CHAPTER 4

RESULT

4.1 Systematic Review

4.1.1 Effects of date palm (*Phoenix dactylifera)* in iron deficiency anaemia

The database search identified 3630 titles of potentially relevant articles. Another 11 titles were located through hand search from the reference section of earlier reviews. A total of 2533articles were retrieved for abstract review (**Figure 4.1**) by using PRISMA guidelines (Moher *et al*., 2009). Screening of the titles and abstracts resulted in the selection of 52 articles for full-text review. Forty-nine of these articles did not meet the inclusion criteria resulting in a final inclusion of only 3 articles. The characteristics of the included studies are shown in **Table 4.1**.

Table 4.1: Characteristics of included studies (in chronological order) on effects of date palm (*Phoenix dactyllifera*) in iron deficiency anaemia

First Author& Year	Extracts Of Dates	Animals model	Types Of Study	Experimental design	Duration of exposure	Sample collection	Parameters	Result
Onuh *et al.*, 2012	• Aqueous extract of dates • Methanolic extract of dates	50 Wistar rats weighing between 100 to 200 grams and aged between 2 to 3 months from Animal House of College of Medicine, University of Nigeria Enugu Campus	Experimental study	• 2 groups were orally administered with dimethyl sulfoxide (DMSO)(n = 10) • 4 groups were orally administered with graded doses of the crude aqueous extract of dates (n = 20) • 4 groups were orally administered with graded doses of the crude methanolic extract of dates (n = 20)	112 days	Blood samples were collected from retro bulbar plexus of the median canthus of the eye	Red blood cell count, haemoglobin, packed cell volume, reticulocyte, platelet count, total white blood cell count, differential white blood cellcount and bonemarrow count.	• Significant increase (p<0.001), in red blood cell count, haemoglobin packed cell volume, reticulocyte and platelet count • Total white blood cell count, differential white blood cellcount and bone marrow count did not differ significantly (p>0.05)

Zen *et al.*, 2013	• Aqueous extract of dates	24 Wistar rats weighing 200 grammes from Laboratory Study Center for Food and Nutrition, University of Gadjah Mada Yogyakarta	Experimental study	• Group 1 was orally administered with standard diet and distilled water (n = 6) • Group 2 were orally administered with low Fe diet and distilled water (n = 6) • Group 3 were orally administered with low Fe diet and distilled water plus date juice at concentration 50% (n = 6) • Group 4 were orally administered with low Fe diet and distilled water plus date juice at concentration • 100% (n = 6)	14 days	Blood samples collected from retro-orbital plexus	Haemoglobin level	• Date extract increases the haemoglobin level (9.25±0.15 g/dL, 10.35±0.14 g/dL) significantly ($p<0.05$) as compared to low iron diet group(7.72±0.17 g/dL)
Abdelsalam *et al.*, 2014	• Aqueous extract of dates	20 mature late-pregnant (≈60 days prepartum) Najdi ewes.	Experimental study	• Group 1 was orally administered with regular commercial pellet plus alfalfa hay (n = 5) • Group 2 were orally administered with palm Sukkary dates aqueous extract (n = 5) • Group 3 were orally administered with probiotic stirred yoghurt (n = 5) • Group 4 were orally administered with palm Sukkary dates aqueous extract and probiotic stirred yoghurt (n = 5)	30 days	Blood sample taken via jugular vein	Red blood cell count,haemoglobin, packed cell volume, reticulocyte, platelet count, total white blood cell count, differential white blood cell count	• Dates extract significantly ($p<0.01$) increase haemoglobin concentration (10.74±0.2 g/dL) • No significant ($p>0.05$) change in red blood cell count, packed cell volume and platelet count • Total white blood cell count did not differ significantly ($p>0.05$)

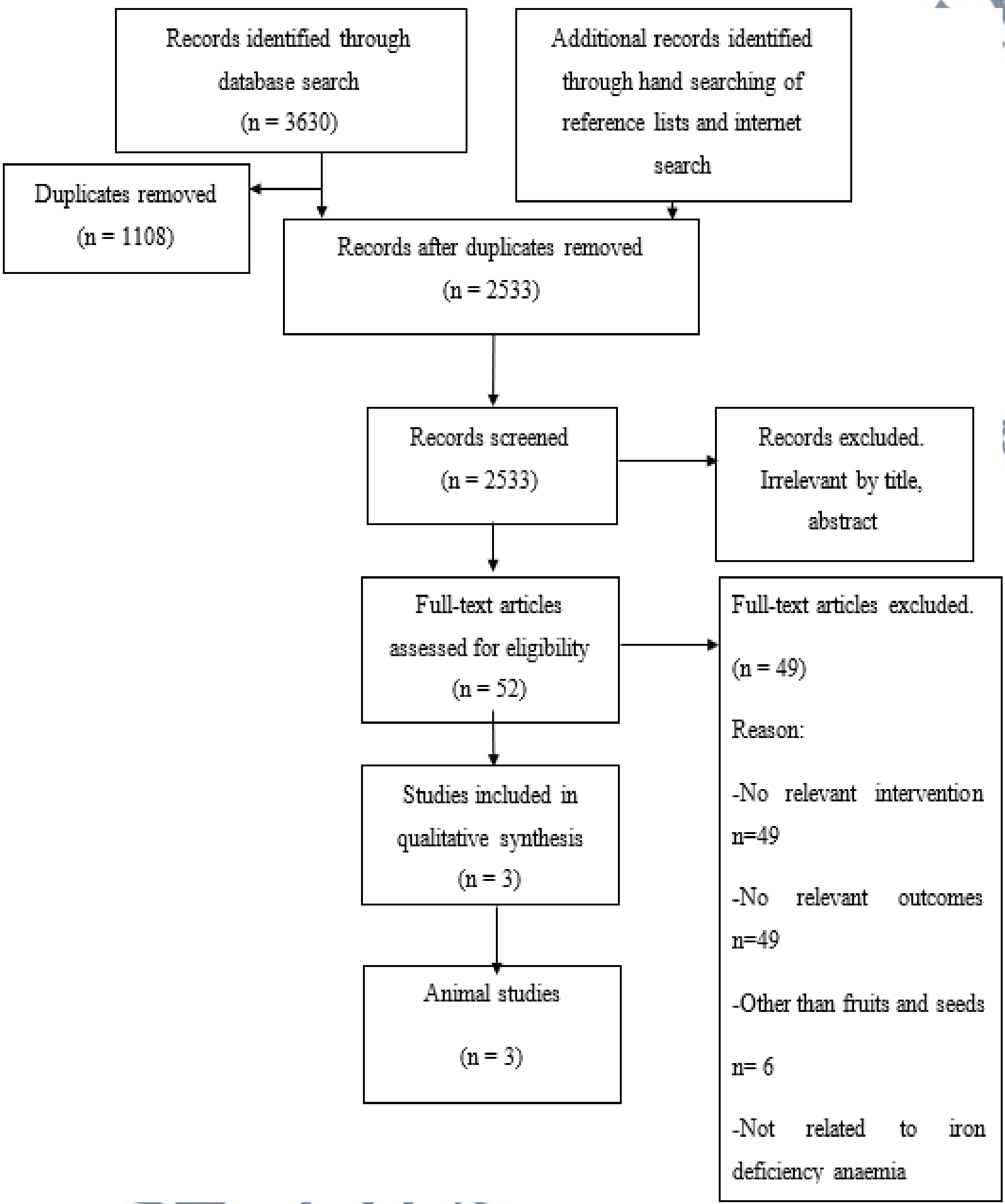

Figure 4.1: Flow chart of search strategy and selection process on effects of date palm (*Phoenix dactylifera*) in iron deficiency anaemia

All of the selected experimental studies involved the use of date palm extract (Onuh *et al.*, 2012; Zen *et al.*, 2013; Abdelsalam *et al.*, 2014). Only one study stated thetype of date palm being used, which is dried Sukkary Dates (Abdelsalam *et al.*, 2014). Aqueous date palm extract has been used in all of these studies which are prepared byremoving the seeds and blending it with distilled water. One of the studies used two types of date palm extract simultaneously which were the aqueous extract and methanolic extract (Onuh *et al.*, 2012). Two studies used only date palm extract in the experiment (Onuh *et al.*, 2012; Zen *et al.*, 2013) while the other study used a combination of date palm extract with probiotic stirred yoghurt from cow's milk in theexperiment (Abdelsalam *et al.*, 2014).

Two of the studies used Wistar rats weighing between 100 to 200 grammes and aged between two to three months as subjects (Onuh *et al.*, 2012; Zen *et al.*, 2013). While the other study involved the use of mature late-pregnant (≈60 days prepartum) *Najdi* ewes as subjects (Abdelsalam *et al.*, 2014). Red blood cell count, haemoglobin, packed cell volume, reticulocyte, platelet count, total white blood cell count and differential white blood cell count were measured as stated by Abdelsalam *et al.*, (2014).Onuh *et al.*, (2012) included the same parameters with an addition of bone marrow count. The third study only involved haemoglobin level measurement using cyanmethemoglobin method (Zen *et al.*, 2013). All of the selected studies clearly statedthe positive effects of date palm towards increasing haemoglobin level and reducing anaemia.

Abdelsalam *et al.*, (2014) demonstrated that date palm extract significantly increases the haemoglobin level by 13% as compared to the control. Zen *et al.*, (2013)and Onuh *et al.*, (2012) also reported a significant increased of haemoglobin level ($p<0.05$) in subjects treated with date palm extract. Treatment with date palm extract shows no effect ($p>0.05$) on total white blood cell (Onuh *et al.*, 2012; Abdelsalam *et al.*, 2014). While reporting a significant increase ($p<0.05$) in red blood cell count, packed cell volume and platelet count, Onuh *et al.*, (2012) however found that the differential white blood cell count and bone marrow findings did not differ significantly ($p>0.05$).

