementation of 500mg calcium to the breakfast meal significantly reduced iron absorption by 53%. The final effect on iron bioavailability, however, relies on numerous factors and was attributed to a balanced combination of both enhancers and inhibitors and also complex interaction with other components of the food matrix.

2.9 Protein related to iron metabolism

2.9.1 Divalent metal transporter (DMT1)

Divalent metal transporter 1 (DMT1) which is also called natural resistance-associated macrophage protein 2 (NRAMP2) and divalent cation transporter 1 (DCT1) are proteins encoded by the *SLC11A2* (solute carrier family 11, member 2) gene located at chromosome 12 (12q13.12) (Vidal *et al*, 1995). DMT1 is a transmembrane protein, essential in transporting iron from endosomes into the cytoplasm. DMT1 also transport numerous other metal ions including copper, zinc, manganese and cadmium via a proton-coupled energy-dependent process (Ludwiczek *et al*., 2007). DMT1 is ubiquitously expressed and tightly regulated by body iron stores to maintain iron homeostasis. Global inactivation of DMT1 gene caused severe and fatal microcytic hypochromic anaemia in $DMT1^{-/-}$ murine model shortly after birth. They also showed progressive postnatal growth retardation, with none surviving more than 7 days. Heterozygous $DMT1^{+/-}$ appeared normal with no significant deformity in the haematological parameter, however, the iron content was lower indicating haploinsufficiency that results from impairment of iron absorption in the small intestine or defects in hepatocyte iron assimilation (Gunshin *et al.*, 2005).

2.9.2 Cytochrome b reductase (Dcytb)

Duodenal cytochrome b reductase (Dcytb) is a transplasma membrane oxidoreductase, that catalyses the reduction of Fe^{3+} to Fe^{2+}, facilitating it to be transported into the small intestine's enterocyte during the iron absorption. Dcytb is encoded by the *CYBRD1* gene in chromosome 2 (2q31.1) and is mainly expressed in the brush border of duodenal enterocytes (Westhuizen *et al*., 2002). Dcytb coming from the cytochromes b561 (CYB561) family, frequently use electrons generated

intracellularly from ascorbic acid. Dcytb is also capable of reducing cupric copper (Cu^{2+}) and ascorbyl radical (AR) (Luo *et al*., 2014). The expression of Cytb is heavily influenced by iron, hypoxia, erythroid activity and increased systemic iron requirement (Asard *et al*., 2013). Dcytb is regulated by iron level through the action of heterodimeric transcription factors known as hypoxia-inducible factor 2- alpha (HIF2α). A low level of iron leads to direct activation of DMT1 and CYBRD1 gene by HIF2α, boosting iron absorption in the small intestine by increasing the expression of apical transporter (Mastrogiannaki *et al*., 2009). While complete ablation of Dcytb in mice results in no apparent defects in iron homeostasis when fed iron-rich or iron-deficient diets, they have less non-heme iron in the spleen and significantly decreased levels of reticulocyte mean corpuscular haemoglobin than control mice (Choi, Masaratana *et al.,* 2012).

2.9.3 Ferroportin

Ferroportin, also known as iron-regulated transporter 1 (IREG1) is a transmembrane protein encoded by the *SLC40A1* gene (solute carrier family 40, member 1), responsible for exporting iron into the bloodstream (Haile, 2000). Ferroportin level is regulated by direct interaction with hepcidin in which it binds to ferroportin and induces its internalization, subsequently decreases the export of cellular iron (Ganz & Nemeth, 2011). The expression of ferroportin is dependent on iron availability, where the expression is downregulated during iron depletion and significantly increased during iron supplementation (Sangokoya et al., 2013). Mutant mice with ferroportin deletion resulted in embryonic lethality while heterozygous mice showed mild disruption of iron homeostasis (Donovan *et al*., 2005). Ferroportin is ubiquitously expressed and most abundant on the basolateral membrane of duodenal enterocytes, hepatocytes, macrophage and adipocytes (Sangokoya *et al*.,2013).