4.1.2 Effects of goat milk in iron deficiency anaemia

The database search identified 5027 titles of potentially relevant articles. Another 8 titles were located through hand search from the reference section of earlier reviews. Atotal of 3624 articles were retrieved for abstract review (**Figure 4.2**). Screening the titles and abstracts resulted in selection of 18 articles for full-text review. Ten of these articles did not meet the inclusion criteria resulting in a final inclusion of only 8 articles.The characteristics of included studies are shown in **Table 4.2.**

Table 4.2: Characteristics of included studies (in chronological order) on effects of goat milk in iron deficiency anaemia

First Author& Year	Type of goat milk	Animals Model	Types Of Study	Experimental design	Duration of exposure	Sample	Parameter	Result
Park *et al.*, 1986	Skim powdered goat milk SGM) and whole goat milk (WGM) of Alpine andNubian breeds goat	63 Sprague Dawley rats, male from Simonsen Laboratories, Gilroy, CA	Experimental study	• Group 1 were orally administered with WGM(n = 9) • Group 2 were orally administered with WGM supplemented with 50 ppm ferrous sulphate (n = 9) • Group 3 were orally administered with WGM supplemented with 100 ppm ferrous sulphate (n = 9) • Group 4 were orally administered with WGM supplemented with 200 ppm ferrous sulphate (n = 9) • Group 5 were orally administered with wholecow milk (n = 9) • Group 6 were orally administered with SGM(n = 9) • Group 7 were orally administered with skim cow milk (n = 9)	10 days	Blood samples collected from retro-ocular capillary of the eye	Total Fe intake(mg), haemoglobin (g/dl), haemoglobin regeneration efficiencies and liver Fe (ppm)	• The respective haemoglobin regeneration efficiencies (HRE) for group fed with goat milk (50.6%, 26.0%) greater thancow milk (13.1%, 13.0%) • The WGM group with highest Fe supplementation had the highest Fe bioavailability

López-Aliaga *et al.*, 2000	Lyophilized goat milk	35 albino Wistar rats, male, with initial body weight 177 grams from University of Granada Laboratory Animal Service	Experimental study	• Group 1 were orally administered with standard diet (n = 11) • Group 2 were orally administered with cow milk diet (n = 10) • Group 3 were orally administered goat milk diet (n = 14)	30 days	Urine, faeces, liver, spleen, both femora, sternum and longissimus dorsi muscle	• Digestive utilization of Fe (food intake (g), apparent digestibility coefficient (%), Fe intake (µg), faecal Fe (µg), absorbed Fe (µg), Fe balance (µg), retention/intake (%) and urinary Fe (µg)) • Fe concentration (µg /g) in organ (liver, spleen and sternum)	• Apparent digestibility coefficient of Fe greater for goat milk (26.64 ± 1.72 %) then cow milk 19.14 ± 1.15 %) • Fe balance,retention of Fe greater in goat milk diet (127.4 ± 9.37 µg) than cow milk diet (111.2 ± 7.77 µg) • The Fe content in the liver and spleen is greater with the goat milk diet (230.3 ± 9.0 and 1284.9 ± • 28.2 µg/g), than cow milk diet (198.3 ± 4.4 and 1102.8 ± 26.7 ****µg/g

Barrionevo *et al.*, 2002	Lyophilized goat milk	69 albino Wistar rats, male,with initial body weight 177 grams from University of Granada Laboratory Animal Service.	Experimentalstudy	• Group T-S: transected rats, standard diet (n = 11) • Group R-S: resected rats,standard diet (n = 13) • Group T-C: transected rats, cow milk diet (n =10) • Group R-C: resected rats,cow milk diet (n = 11) • Group T-G: transected rats, goat milk diet (n =14) • Group R-G: resected rats,goat milk diet (n = 10)	30 days	Urine, faeces, kidney, liver, spleen,both femora, sternum and heart	• Digestive utilization of Fe(food intake (g), apparent digestibility coefficient (%), Fe intake (µg), faecal Fe (µg), absorbed Fe (µg), Fe balance(µg), retention/intake (%) and urinary Fe (µg)) • Fe concentration inorgan (µg /g) (liver, spleen,heart, femur and sternum)	• In the transected and resected rats, the • Apparent digestibility coefficient of Fe was highest with the goatmilk diet (26.6 ± 1.7 %, 16.1 ± 1.2 %), followed by the standard diet (22.1 ± 1.1 %, 12.5 ± 1.1 %) and lowest with thecow milk diet (18.1 ± 1.1 %, 9.2 ± 0.7 %). • Transected and resected rats fed with goat milk diet, the deposit of Fe in the organs were greater than with those fed the cow milk diet and standard diet

Alférez et al., 2006	Lyophilized goat milk	59 albino Wistar rats, male, with age ~3 week from University of Granada Laboratory Animal Service	Experimental study	• Anaemic group administered withstandard diet • Anaemic group administered with goatmilk diet • Anaemic group administered with cowmilk diet • Control group administered withstandard diet • Control group administered with goatmilk diet • Control group administered with cowmilk diet	14 days	Blood from caudal vein, urine, faeces, liver, spleen, both femora and sternum	• Digestive utilization of Fe(food intake (g), apparent digestibility coefficient (%), Fe intake (µg), faecal Fe (µg), absorbed Fe (µg), Fe balance (µg), retention/intake (%) and urinary Fe (µg)) • Fe concentration in organ (µg /g) (liver, spleen, femur and sternum) • Haemoglobin regeneration efficiency (HRE) • Haematological parameters(serum Fe , red blood cell, packed cell volume and platelet)	• Goat milk improvedFe metabolism in Fe-deficient rats, leading to a higher Fe content in the spleen (718.8 ± 68.5mg/kg), liver (367.8 ± 38.6 mg/kg) , sternum (111.3 ±2.7 mg/kg) and femur (120.9 ± 8.1 mg/kg)in comparison with cow milk (spleen = 405.0 ± 54.3 mg/kg;liver = 338.9 ± 19.7mg/kg; sternum = 78.9 ± 2.5 mg/kg; femur = 80.4 ± 2.9mg/kg) . • Higher HRE (35.8 ± 1.6 %) , serum Fe (1731 ± 90 µg/L), red blood cells (8.24± 0.19x1012 /L) and packed cell volume levels (42 ± 1.4 %) and lower platelet count (886 ± 50 %) after anaemic rat treated with goat milk.

Nestares *et al.*, 2008	Lyophilized goat milk	60 albino Wistarrats, male, withage ~3 weekfrom Universityof Granada Laboratory Animal Service	Experimental study	• Anaemic group administered withstandard diet • Anaemic group administered with goatmilk diet • Anaemic group administered with cowmilk diet • Control group administered withstandard diet • Control group administered with goatmilk diet • Control group administered with cowmilk diet	14 days	Blood from abdominal aorta, liver, spleen, both femora and sternum	• Digestive utilization of Fe(food intake (g), apparent digestibility coefficient (%), Fe intake (µg), faecal Fe (µg), absorbed Fe (µg), Fe balance(µg), retention/intake (%) and urinary Fe (µg)) • Fe concentration in organ (µg /g) (liver, spleen, femur and sternum) • Haemoglobin regeneration efficiency (HRE) • Haematological parameters (serum Fe , serum ferritin, transferrin saturation, total Fe binding capacity, red blood cell, haematocrit, haemoglobin concentration and platelet)	• Apparent digestibility coefficient (52.6± 0.9 %) and the Fe retention/intake (R/I) ratio (51.5± 0.8 %) higher inanaemic rats fed goat milk diet despite high-Ca content. • Fe-deficient rats with respect to their controls, both fed with double-Ca, showed higher values of RBC (7.25 ± 0.20 $x10^{12}$ /L) and haematocrit (36.6 ± 1.1 %) for goat milk diets • Ca enrichment decreased Fe stores in liver and sternumin anaemic rats fed cow milk diet, however goat milk diet did not modifyFe content in the organs studied in anaemic rats. • Serum ferritin (98.0 ± 2.0, 90.2 ± 2.6 µg/L) was always higher in rats fed goat milk diet in anaemic rats fed either normal or Ca-enriched diets.

López-Aliaga *et al.*, 2009	Lyophilized goat milk	40 albino Wistarrats, male, with age ~3 week from Universityof Granada Laboratory Animal Service	Experimental study	• Anaemic group administered withstandard diet • Anaemic group administered with goatmilk diet • Anaemic group administered with cowmilk diet • Control group administered withstandard diet • Control group administered with goatmilk diet • Control group administered with cowmilk diet	14 days	Blood from abdominal aorta,urine andfaeces	• Digestive utilization of Fe(apparent digestibility coefficient (%), Fe intake (µg), faecal Fe (µg), retention/intake (%) urinary Fe (µg) and Fe retention/intake ratio (R/I)	• ADC (54.47 ± 1.0 %) and Fe R/I ratio(55.02 ± 1.0 %) were higher in the anaemic rats fed thegoat milk diet than among those fed thecow milk diet (ADC = 37.62 ± 1.6 %; Fe R/I ratio = 37.09 ± 1.6 %)

Diaz-Castro *et al.*, 2011	Lyophilized goat milk	180 albino Wistar rats, male, with age ~3 week from University of Granada Laboratory Animal Service	Experimental study	• Anaemic group administered with goatmilk and normal Fe content • Anaemic group administered with goat milk diet and Fe overload • Anaemic group administered with cow milk and normal Fe content • Anaemic group administered with cow milk diet and Fe overload • Control group administered with goat milk and normal Fe content • Control group administered with goat milk diet and Fe overload • Control group administered with cow milk and normal Fe content • Control group administered with cow milk diet and Fe overload	50 days	Blood from caudal veinand abdominal aorta, both femur and sternum	• Mineral contentin bone tissue (sternum and femur)	• Increase of Fe in sternum, which indicates that the hematopoietic process essentially recovered earlier onthe goat milk diet compared with the cow milk diet

Diaz Castro *et al.*, 2014	Lyophilized goat milk	60 albino Wistarrats, male, with age ~3 weekfrom Universityof Granada Laboratory Animal Service	Experimental study	• Anaemic group administered withstandard diet • Anaemic group administered with goatmilk diet • Anaemic group administered with cowmilk diet • Control group administered withstandard diet • Control group administered with goatmilk diet • Control group administered with cow milk diet	30 days	Blood from abdominal aorta and liver	• Haematology parameter (haemoglobin concentration, RBC, haematocrit, mean cell volume, platelets,serum Fe , total Fe binding capacity, transferrin saturation, serumferritin, serum hepcidin) • Liver Fe content • Divalent metal transporter 1 (DMT1)	• Goat milk increasedthe red blood cell count ($7.71 \pm 0.28 \times 10^{12}$/L and serum hepcidin ($14.30 \pm 0.70$ ng/mL) in anaemic rat • Goat milk restore Fe content (309.91 ± 28.35 µg/g) inliver and hematological parameters in anaemic rat. • Goat milk significantly ($P < 0.05$) increase expression of DMT1 in anaemic rat (145.26%)

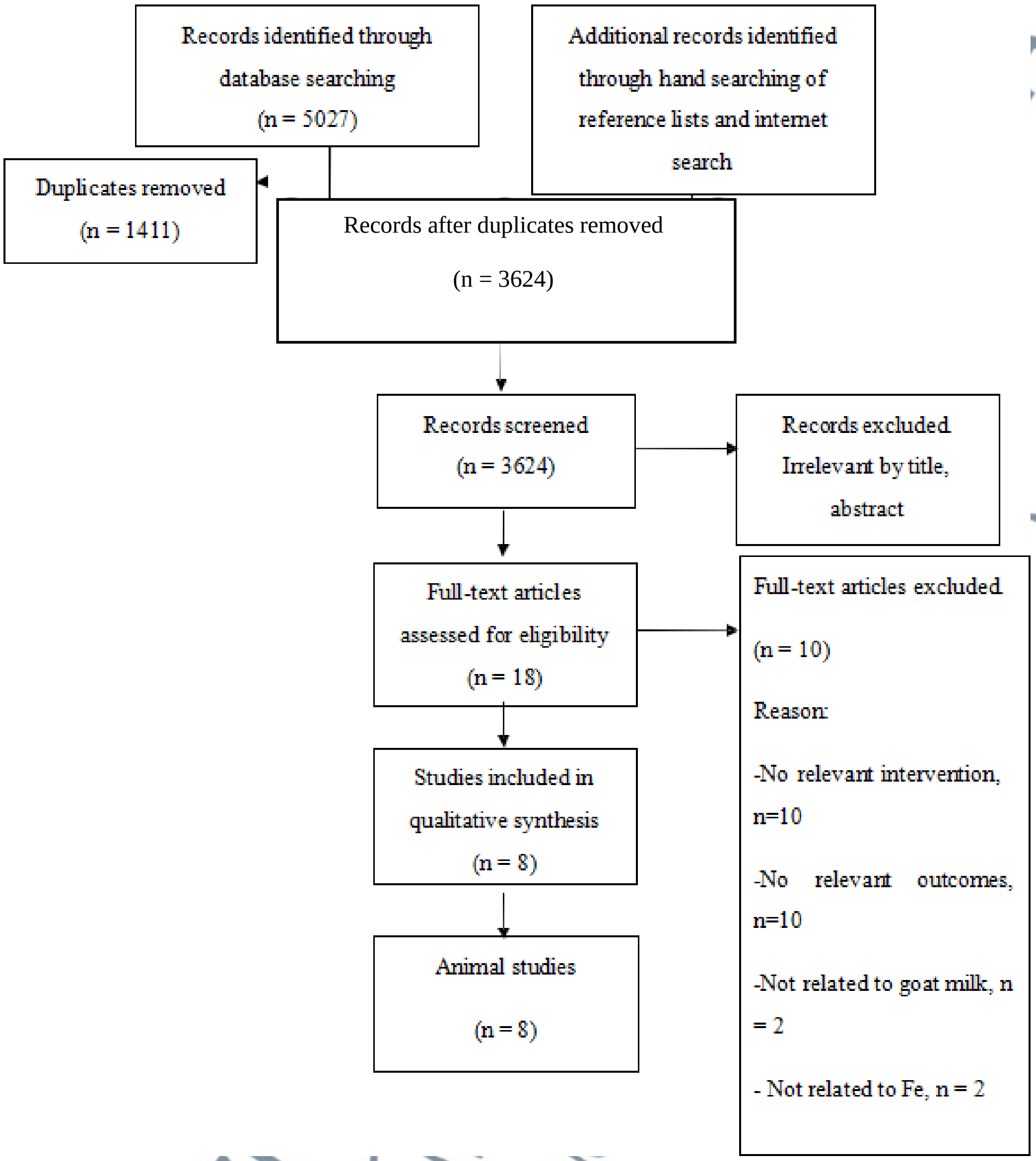

Figure 4.2: Flow chart of search strategy and selection process on effects of goat milk in iron deficiency anaemia

The selected experimental studies generally involved the inclusion of goat's milk, cow's milk and standard diets recommended by American Institute of Nutrition (AIN) to study the iron metabolism in rats. Park *et al.*, and Diaz-Castro *et al.* however, implemented the usage of cow's milk and goat's milk respectively, with and without iron overload. Park *et al.* used ferrous sulphate while Diaz-Castro *et al.*, used ferric citrate as the iron source. Of the eight selected articles, one study used skim powdered milk and whole goat's milk while the others commonly used lyophilized goat's milk.

Albino Wistar rats with initial weight of approximately 177 gram was used by two studies, five studies used albino Wistar rats with age of approximately 3 weeks while one studyused Sprague-Dawley rats. Total iron intake, haemoglobin level, haemoglobin regeneration efficiency and concentration of liver iron are among the parameters included in the study conducted by Park *et al.*

López-Aliaga *et al.*, Barrionuevo *et al.*, Alférez *et al.*, Nestares *et al.*, López-Aliaga *et al.*, and Diaz-Castro *et al.* included total iron intake with other additional parameters such as digestive utilization of iron, apparent digestibility coefficient, faecal iron, absorbed iron, iron balance, retention/intake and urinary iron while observing the iron concentration in specific organ suchas liver, spleen, heart, femur and sternum. Haemoglobin regeneration efficiency and haematological parameters such as serum iron, red blood cell count, packed cell volume, platelet serum iron, total iron binding capacity, transferrin saturation, serum ferritin, and serumhepcidin were also included.

Park *et al.*, and Alférez *et al.* reported that iron deficient rats treated with goat's milk showed higher haemoglobin regeneration efficiencies than those treated with cow's milk or normal diet. This was evident by the increased serum haemoglobin level, red blood cell count,packed cell volume, haematocrit and mean cell volume. Diaz *et al.,* found that the divalent metal transporter -1 (DMT-1) receptor which plays an important role in iron absorption in the small intestine, was significantly up-regulated in anaemic rats treated with goat's milk compared with cow's milk or normal diet. This indicates an early induction of erythropoiesis by goat's milk. These findings were more significant when whole goat's milk was compared with skim powdered goat's milk.

Goat's milk supplementation in iron deficient rats treated with iron therapy did not affect iron bioavailability. However, it was observed that iron bioavailability is low when these rats were treated with cow's milk.

The levels of serum iron, ferritin, hepcidin and iron stores in liver, spleen and bone marrow of iron deficient rats were significantly improved when treated with goat's milk. These findings were less significant in groups treated with cow's milk or standard diet. In normal rats, iron stores were reduced in the group treated with high calcium diet and cow's milk. However,the iron stores were not affected in subjects supplemented with high calcium diet and Goat milk.

4.2 Nutritional analysis

Both types of dates and goat milk were analyzed for sugar profile, vitamin c and minerals asdescribed in **Section 3.4** by using High Performance Liquid Chromatography (HPLC) for sugar profile and vitamin C while minerals using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). **Table 4.3** shows the results of nutritional analysis of two varieties of date palm and goat milk.

Table 4.3: Nutritional analysis of date palm and goat milk

Parameters	**Result**			
	Ajwa Date	**Mariami Date**	**Goat Milk**	**Goat Milk Fed Ajwa Seed**
Glucose	31.60 g/100g	32.90 g/100g	ND (<0.50)	ND (<0.50)
Sucrose	ND (<0.50)	ND (<0.50)	ND (<0.50)	ND (<0.50)
Galactose	ND (<0.50)	ND (<0.50)	ND (<0.50)	ND (<0.50)
Fructose	29.50 g/100g	31.20 g/100g	ND (<0.50)	ND (<0.50)
Lactose	ND (<0.50)	ND (<0.50)	3.38 g/100ml	4.63 g/100ml
Maltose	ND (<0.50)	ND (<0.50)	ND (<0.50)	ND (<0.50)
Iron	10.70 mg/kg	7.47 mg/kg	0.90 mg/L	0.28 mg/L
Manganese	5.23 mg/kg	5.42 mg/kg	ND (<0.10)	0.20 mg/L
Zinc	2.39 mg/kg	3.40 mg/kg	0.99 mg/L	3.58 mg/L
Copper	ND (<0.10)	2.39 mg/kg	ND (<0.10)	ND (<0.10)
Calcium	536.00 mg/kg	482.00 mg/kg	72.2 mg/100ml	86.0 mg/100ml
Magnesium	418.00 mg/kg	451.00 mg/kg	10.00 mg/100ml	9.29 mg/100ml
Vitamin C	9.80 mg/100g	5.49 mg/100g	ND (<0.10)	ND (<0.10)

ND indicate not determined

a) Sugar content

Results showed that both date palm varieties were rich in reducing sugar (glucose and fructose). The values were 31.60g/100g (glucose) and 29.5g/100g (fructose) for Ajwa date and 32.90g/100g (glucose) and 31.2g/100g (fructose) for Mariami date. At the same time, they presented a weak content of sucrose, galactose, lactose and maltose (all < 0.50g/100g). Goat milk fed Ajwa seed showed poor reducing and non-reducing sugar levels below < 0.50g/100ml except for lactose. The value was respectively 3.38g/ml for goat milk and 4.63g/ml for goat milk fed Ajwa seed.

b) Mineral content

The result shows that date palm and goat milk are rich in calcium and magnesium. The calcium content in Ajwa date (536.00 mg/kg) is higher than Mariami date (482.00 mg/kg), while magnesium content in Mariami date (451.00 mg/kg) is higher than Ajwa date (418.00 mg/kg). Goat milk fed Ajwa seed contains higher calcium but lower magnesium than regular Goat milk. Iron content in Ajwa date was higher than Mariami date, which is 10.70mg/kg and 7.47mg/kg, respectively. Both Goat milk shows a low level of iron which are 0.90mg/L for Goat milk and 0.28mg/L for Goat milk fed Ajwa seed. Mariami date shows higher copper and zinc than Ajwa date. Goat milk fed Ajwa seed shows higher zinc content than goat milk.

c) **Vitamin C**

The result shows that vitamin C content varies according to the varieties of dates. Vitamin C content in Ajwa date was higher than Mariami date, which is 9.80mg/100g and 5.49mg/100g, respectively. Both goat milks showed poor content of vitamin C (< 0.10mg/100ml).