2.9.4 Ferritin

Exist as a hallow globular protein, ferritin is an intracellular iron carrier and storage, comprised of two subunits namely heavy and light subunit. Heavy subunit is encoded by the *FTH1* gene in chromosome 11 (11q12.3), important for iron uptake into the protein, while light subunit, encoded by the *FTL* gene in chromosome 19 (19q13.3-13.4) is responsible for facilitating the formation of iron mineralization inside the protein core (Drysdale, 1988). Ferritin has a molecular weight of 474kDa and can accommodate up to 4500 iron atoms (Pantopoulos *et al.*, 2012; Ferreira *et al.*, 2000). Iron enters ferritin with the help of iron chaperon protein and when required, iron is released via ferritin degradation performed by either lysosomal or proteasomal pathways (Zhang *et al*., 2010). According to Ferreira *et al*., (2000), complete abolishment of *the Fth1* gene resulted in the increased intracellular labile iron pool and tissue damage that further led to embryonic lethality at 3.5 and 9.5 days in Fth $^{-/-}$ mice. Meanwhile, conditional Fth1 ablation triggers iron-induced oxidative damage in hepatocytes due to an inability to sequester and detoxify iron (Arosio & Levi, 2010). Even though ferritin is mostly found in the cytosol, some are being secreted into the serum, where it acts as an iron carrier. The production of ferritin is majorly triggered by the presence of iron. Hence, plasma ferritin is frequently utilised as an indication of total body iron to diagnose and monitor disease associated with both iron overload and iron deficiency. Serum ferritin level increases in response to systemic iron load or infection (Pantopoulos *et al*., 2012; Wang *et al*., 2010).

2.9.5 Transferrin

In the body, all circulating plasma iron is bound to blood plasma glycoprotein called transferrin. Transferrin is highly conserved with a molecular weight of 76kDa. In humans, transferrin is encoded by the *TF* gene located in chromosome 3 (3q22.1) (F Yang et al., 1984). Transferrin is mainly produced in the liver, however, some organs including the brain and testes also produce transferrin for local use. Transferrin exists as a bi-lobal protein consisting of a polypeptide chain with 679 amino acids and two carbohydrate chains called N- and C- termini lobes. Each lobe has a distinct affinity for binding a certain metal ion such as iron, gallium, aluminium, zinc, or cadmium. Transferrin binds to iron tightly yet reversibly and can be found in serum as non-bound (apo-Tf), monomeric or diferric form (holo-Tf) (Kawabata, 2019; Macedo & de Sousa, 2008). The main role of transferrin is to deliver and transfer iron to all tissues. It mainly delivers iron from the small intestine where iron absorption took place and from the macrophage, where iron is being recycled from senescent red blood cells. Iron bound Transferrin is internalised by receptor-mediated endocytosis via clathrin-coated pit. As free iron is toxic, transferrin chelates the free iron thus also acting as a protective scavenger and prevent the formation of reactive oxygen species (ROS). Severe mutations in transferrin lead to atransferrinaemia, a rare hereditary disorder characterised by iron overload in non-haemopoietic tissue while continues to have severe iron deficiency anaemia. This mutation has been detected in both humans and rats (Beutler *et al*., 2000; Trenor *et al*., 2000).

2.9.6 Transferrin receptor

Transferrin receptor (TfR) is a cell surface receptor, regulates the uptake of iron into cells via receptor-mediated endocytosis of iron-loaded transferrin. Encoded by the *TFRC* gene located at chromosome 3 (3q29), TfR is a homodimeric transmembrane glycoprotein. Once the halo-transferrin complex binds to TfR in the cell surface, the internalization thru clathrin-mediated endocytosis occurs, followed by the dissociation of iron from transferrin. The transferrin/TfR complex in the endosome is transported back into the cell surface and can be recycled for another cellular iron uptake. The uptake of transferrin-bound iron through TfR is the primary source of iron for almost all cells as transferrin is abundantly available and mostly unsaturated. Hence, it can sustains a relatively large amount of iron in the circulation (Recalcati *et al.*, 2017). TfR is ubiquitously expressed in all tissue at low level, with elevated level of TfR is regularly found on numerous cancer cells and during inflammation (Shen *et al*., 2018). Levy *et al.*, (1999) was the first to demonstrate that complete disruption of mouse $TfR^{-/-}$ caused embryonic lethality due to defects in erythropoietic and neurologic development while targeted ablation of $TfR^{+/-}$ is associated with microcytic hypochromic anaemia. Wang *et al.*, (2020) showed that loss of TfR selectively in hemopoiesis stem cell (HSC) significantly impaired iron uptake and markedly impairs the expansion of functional HSC in the bone marrow in mice. Mice lacking TfR in muscle also found to exhibit iron deficiency in muscle, adipose tissue and liver before rapidly developing a dramatic and lethal phenotype, while point mutations of the TfR caused microcytic anaemia that does not respond to iron therapy (Barrientos *et al*., 2015 ;Conway *et al*., 2018). TfR is not only important for iron import, but also essential in intestinal homeostasis. Inactivation of TfR in mice intestinal epithelium cells caused severe disruption in epithelial barrier and early death (Chen *et al*., 2015).