4.3 Iron Bioavailability Study

4.3.1 Cell viability

Cell-based assays are used to determine if the test compounds have effects on cell proliferation or show direct cytotoxic effects that eventually lead to cell death. Cell viability is defined as the number of cells that are living based on a total cell sample. Date palm extract and Goat milk were obtained as described in **Section 3.3.4**. This extract was used to determine the cell viability of Caco-2 human epithelial cells. The Caco-2 cell line was grown in DMEM medium supplemented with 16% FBS and 1% penicillin-streptomycin (**Section 3.3.3.2**) and cytotoxicity assay were performed using Cell Titer 96® $_{\text{Aqueous}}$ One Solution Cell Proliferation assay (Promega, USA) as described in **Section 3.3.6**. Cytotoxicity effect of the extract and cell viability were observed at 24th, 48th and 72nd hours.

4.3.1.1 Date palm

The cell viability was determined by cytotoxicity assay as described in (**Section 3.3.6)**. Cell viability is inversely proportional to the concentration of date palm extract at 24^{th}, 48^{th} and 72^{nd} hours of treatment, as shown in **Figure 4.3**. Both date palm extracts show the same trend.

Mariami and Ajwa date palm extracts show high cytotoxic to the cell at a concentration of 1000 mg/ml at 24^{th}, 48^{th} and 72^{nd} hours of treatment. After 24^{th} hours of treatment, cell viability or Mariami and Ajwa date palm extracts were 32.84% and 23.04% respectively. At 48^{th} hours of treatment, cell viability keeps on decreasing to 24.84% for Mariami date and 10.17% for Ajwa date. Three days after treatment, the cell viability for both date palm decrease to 12.62% and 6.41% respectively.

The Mariami and Ajwa date palm extracts showed the maximum non-toxic dose at concentration of 100 mg/ml with cell viability of 77.65% and 73.83% respectively at 24^{th} hours of treatment, 68.88% and 66.28% respectively at 48^{th} hours of treatment and 59.53% and 52.06% respectively at 72^{nd} hours of treatment. Therefore, date palm with 100 mg/ml concentration was chosen for the iron bioavailability assay.

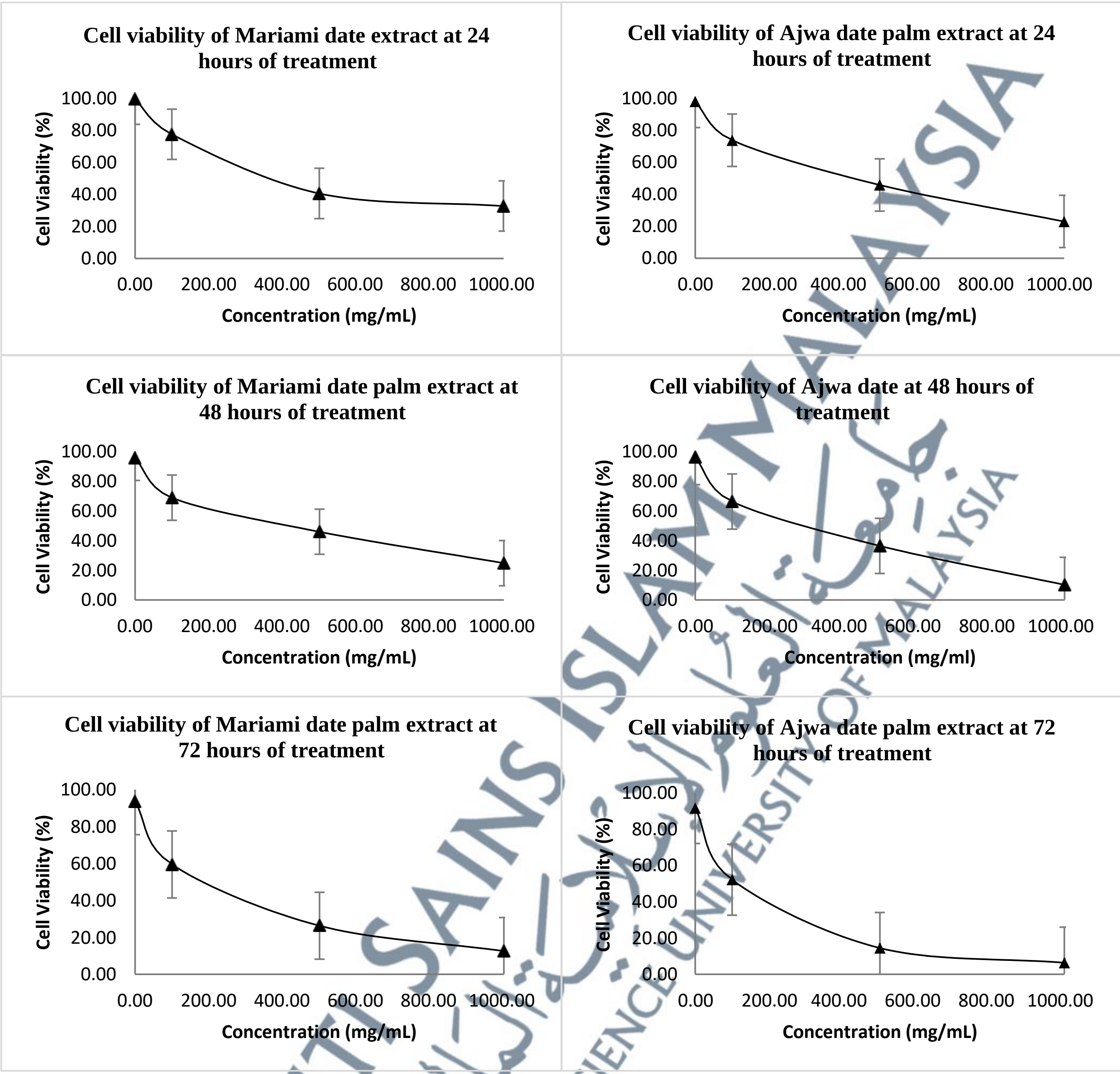

Figure 4.3: The percentage of cell viability of Caco-2 cell line at 24th, 48th and 72nd hours after treatment with two types of date palm extract. The data are shown as mean ± S.D. performed in triplicates.

4.3.1.2 Goat milk

The cell viability was determined by cytotoxicity assay as described in (**Section 3.3.6)**. Cell viability shows directly proportional to concentration of Goat milk at 24^{th}, 48^{th} and 72^{nd} hours of treatment as shown in **Figure 4.4**. Both Goat milks showed the same trend. Goat milk and Goat milk fed Ajwa seed increase cell proliferation. After 24^{th} hours of treatment, cell viability of Goat milk and Goat milk fed Ajwa seed at 100v/v% were 160.65% and 159.86% respectively.At 48^{th} hours of treatment, cell viability keeps on increasing to 207.72% and 190.08%. Three days after treatment, the cell viability for both goat milk increase to 223.31% and 237.73% respectively.

The Goat milk and Goat milk fed Ajwa seed showed the maximum non-toxic dose at concentration of 100 v/v%. Therefore, goat milk with 100 v/v% concentration was chosen in the iron bioavailability assay.

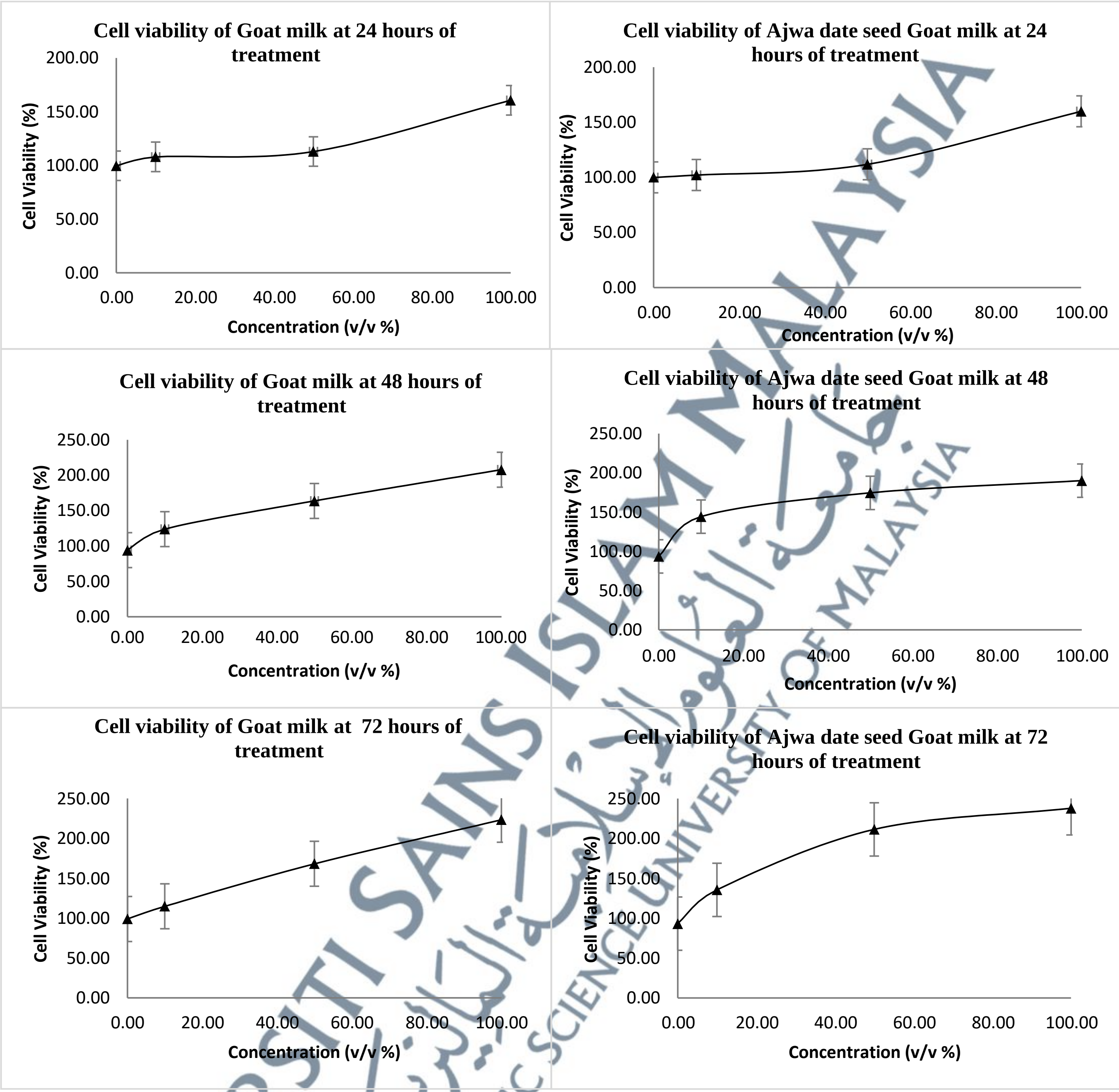

Figure 4.4: The percentage of cell viability of Caco-2 cell line 24th, 48th and 72nd hours after treatment with two types of Goat milk. The data are shown as means ± S.D. performed in triplicates.

4.3.2 Iron bioavailability in Caco-2 cell culture model

Both types of date palm extracts, goat milk samples and ferrous sulphate standards were prepared as described in **Section 3.3.5 and 3.3.3.6**. These test samples and standards were used to determine the iron bioavailability using Caco-2 cell model as described in **Section 3.3.7**. The standard curve was plotted using iron concentration and ferritin/protein. Iron bioavailability of the tested sample were estimated by comparing to ferrous sulphate standard with similar iron concentration in the standard curve as described in **Section 3.3.10. Figure 4.5** demonstrates the standard curve with linear equation as follows:

Ferritin (ng)/ protein (mg) = (16.519 X sample Fe) + 16.255, R^2 *= 0.974.*

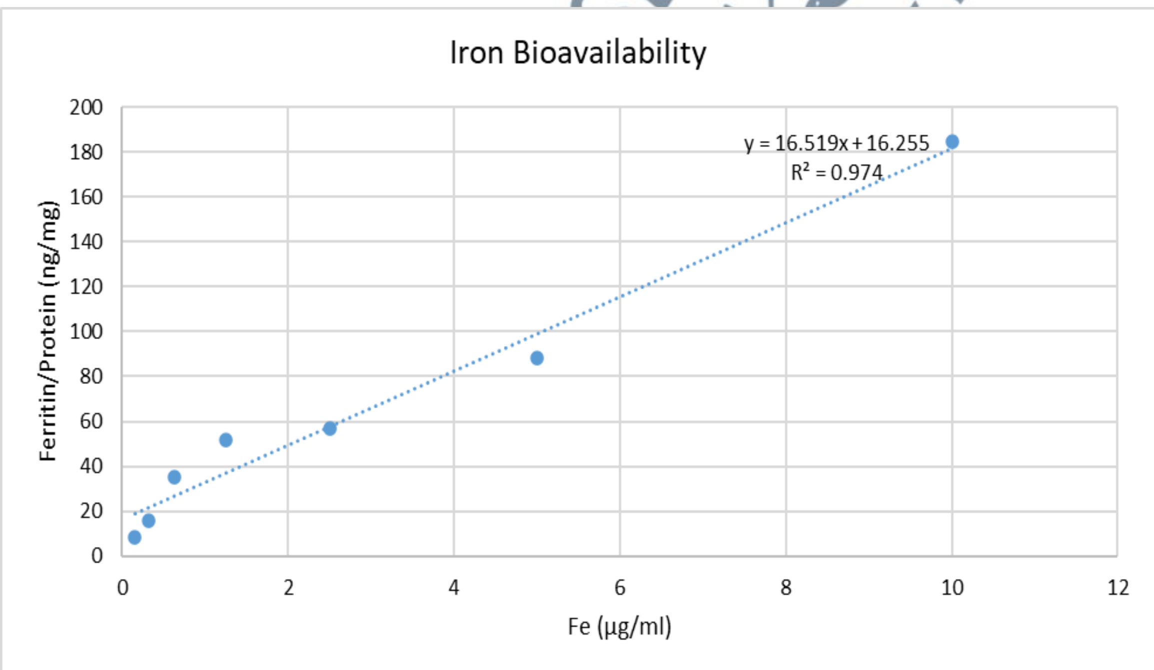

Figure 4.5: Standard curve of iron bioavailability

The results of iron bioavailability at 30 minutes of treatment are shown in **Table 4.4**. The reading was deducted with baseline reading (at 0 minute), as shown in **Table 4.4**. By using the equation above (**Figure 4.5**), the bioavailability of iron from mixture of Mariami date and Goat milk fed Ajwa seed shows the highest value at 30 minutes (7.58 ± 5.49% of ferrous sulphate) as compared to other samples tested. It is followed by Mariami date + goat milk (43.20 ± 4.16% of ferrous sulphate), Mariami date (39.92 ± 2.50% of ferrous sulphate), Ajwa date + Goat milk (27.46 ± 5.81% offerrous sulphate), Ajwa date + Goat milk fed with Ajwa seed (24.60 ± 1.17% of ferrous sulphate), Ajwa date (22.78 ± 1.46% of ferrous sulphate), Goat milk fed ajwa (8.26 ± 1.40% offerrous sulphate) and Goat milk (2.50 ± 1.11% of ferrous sulphate).

Table 4.4: Iron bioavailability at 30, 60 and 120 minutes of treatment in Caco-2 cell line.

	Iron Bioavailability (% of ferrous sulphate)		
Treatment	**30 minutes**	**60 minutes**	**120 minutes**
Ajwa Dates	22.78 ± 1.46	26.48 ± 1.32	25.72 ± 2.14
Mariami Dates	39.92 ± 2.50	49.08 ± 2.54	43.69 ± 2.12
Goat milk fed ajwa seed	8.26 ± 1.40	9.19 ± 0.94	6.43 ± 1.27
Goat milk	2.50 ± 1.11	0.87 ± 1.14	1.70 ± 0.96
Ajwa date + Goat Milk Fed Ajwa Seed	24.60 ± 1.17	45.22 ± 2.39	36.84 ± 2.85
Ajwa date + Goat Milk	27.46 ± 5.81	42.56 ± 8.61	31.36 ± 3.10
Mariami + Goat Milk Fed Ajwa Seed	57.58 ± 5.49	78.01 ± 5.26	99.78 ± 4.53
Mariami + Goat Milk	43.20 ± 4.16	61.87 ± 3.70	60.60 ± 3.89

Iron bioavailability at 60 minutes of treatment is shown in **Table 4.4**. The reading was deducted with baseline reading (at 0 minute) as shown in **Table 4.4**. By using the equation above (**Figure 4.5**), the bioavailability of iron from a mixture of Mariami date and Goat milk fed Ajwa seed shows the highest (78.01 ± 5.26% of ferrous sulphate) as compared to other samples tested. Itis followed by Mariami date + goat milk (61.87 ± 3.70% of ferrous sulphate), Mariami date (49.08 ± 2.54% of ferrous sulphate), Ajwa date + Goat milk fed with Ajwa date seed (45.22 ± 2.39% of ferrous sulphate), Ajwa date + Goat milk (42.56 ± 8.61% of ferrous sulphate), Ajwa date (26.48 ± 1.32% of ferrous sulphate), Goat milk fed ajwa seed (9.19 ± 0.94% of ferrous sulphate) and Goat milk (0.87 ± 1.14% of ferrous sulphate).

Iron bioavailability at 120 minutes of treatment is shown in **Table 4.4**. The reading shown was deducted with baseline reading (at 0 minutes). By using the equation as shown in **Figure 4.5**, the bioavailability of iron from a mixture of Mariami date and goat milk fed Ajwa seed shows the highest (99.78 ± 4.53% of ferrous sulphate) as compared to other samples tested. It is followed by ferrous sulphate (74.00 ± 1.80% of ferrous sulphate), Mariami date+ goat milk (60.60 ± 3.89% of ferrous sulphate), Mariami date (43.69 ± 2.12% of ferrous sulphate), Ajwa date + Goat milk fed with Ajwa date seed (36.84 ± 2.85% of ferrous sulphate), Ajwa date + Goat milk (31.36 ± 3.10% of ferrous sulphate), Ajwa date (25.72 ± 2.14% of ferrous sulphate), Goat milk fed ajwa (6.43 ± 1.27% of ferrous sulphate) and Goat milk(1.70 ± 0.96% of ferrous sulphate).

Results shown in **Table 4.4** demonstrates that the iron bioavailability was increased starting from 0 minute until 60 minutes of treatment. At 120 minutes, iron bioavailability starts to decrease for all the treatment except for Mariami date + Goat milk fed Ajwa seed which the bioavailability keeps increasing. The result above, shows that the mixture of date palm with goat milk increased the iron bioavailability when compared with date palm and goat milk treatment alone.

4.3.3 Iron bioavailability in a different time intervals of treatment

Iron bioavailability values from each sample treatment was used for statistical analysis using one-way ANOVA followed by post hoc test to compare the means between times of treatment. Iron bioavailability using Caco-2 cell model were carried out in three times independently, with each treatment performed in triplicate. The reading shown at 30, 60 and 120 minutes were deducted with baseline reading (at 0 minutes).

Figure 4.6 shows the statistical result of iron bioavailability of date palm and goat milkat different duration of treatment. Ajwa date and Goat milk fed Ajwa seed shows 20% increase in iron uptake from 30 minutes to 60 minutes of treatment, but it starts to decrease at 120 minutes. Additionally, the Mariami date treatment rise in iron bioavailability, exhibiting a substantial 22.95% increase from 30 minutes to 60 minutes. However, there was a subsequent decline of 10.98% observed at the 120 minutes treatment. In contrast, when considering the goat milk treatment, there was a significant 65.2% reduction in iron uptake during the initial 30 minutes to 60 minutes period. Nevertheless, a reversal occurred, showcasing an upward trend in iron uptake at the 120-minute interval.