2.9.7 Hepcidin

Hepcidin is encoded by *HAMP* (hepcidin antimicrobial peptide) gene on chromosome 19 (19q13.12) and was originally known as Liver- Expressed Antimicrobial Peptide 1 (LEAP1). Hepcidin is highly expressed in the liver followed by a fair expression in the heart and brain. Hepcidin is a major regulator of systemic iron homeostasis, and its dysregulation leads to the pathogenesis of a variety of iron related disorders. Hepcidin inhibits iron circulation into the plasma by binding to ferroportin on the cell's plasma membrane, inducing lysosomal degradation. Hepcidin expression is strictly controlled at the transcriptional level and is increased by the presence of iron. Inactivation of ferroportin causes intracellular iron retention, which directly restricts iron absorption and bioavailability in the circulation (Pantopoulos *et al*., 2012). Hepcidin prevents the iron flow into blood circulation after duodenal absorption, inhibits iron release from macrophages and immobilize deposited iron in hepatocytes (Girelli *et al*., 2016). In mice, targeted mutation of the hepcidin gene resulted in severe multivesicular iron overload with elevated serum iron and ferritin levels (Lesbordes-Brion *et al*., 2006). Hepcidin KO mice also developed chronic pancreatitis (Lunova *et al*., 2017). As a hormone, hepcidin is also influenced by various stimuli. RBC transfusion, chronic kidney disease, repleted iron store, genetic factor, inflammation, and oral iron administration increases hepcidin level. Meanwhile, ineffective erythropoiesis, IDA, hypoxia, chronic liver disease, hereditary hemochromatosis decrease hepcidin level to ensure enough iron supply for erythropoiesis (Girelli *et al*., 2016). Hepcidin is influenced by many different stimuli, several stimuli may be present simultaneously thus, its production is depending on the relative strength of each other. For example, in severe iron deficiency, the hepcidin output tends to be low even though there is an inflammation (Jonker *et al*., 2013).

2.10 Date palm *(Phoenix dactylifera)*

Phoenix dactylifera, generally known as date palm is a monocotyledon plant that predominantly cultivated in the Middle East. The word *dactylifera* means "finger-bearing', referring to the clustered produced by this plant. *Dactylifera* is derived from the Greek term '*dactylus'*, which means finger and the Latin term '*ferous'* means bearing (Al-Alawi *et al*., 2017). As the authentic wild ancestor is still yet to be identified, the origin and the domestication of today's cultivated date palm remain obscure. Currently, about 5000 date varieties are grown around the globe and has been consumed as a staple food in the Middle East region and some African countries. Different regions produce different date palms variety that differ in their organoleptic, chemical and physical properties. Ajwa, Assel, Zahidi, Medjool, Deglet Nour, Mabrook, Halawi, Mariami and Zahidi are among the most commonly consumed date palm (Khalid *et al*., 2017). Date palm trees start fruiting at the age of 5 years and continue to produce until 60 years. In 2020, data from the Food Agriculture Organisation (FAO) recorded that the global production of date palm amounted to about 9.45 million metric ton, up from 9 million metric tons in 2019.

Date palm flourish in areas characterized by hot and low humidity and can be grown in a diverse type of soils with varying levels of organic and mineral nutrient. Date palm are known to be able to tolerates soil salinity better than other cultivated fruit crop. It's also confer different external (colour, texture) and internal (sweetness, chemical composition) characteristics during growth, depending on the maturity and ripeness stages (Al-Alawi *et al*., 2017). Date palm goes through five stages of formation and ripening by their Arabic names; Hababuk (post- pollination), Kimri (green, unripe), Khalal (full-size, crunchy), Rutab (ripe, soft) and Tamer (ripe, sun-dried) (Al-Mssallem *et al*., 2013) (figure 6). Date palm is typically harvested at the Rutab and Tamer stage

where they become edible as a result of decreased bitterness, increased sweetness and improves tenderness and succulence. Ripe date palm especially at the Tamer stage have considerable economic value due to their good storability which make them available for consumption in all season.

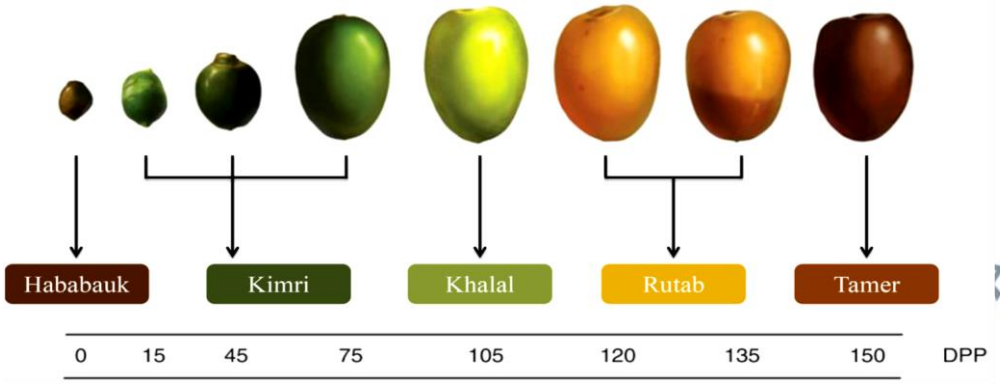

Figure 6: The growth stage of date palm fruit. The different growth stages of date palm fruit after days post pollination (DPP). (Al-Mssallem *et al*., 2013)

For Muslims, date palms are of religious value and are mentioned by the Quran and prophetic Sunnah as the ultimate superfood for the preservation of health as stated in the Al-Quran in surah An-Nahl,

"And from the fruit of the palm trees and grapevines you take intoxicant and good provision. Indeed, in that is a sign for a people who reason" (Al-Quran, 16:67).

The date palm was addressed more than 20 times, more than any other fruit plant in the Al-Quran, therefore it holds a special place in Islamic tradition and culture. The Prophet Muhammad (ﷺ) has been given high preference and significance to the date palm, urging Muslims to eat dates and maintain date orchards. The Prophet Muhammad (ﷺ) said that

"There is a tree among the trees which is similar to a Muslim (in goodness), and that is the date palm tree." (Sahih al-Bukhari, 65:359).

Due to its high natural sugar contents, date palm is considered a naturally high energy food for a quick boost. Hadith narrated by Aisha reported Prophet Muhammad (ﷺ) as saying

"A family which has dates will not be hungry" (Sahih Muslim, 23:5078).

As date palm flesh contains a predominance amount of reducing sugar that is easily digested and absorbed, it is customarily used to break the fast throughout the month of Ramadhan. The Prophet Muhammad (ﷺ) reported as saying

"If anyone of you is fasting, break your fast with dates for that is a blessing; if there are no dates, then break the fast with water as it is purifying" (Al-Tirmidhi, 975).

It is also narrated by Anas ibn Maalik that the Prophet Muhammad (ﷺ) used to break his fast with fresh dates before praying. If there were no fresh dates, then with the dried dates and if no dried dates, then with a few sips of water (Ahmad, 12265; Abu Dawood, 2356). By eating dates after fasting, the level of blood glucose will quickly return to normal, and help prepared the stomach to receive food thus preventing overeating. In Islamic tradition, the consumption of date palm has also been emphasized during pregnancy and after childbirth. According to Surah Maryam, Allah also instructed Maryam to consume freshly ripened date palm while she was experiencing pain and discomfort during the late stages of her pregnancy,

"Shake towards yourself the trunk of the palm-tree, and fresh ripe dates will come tumbling upon you. Eat and drink, and delight your eyes" (Al-Quran, 19:25).

Muslims are recommended to consume an odd number of date palms as narrated by Anas Bin Malik: The Prophet Muhammad (ﷺ) used to eat odd numbers of dates (Sahih Bukhari, 15:73). However, it is more advisable for Muslims to eat 7 date palms as The Prophet Muhammad (ﷺ) said:

"He who eats seven Ajwa dates every morning, will not be affected by poison or magic on the day he eats them". (Sahih al-Bukhari, 70:74).

The Prophet Muhammad (ﷺ) consumed varieties of date palms, however, favoured the variety known as Ajwa. Ajwa date palm is indigenously grown in Medina, Saudi Arabia. It is characterised by an oval-shaped, medium-sized date with skin colour ranging from a dark brown to almost black.

2.10.1 Biochemical composition of date palm

Depending on cultivar, stage of ripening and water content, date flesh is comprised of 50-88% sugar, 5-20% water, 3-18% crude fibre, 3% polyphenol, 1-7% protein, 1-4% pectin, 1-2.5% ash and 0.1-0.5% fat (Ashraf & Hamidi-Esfahani, 2011). Each kilogram of date palm provides approximately 1570 – 3000 calories, making it a natural energy-rich food. Date palm is a high-energy food owned to its high content of carbohydrates. In the early stages of maturity, date palm contains high sucrose which then is converted into simple reducing sugar, glucose and fructose during the ripening process which makes it very sweet (Ali *et al.*, 2012). Despite the low quantity of protein found in date palm, the protein contains amino acids vital to the human body. Date palm contains 18 types of essential amino acids such as arginine, cysteine, glycine, histidine, isoleucine, phenylalanine and proline (Hussain *et al.*, 2020). Amino acid are the building block of proteins and many other biomolecules like neurotransmitter and hormones, and play important role in cellular processes. Kwashiorkor and marasmus are among clinical disorder caused by inadequate amino acid intake (Khan *et al*., 2018). Date palm is also enriched with dietary fibre of both soluble and insoluble fibres. The primary components are cellulose, hemicellulose, pectin, lignin and insoluble proteins. The higher content of dietary fibre in date palm promotes satiety and has a laxative

effect due to its bulking capacity. Dietary fibre possess numerous therapeutic effects and has been linked to decreased incidence of constipation, helped in maintaining a healthy weight, supported the growth of intestinal microflora and lowered the risk of diabetes and heart disease (Li & Komarek, 2017).