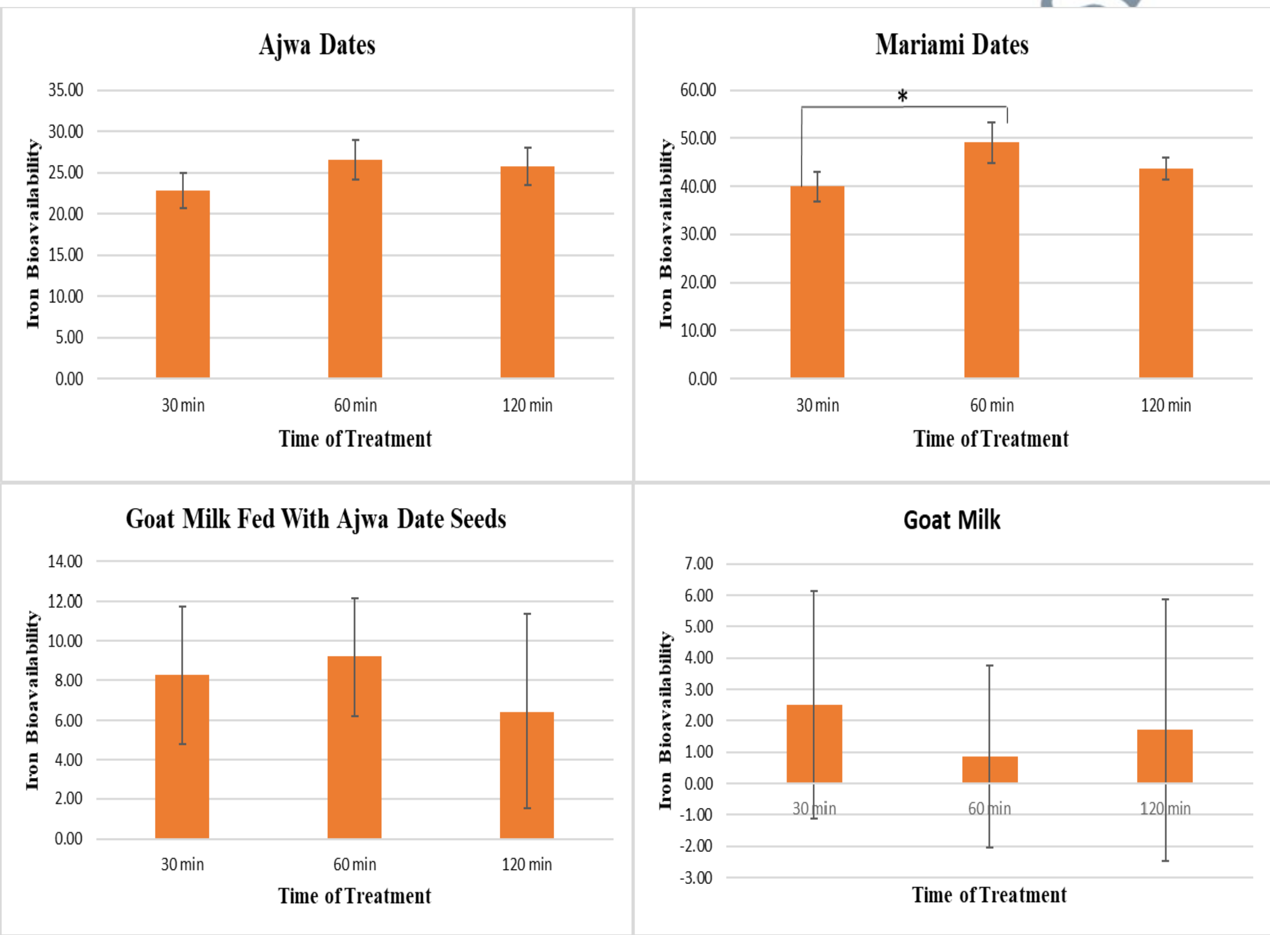

Figure 4.6: Iron bioavailability of date palm and goat milk between times of treatment. The data are shown as means ± SD. Significant at p<0.05 and n=9 between two groups.

Figure 4.7 shows the statistical result of mixture of date palm and goat milk at different duration of treatment. The combination of Ajwa date with goat milk fed Ajwa seed, yielded a marked and statistically significant rise ($p < 0.05$) in iron absorption, shows an impressive 83.82% increase from the 30 minutes to the 60 minutes point. However, there was a subsequent decrease of 18.53% observed in iron absorption from the 60 minutes interval to the 120 minutes interval. Ajwa date mixed with Goat milk also shows the same trend as mixture of Ajwa date with goat milk fed Ajwa seed. The mixture of Mariami date with goat milk resulted in a noteworthy and statistically significant rise ($p < 0.05$) in iron absorption. Specifically, there was a substantial 43.22% increase observed from the 30-minute mark to the 60-minute mark, and a comparable increase of 40.28% was observed when considering the entire treatment duration from the initial 30 minutes to the 120-minute point. Meanwhile, mixture of Mariami date with goat milk fed Ajwa seed shows significant increase in iron absorption. This enhancement was particularly evident within specific time intervals. Within the first 30 minutes to 60 minutes, there was a substantial 35.48% increase in iron absorption. When extending the observation period from 30 minutes to 120 minutes, the increase in iron absorption was even more remarkable, showing a boost of 73.29%. Furthermore, when comparing the 60-minute mark to the 120-minute mark of the treatment, the iron absorption increased by 27.90%. Mariami date mixed with goat milk shows significant increase in iron absorption from 30 minutes to 60 minutes and 30 minutes to 120 minutes of treatment.

To conclude, the results demonstrates that the iron bioavailability was increased starting from 30 minutes until 60 minutes of treatment. However, the iron bioavailability starts to decrease at 120 minutes for all the treatment except for combination of Mariami date and goat milk fed Ajwa seed treatment which they keep increasing.

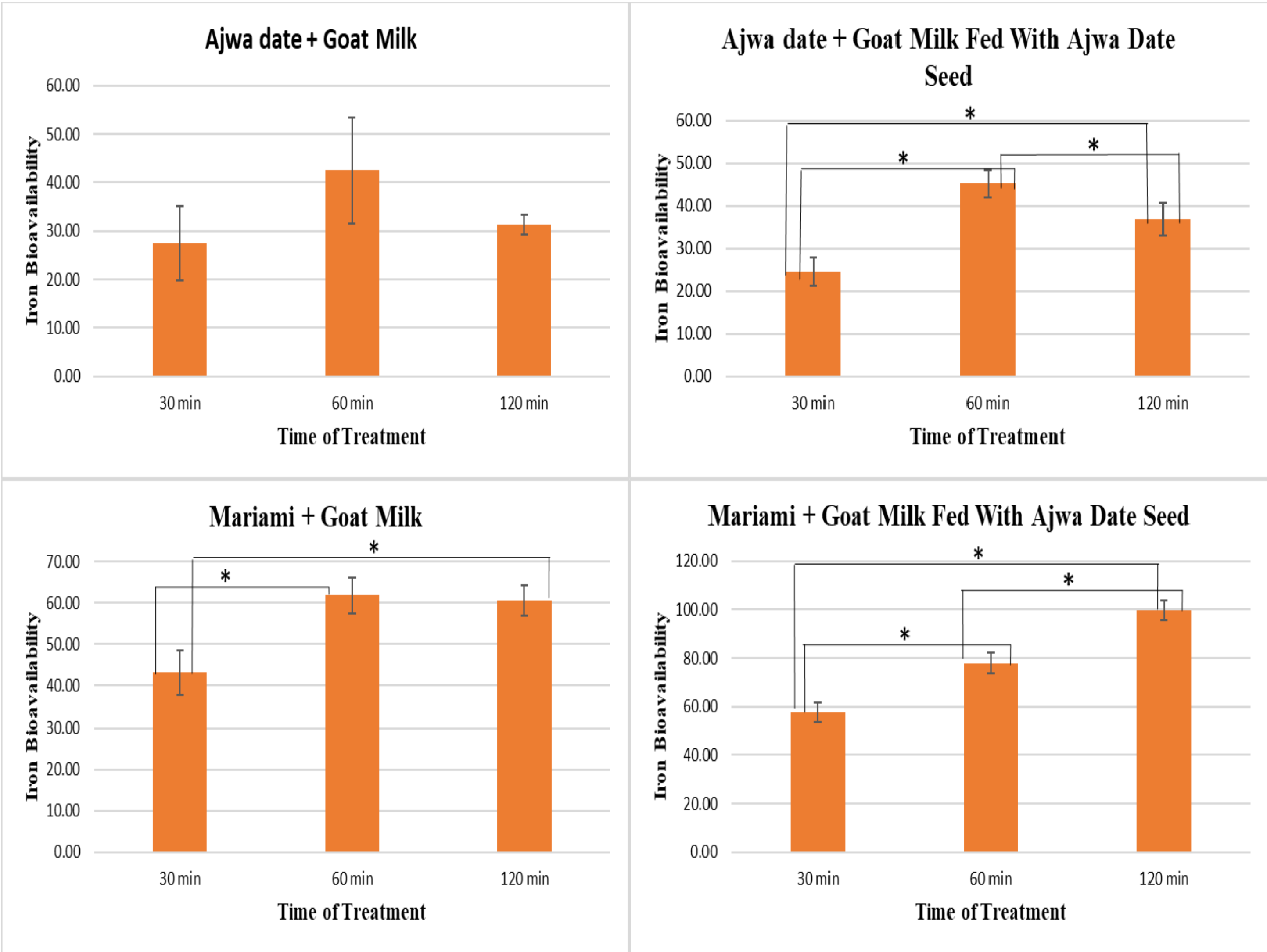

Figure 4.7: Iron bioavailability of mixture of date palm and goat milk between times of treatment. The data are shown as means ± SD. Significant at p<0.05 and n=9 between twogroup

4.3.4 Comparison of iron bioavailability between groups of treatment

Iron bioavailability value from each treatment sample was used for statistical analysis using one-way ANOVA followed by post hoc test to compare means between groups of treatment. Iron bioavailability using Caco-2 cell model were carried out in three times independently, with each treatment performed in triplicate. Iron bioavailability between groups at 30 minutes of treatment are as shown in **Figure 4.8** below. Iron absorption of Mariami date is higher than Ajwa date as shown in **Figure 4.8 (a), (b)** and **(c)** although it is not significant**. Figure 4.8 (a)** and **(b)** showed that there is no significant different in iron absorption when Ajwa and Mariami date mixed with both type of goat milk. Even though it is not significant, but the improvement can be seen when mixture of date palm and goat milk shows higher in iron bioavailability when compared with date palm and goat milk alone. But there is a significant different between Ajwa date treatment and mixture of Mariami date with both type of Goat milk. Iron absorption of date palm was higher than goat milk as shown in **Figure 4.8 (c).** Besides that, iron absorption of Ajwa seed fed goat milk is higher than Goat milk as shown in **Figure 4.8 (c), (d)** and **(e)** although it is not significant**.**

Figure 4.8 (d) shows higher value of iron absorption for the mixture of both date palm with goat milk than goat milk alone**.** Besides that, mixture of date palm with goat milk also shows significantly higher value than Ajwa seed fed goat milk. **Figure 4.8 (f)** shows higher value of iron bioavailability for mixture of Mariami date with Goat milk fed Ajwa seed when compared to both mixture of Ajwa date with Ajwa seed fed goat milk and Ajwa date with Goat milk.