Date palm also contains numerous minerals and vitamins. Minerals present in date palm includes potassium, phosphorus, magnesium, sodium and iron. The date palm also contains calcium, cobalt, copper, fluorine, manganese and zinc (Dghaim *et al.*, 2021). These elements are essential to the human body. Magnesium and calcium are significance for the development of healthy bone, potassium and phosphorus are important for energy metabolism, sodium is involved in the regulation of fluid and electrolyte balance, fluorine is useful for preventing tooth decay, and iron is essential for red blood cell production (Soetan *et al*., 2010). Date palm also supplies at least 6 vitamins; thiamine, vitamin A, riboflavin, pyridoxin, niacin and ascorbic acid that essential for normal human growth and development (Mirza *et al.*, 2019). Date palm also rich in phytochemicals like carotenoids, phytosterols, phenolic acids, sterols, tannin and flavonoids. Phytochemical is a plant-derived compound that has therapeutic properties that may provide beneficial effects when used as medicine or as part of one's everyday diet (Al-Alawi *et al*., 2017).

2.10.2 Health benefit of date palm

Scientific evidence proved that there is wisdom behind the prophetic practices of consuming date palm. Date palm brings about tremendous medicinal values, that are beneficial to human health. Date palm flesh possesses high sugar content that provides instant energy and is readily accessible. Consumption of date palm in late pregnancy also increased mean cervical dilatation resulting in reduced labour length process and a

significant decreased in labour augmentation (Al-Kuran *et al*., 2011; Kordi *et al.*, 2017; Kordi *et al*., 2014). A study by Razali *et al.*, (2017) demonstrated that even though there was no significant difference in the mean cervical dilatation in control and date consumed groups, the consumption of date palm indeed significantly reduced the need for labour augmentation with oxytocin. According to Royani *et al*., (2019), daily consumption of 7 Ajwa date during pregnancy has remarkable potential in decreasing the mean arterial pressure (MAP) and roll-over test (ROT) in pregnant women at risk of developing preeclampsia.

Date palm has been shown to have anti-tumour properties as it contains flavonoids, namely polyphenols, steroids and quercetin. Eid *et al*., (2014) reported that date palm extract is effective in inhibiting the growth of colon adenocarcinoma cell *in-vitro.* Consumption of date palm also increases the growth of beneficial bacterial in the colon such as bifidobacteria, thus may contribute in the maintenance of healthy bowel. A study by Siddiqui *et al.,* (2019) discovered that date palm extract was effective against human hepatocellular carcinoma HepG2 cell line. The study found that ethanolic extract of Ajwa date pulp containing active compound β-D-glucan mediated anti-proliferative effects against HepG2 cells. Several studies reported that methanolic extract of Ajwa date exhibited antimicrobial and antibacterial activity against various bacteria strain such *S. aureus, B. Cereus, S. marcescens, S. typhi, V. cholera, E. faecalis, K. pneumoniae and E. coli*. (Anwar *et al*., 2022; Abdullah *et al*., 2019; Samad *et al*., 2016). Belmir *et al.,* (2016) found out that the therapeutic index of amphotericin B, an antifungal drug, improved significantly with the date palm extract. Moreover, the aqueous extract of date palm also shown to protect human red blood cells against cytotoxicity induced by amphotericin B.

Numerous studies demonstrated organ protective effects and anti-inflammatory activity of date palm against toxicity caused by natural toxins, industrial chemicals or due to complications of drug *in vivo* and *ex vivo*. It was revealed that date palm extract had hepatoprotective effects against envenomation with *Cerastes cerastes* venom in albino rats. Rats treated with date palm extract pre or post-injection of a single dose of venom showed a significant decrease in liver injury parameters compared to venom groups without date palm (Bashandy *et al*, 2016). A study by Abdu (2018) reported that Ajwa aqueous extract has a therapeutic effects against fungal toxin ochratoxin A, in which the bilirubin and ALT enzyme activity significantly improved post-treatment. Meanwhile, daily treatment of acute diclofenac toxicity with 2g/kg orally administered Ajwa date extracts for 4 days resulted in notable improvement and reversal of diclofenac-induced pathological changes in both lung and liver in the rats (Aljuhani *et al.*, 2019). Treatment with 300mg/kg aqueous and methanolic extract of date palm for 7 days showed a marked reduction in histopathological alterations of liver tissues in rabbits subjected to azithromycin toxicity (Ahmad *et al.*, 2018). Ragab *et al.*, (2013) demonstrated that biochemical profile, serum antioxidant enzymes and histopathological changes were significantly improved after 300mg/kg of date palm extract was given to rabbits induced with lead acetate toxicity for 14 days. A study by Al-Yahya *et al.*, (2016) showed that date palm not only attenuated the cytotoxicity of dichlorofluorescein in cardiomyoblast cell lines (H9C2) but also promoted cell recovery and proliferation. The study also showed that date palm extract downregulated the expression of the proinflammatory cytokines and apoptotic markers in myocardial infarction (MI) induced rats with histopathological analysis revealed reversed myocardial membrane damage. Zhang *et al.*, (2013) showed that date palm extract was

able to inhibit lipid peroxidation (LPO), and cyclooxygenase enzymes, COX-1 and COX-2 that are responsible for initializing the inflammatory process in the body.