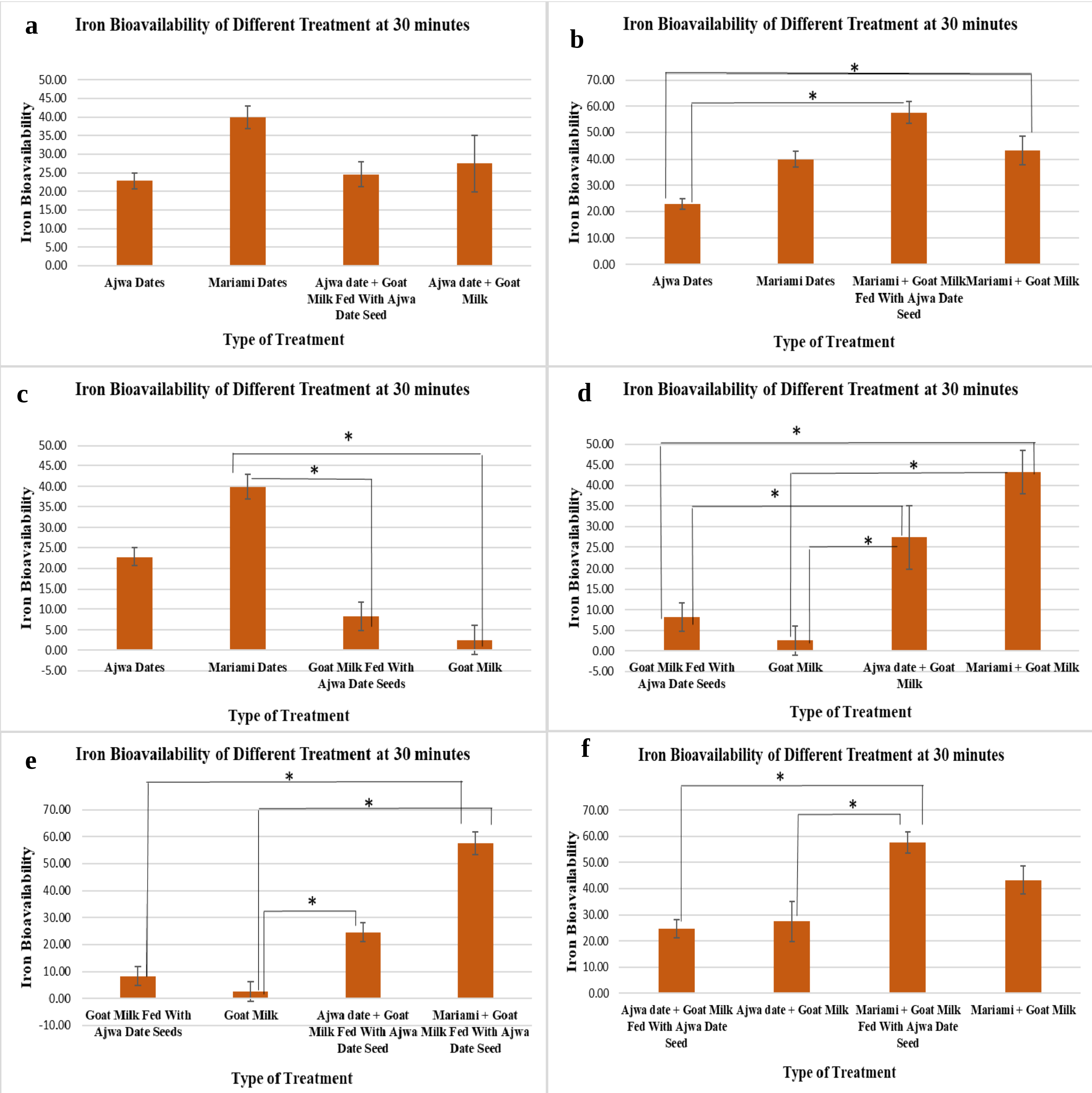

Figure 4.8: Iron bioavailability between groups of different treatment combinations at 30 minutes. The data are shown as means ± SD. *Significant at p<0.05, and n=9

Iron bioavailability between groups at 60 minutes of treatment are shown in **Figure 4.9** below. Iron absorption of Mariami date is significantly higher than Ajwa date as shown in **Figure 4.9 (a).** The result shows in **Figure 4.9 (a)** and **(b)** shows that there is no significant different in iron absorption when Ajwa and Mariami date mixed with both type of goat milk except for Mariami date and mixture of Mariami date with Goat milk fed Ajwa seed that shows a significant improvement in iron absorption. Even though it is not significant, but the improvement yet can be seen when mixture of date palm and goat milk shows higher in iron bioavailability when compared with date palm and goat milk alone. But there is a significant different between Ajwa date treatment and mixture of Mariami date with both type of Goat milk. Iron absorption of date palm was higher than goat milk as shown in **Figure 4.9 (c)**. Besides that, iron absorption of Goat milk fed Ajwa seed is higher than Goat milk as shown in **Figure 4.9 (c), (d)** and **(e)** although it is not significant**.**

Figure 4.9 (d) shows a significant higher in iron absorption for both date palm when mixed with goat milk than treatment with goat milk and goat milk fed Ajwa seed alone**.** Iron bioavailability results shows a significant higher for both date palm when mixed with goat milkfed Ajwa seed than treatment with goat milk and goat milk fed Ajwa seed alone as shown in **Figure 4.9 (e).**

Figure 4.9 (f) shows a significant higher of iron bioavailability for mixture of Mariamidate with Goat milk fed Ajwa seed when compared to both mixture of Ajwa date with Goat milk fed Ajwa seed and Ajwa date with Goat milk.

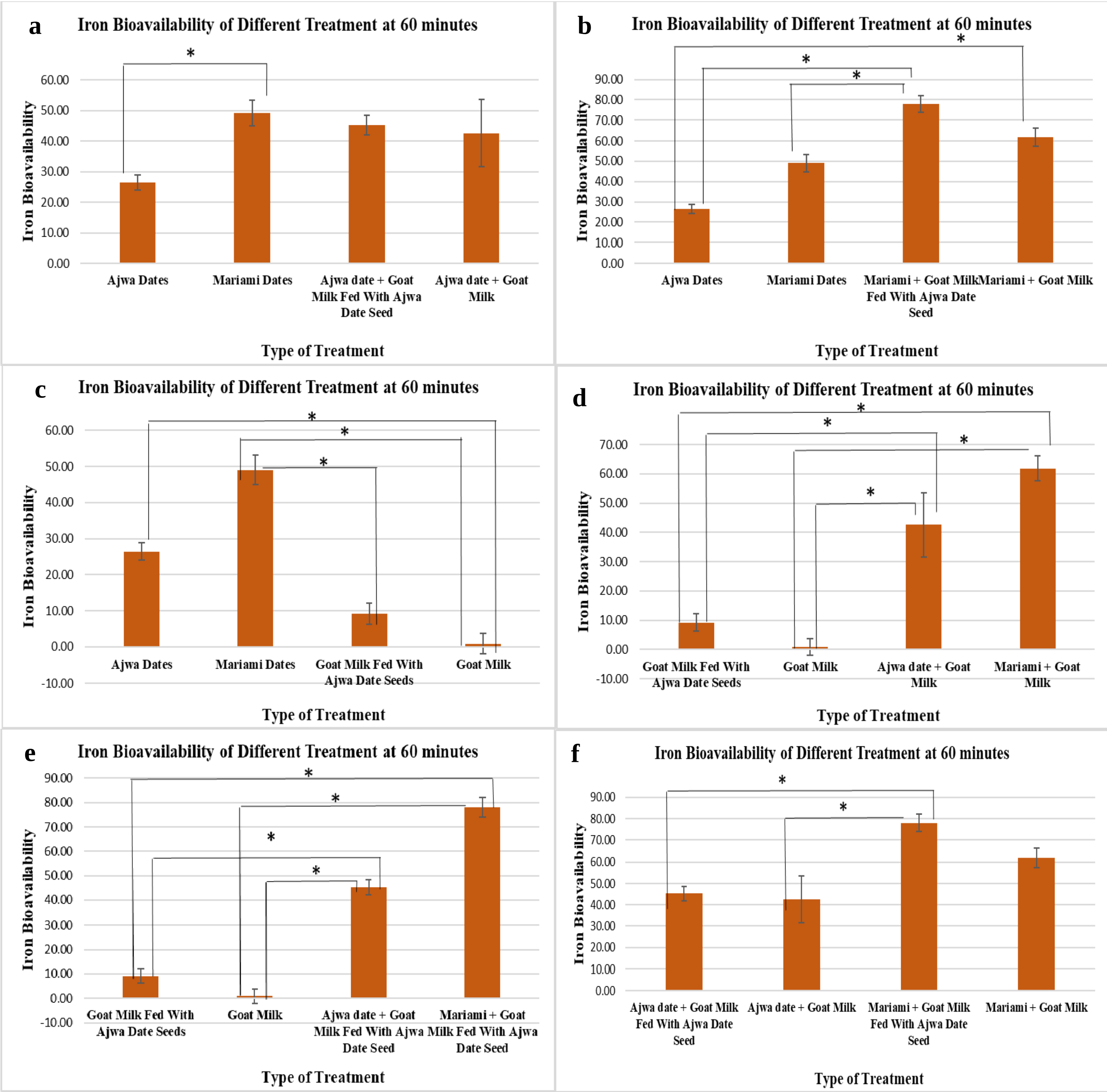

Figure 4.9: Iron bioavailability between groups of treatment at 60 minutes. The data are shown as means ± SD. *Significant at p<0.05 and n=9

Iron bioavailability between groups at 120 minutes of treatment are shown in **Figure 4.10** below. Iron absorption of Mariami date is significantly higher than Ajwa date as shown in **Figure 4.10 (a).** The result shows in **Figure 4.10 (a)** shows that there is no significant different in iron absorption when Ajwa date mixed with both type of goat milk. Even though it is not significant, but the improvement yet can be seen when mixture of Ajwa date with bpth type ofgoat milk shows higher in iron bioavailability when compared with Ajwa date and goat milk alone. **Figure 4.10 (b)** shows Mariami date and mixture of Mariami date with both types of Goat milk shows a significant improvement in iron absorption. Significant different also shows in comparison of Ajwa date with mixture of Mariami date with both types of Goat milk.