2.10.3 Beneficial effect of date palm on IDA

Earlier studies have proven that date palm offers anti-anaemic potential. According to Onuh *et al.,* (2012), crude methanolic and crude aqueous extracts of date palm improved RBC, Hb, PCV, reticulocytes, and platelet count in anaemic rats, suggesting that tannin, ascorbic acid and phenol contained in date palm extract may have stimulatory effects on the bone marrow to support haemopoiesis. Zen *et al.*, (2013) showed that date palm juice significantly increased Hb level in rats fed on a low iron diet. Abdelsalam *et al.*, (2014) showed a significant increase in Hb concentration in anaemic late pregnant Najdi ewes when supplemented with date palm extract. The supplementation of date palm also resulted in a high percentage of surviving lamb at birth with significant increased mean litter weight. Iron is indispensable during pregnancy for foetus development, thus providing date as high energy food for the mother would be of great beneficial impact on the foetal growth, survival, and postnatal health status. In human, numerous studies has been conducted to investigate the effect of date palm on anaemia indices. According to Irandegani *et al*., (2019), consumption of date palm in 2 months successfully increased Hb, haematocrit and serum ferritin levels in primary school children with IDA. Abdel-Rahman *et al.,* (2008) had similar finding in which consumption of 100g dates daily for 7 weeks increased Hb, iron and ferritin levels in anaemic Egyptian women. Sari *et al.,* (2018) revealed that incorporating date palm to iron supplementation raises the Hb level of teenage girls with anaemia. Meanwhile, studies by Widowati *et al*., (2019) and Mawaddah, (2020), demonstrated that the supplementation of date palm extract increases the Hb level in

anaemic pregnant women and teenage girls, respectively. These findings suggest that date palm, regardless of the variety and type of extraction is rich in beneficial nutritional contents, that supports haemoglobin and erythropoietic synthesis.

2.11 Goat milk

Goat (*Capra hircus*) was one of the earliest domesticated animals and is one of the primary suppliers of milk and meat products for human use. According to Food and Agriculture Organization of the United Nations (FAO) data, the worldwide dairy goat population was predicted to be 218 million in 2017, with a goat milk volume of roughly 18.7 million tons, accounting for 2% of total global milk production (Miller & Lu, 2019). The majority of dairy milk comes from Anglo-Nubian, British Alpine, Toggenburg and Saanen breeds and is commonly processed into cheese, butter, ice cream, yoghurt and other products. Milk in general has been consumed by many Muslims, following the sunnah of The Prophet Muhammad (ﷺ). Milk was mentioned several times in Quran and they are promised in abundance in the Paradise Hereafter;

"And indeed, for you in grazing livestock is a lesson. We give you drink from what is in their bellies-between excretion and blood- pure milk, palatable to drinkers" (Al-Quran, 16:66)

"Is the description of Paradise, which the righteous are promised, wherein are rivers of water unaltered, rivers of milk the taste of which never changes, rivers of wine delicious to those who drink, and rivers of purified honey, in which they will have from all [kinds of] fruits and forgiveness from their Lord, like [that of] those who abide eternally in the Fire and are given to drink scalding water that will sever their intestines?" (Al-Quran, 47:15)

Milk is packed with excellent constituents and has favourable effects on human health considering the total solid, fat, protein, mineral and vitamin, supplying energy and nutrients required for growth and development. Narrated Abdullah ibn Abbas:

The Prophet Muhammad (ﷺ) said*: "When one of you eats food, he should say: O Allah, bless us in it, and give us food (or nourishment) better than it. When he is given milk to drink, he should say: O Allah! bless us in it and give us more of it, for no food or drink satisfies like milk".* (Abu Dawud, 20:3721)

The Prophet Muhammad (ﷺ) regularly drinks milk, especially when food was scarce. Raw milk from camel, goat and sheep were consumed regularly by The Prophet Muhammad (ﷺ) (Akgun & Ozturk, 2017). Abu Bakr Siddiq narrated,

"As we went along with Allah's Messenger (ﷺ) *from Mecca to Medina, we passed by a shepherd and Allah's Messenger* (ﷺ) *was feeling thirsty. He (Abu Bakr Siddiq) said: I milked for him a small quantity of milk (from his goat) and brought it to him (Prophet Muhammad* (ﷺ)*, and he drank it and I was very happy".* (Sahih Muslim, 36:112)

2.11.1 Biochemical composition of goat milk

The physical characteristics and composition of goat milk varied according to breed, lactation periods, age, the health status of the udder, diet and environmental conditions. Physically, fresh goat milk is white, opaque, and has a hint of sweet taste and alkaline in nature. Goat milk has an average of 13.2% total solids, which includes 4.5% fat, 3.6% protein, 4.3% lactose, and 0.8% minerals. (Turkmen, 2017). Lipid is one of the most essential components in milk since it influences the price, nutritional value, physical and sensory aspects of dairy products. The fat present in milk is dispersed in a form of emulsified globules of 3.5μm in size, smaller than that of 4.5 μm cow's milk. The fat globules with smaller size give a better dispersion and more homogenous

mixture of fat, giving a smoother texture in goat milk's derived products (Park, 2017). The chemical and physical features of goat milk fat globules are comparable to those of cow milk, but it lacks agglutinin, a protein that causes fat globules to coagulate and aggregate, particularly at a low temperature. Goat milk fat is enriched in short and medium-chain fatty acids (FA) like butyric, lauric, linoleic acid, caproic, caprylic and capric. These short and medium-chain fatty acids contribute to a larger surface area for digestive action by lipase, allowing an easy digestion and absorption of milk fat. They are also the reason for the 'goaty' odour characteristic (Kalyankar *et al.*, 2016). Goat milk contains a greater concentration of conjugated linoleic acids than cow milk, which has been shown in animal studies to offer health benefits such as antioxidants, anti-inflammatory, anti-carcinogenic and anti-hypertensive. Goat milk is also enriched in medium-chain triglycerides (MCT), which consists of saturated fatty acids with 6-10 carbon chain, that is rapidly metabolized to provide energy (Turkmen, 2017).

Lactose is the primary carbohydrate in goat milk, produced in the mammary gland and is made up of glucose and galactose. Goat milk contains about 0.2-0.5% less lactose content than that of cow milk. Other small amounts of carbohydrates found in goat milk include oligosaccharides, glycopeptides, glycol-proteins, and nucleotides sugar. Milk oligosaccharide has been widely recognised for its antigenic characteristics and is valuable in stimulating the growth of beneficial gut flora (Kalyankar *et al.*, 2016). Protein in goat milk is composed of casein and whey protein. Casein is a family of related phosphoproteins and comprised of four different fractions namely αS1-casein, αS2- casein, κ- casein and β- casein. Meanwhile, the portion of milk proteins that remains soluble in milk serum following casein precipitation at 20°C, pH 4.6 is called whey protein. It is also known as the by-product of cheeses or casein manufacturing. A major component of this fraction are β-lactoglobulin, α-lactoalbumin, serum albumin

and protease-peptone fraction and immunoglobulin (Ig) (Farrell *et al.,* 2004). Goat milk has more calcium, potassium, chlorine and phosphorus than cow or human milk. Although the micromineral level may not fluctuate substantially, the amount can vary according to breed type, diet, and lactation stages. Goats convert all β-carotene in milk into Vitamin A, resulting in milk that is entirely white and containing more Vitamin A than cow milk. Additionally, it also provides ample amount of micronutrient such as niacin, thiamine and riboflavin that are crucial for energy metabolism (Turkmen, 2017).

2.11.2 Health Benefit of goat milk

The most intriguing aspect of goat milk is its exceptional digestibility. The high proportion of small size fat globules in goat milk contributes to greater fat dispersion and a more homogeneous mixing of fat in the milk, resulting in efficient digestibility. This smaller size, paired with the lack of agglutinin, a protein that causes fat molecules to coagulate together, increases the surface area of globules that come into touch with pancreatic lipase, resulting in more efficient digestion and enhanced lipid metabolism (Park, 2017). Goat milk also contains a high proportion of medium-chain triglycerides (MCT). MCT is a form of saturated fatty acids containing 6-12 carbons atoms that are easily digested and effectively burned by the body for energy. Due to its small size, MCT is absorbed intact directly into the bloodstream, and rapidly transported to the liver mitochondria for β- oxidation to produce a rapid energy discharge (Neha & Berkeley, 2017). Cow milk allergy is the most frequent food allergy in the paediatric population (Høst, 2002; Lifschitz & Szajewska, 2015). It is caused by the allergens αS1-casein and β -lactoglobulin, which trigger the body to develop IgE antibodies, which then initiate an immunological response that produces symptoms including dermatitis, asthma, wheezing, gastrointestinal issues, or anaphylaxis (Hochwallner et al., 2014).

Even though heat denaturation can destroy some of β-lactoglobulin allergy-causing properties, αS1- casein, however, can retain its binding capability to IgE even after a strong denaturing process. Thus, due to its naturally low αS1- casein level, goat milk has been suggested as an alternative for new-borns and children who are sensitive to S1-casein in cow milk.

Goat milk is rich in oligosaccharides with a structure similar to human milk. Oligosaccharides are resilient to gastric acidity and enzymes hydrolyses in the colon, and hence may be fermented by intestinal biota, influencing the composition, activity and growth of intestinal bacteria. Daddaoua *et al.*, (2006) showed the anti-inflammatory effects of goat milk oligosaccharides in colonic inflammation induced by the trinitro benzenesulfonic acid (TNBS) in rats. The supplementation of 500mg/kg goat milk oligosaccharides for 6 days significantly reduced the expression of predominant cytokines iNOS, COX2 and IL-1β. Additionally, Medeiros *et al.*, (2018), demonstrated that dried goat whey successfully modulate the memory and cortical spreading depression in rats. They suggested that salicylic acid, taurine, and linoleic acid in dried goat whey played a critical role in supporting brain development and body weight improvement in malnourished rats.

Raw goat milk has a very rich and complex autochthonous microbiota. Milk in the healthy udder cells are sterile but thereafter quickly colonised by microorganisms through various resources, from teat apex, milking equipment, feeding and environments (Quigley *et al.*, 2013). These microbes can either promote health and enhance food safety or cause milk spoilage due to improper handling. Goat milk contains several bacteria families renowned for their probiotic benefits such as *Lactobacillus, Lactococcus* and *Leuconostoc* (Quigley *et al.*, 2013). Cavicchioli *et al.*, (2018) revealed that bacteriocins from *L. lactis subsp lactis* and *E. durans* strain isolated

from goat milk possess antiviral activity that can inhibit the poliovirus (PV-1) and herpes simplex virus 1 (HSV-1). Goat milk and its derivatives exert a protective effects against parasites *H. contortus* (Alimi *et al.*, 2018) and wide range of bacteria including *S. aureus, B. cereus, E. coli, K. pneumoniae, S. marcescens, L. monocytogenes, P. aeruginosa, L. inoccua, , S. flexneri, and E. cloacae* (Hernández-Saldaña *et al*.,2016).

2.11.3 Beneficial effect of goat milk on IDA

Díaz-Castro *et al.,* (2014) reported that the iron transporter, divalent metal transporter-1 (DMT1) was significantly upregulated in anaemic rats treated with goat milk compared with cow milk and normal diet. A study by López-Aliaga *et al*., (2018) showed that consumption of fermented goat milk lowered the expression of pro-inflammatory cytokines and improves the haematological status of anaemic rats during anaemia recovery. According to Alférez *et al.,* (2019), fermented goat milk significantly improves haematological parameters and promotes positive metabolic responses that may minimise cardiovascular risk and vascular damage during anaemia recovery and iron overload. Moreno-Fernández *et al*., (2019) demonstrated that goat milk may also exerts a potential neuroprotective effect as consumption of fermented goat milk during anaemia recovery improved brain molecular parameters in IDA rats.

Milk is rich in calcium that is beneficial for bone mineralization, preventing osteoporosis. Calcium, however, may have a possible detrimental effect on other divalent metals as calcium interferes with dietary minerals absorption. Even though the exact mechanism is yet to be elucidated, several studies have demonstrated that the inclusion of calcium in the diet reduces iron bioavailability (Candia *et al*., 2018; Mónica *et al*., 2018; Thompson *et al*., 2010). However, a study by López-Aliaga *et al.,* (2009) revealed that the consumption of calcium-supplemented goat milk by anaemic rat had

no deleterious impacts on iron absorption. They find that, despite its high calcium content, goat milk minimises calcium-iron interaction and so has no negative effect on iron absorption. Similar results were reported by Díaz-Castro *et al*., (2011), in which they discovered that not only does calcium-fortified goat milk favour iron deficiency anaemia recovery, but it also increases the bioavailability of copper, a mineral essential for erythropoiesis. Prior studies have shown that goat milk provides a better use of iron, which minimizes possible interaction between iron and other minerals and hence, improve the digestion and utilization of copper, zinc, magnesium, phosphorus and selenium in animal models (Barrionuevo *et al*., 2002, 2003; Campos *et al*., 2003).

Numerous studies have revealed the beneficial effects of date palm and goat milk both *in- vitro* and *in- vivo*. Consequently, researches on the disease curing potentials of both date palm and goat milk are has been steadily rising in recent decades. The purpose of this research is to investigate the potential value of both date palm and goat milk, with a focus on the benefit of iron deficient anaemia.