Iron absorption of date palm was higher than goat milk as shown in **Figure 4.10 (c)** where Mariami date shows a significantly higher than Goat milk and Goat milk fed Ajwa seed while Ajwa date also shows a significant higher than Goat milk. Besides that, iron absorption of Goat milk fed Ajwa seed is higher than Goat milk as shown in **Figure 4.10 (c), (d)** and **(e)** although it is not significant**.**

Figure 4.10 (d) shows a significant higher in iron absorption for both date palm when mixed with goat milk than treatment with goat milk and goat milk fed Ajwa seed alone**.** Iron bioavailability results shows a significant higher for both date palm when mixed with goat milkfed Ajwa seed than treatment with goat milk and goat milk fed Ajwa seed alone as shown in **Figure 4.10 (e).**

Figure 4.10 (f) shows a significant higher of iron bioavailability for mixture of Mariamidate with Goat milk fed Ajwa seed and Mariami date with Goat milk when compared to both mixture of Ajwa date with Goat milk fed Ajwa seed and Ajwa date with Goat milk.

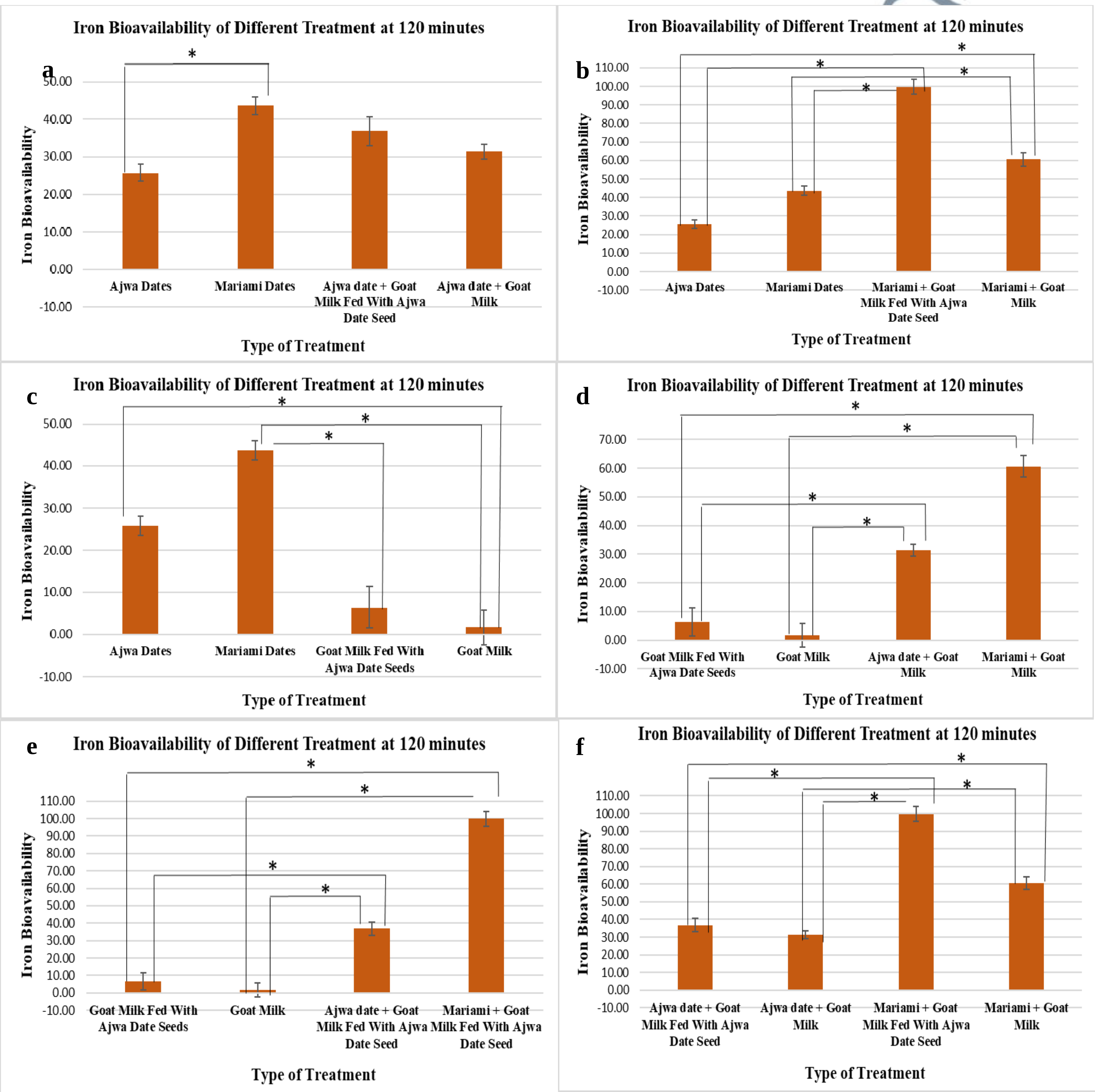

Figure 4.10: Iron bioavailability between groups of treatment at 120 minutes. The data are shown as means ± SD. *Significant at p<0.05 and n=9

4.4 Human study

Human subjects with mild iron deficiency anaemia was selected by random sampling as shown in **Figure 4.11**. Female adult aged 20 to 45 years old were screened for haemoglobin level as described in **section (3.4.10.1)**. Out of 178 subjects, only 99 (55.65%) were found to have haemoglobin level of less than 12 g/dL. Sixty-five (36.53%) subjects were found to have ferritin level below 30 ng/ml and among them, 5 (7.69%) subjects were positive for thalassaemia trait. Sixty subject which were eligible to be subjects in this study were assigned with different treatment. Throughout the experiment, 3 subjects dropped out due to personal commitment.

The red blood cell parameters and iron profile data from mild iron deficiency anaemia subject was selected as illustrated in **Figure 4.11.** The data was used for statistical analysis to compare median before and after given a treatment by using Wilcoxon Signed Rank testas described in **Section 3.5**. The control group were not given any treatment throughout the experiment.

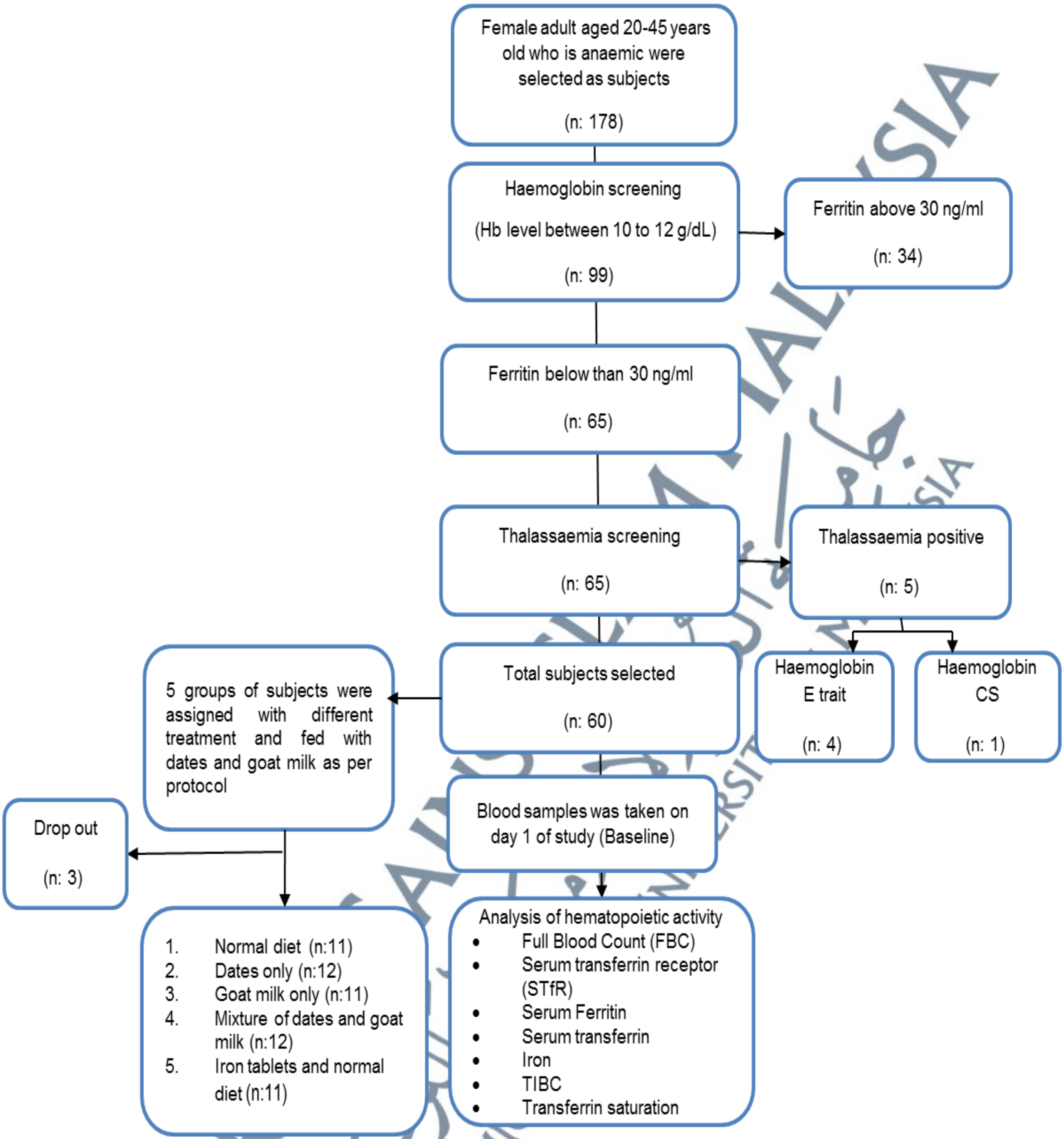

Figure 4.11: Flow chart in subject selection

4.4.1 Control Group

The control group were not given any treatment throughout the experiment. Blood samples wastaken on day 1 of study and repeated at week 4, week 8 and week 12 of study. The haemoglobin parameter and iron profile data from control subject was used for statistical analysis to compare median at baseline, week 4, week 8 and week 12 of treatment by usingWilcoxon Signed Rank test as described in **Section 3.5**

Result of statistical analysis and descriptive statistics was shown in **Table 4.5.** The results show a mean (SD), median (IQR) and P value for haemoglobin parameters and ironprofile. The result demonstrates p-value of more than 0.05 ($p > 0.05$) for all the parameters at week 4 of experiment. At week 8 of experiment there is a significant increase in reticulocyte count and transferrin while at week 12 of experiment, there is a significant increase in red blood cell count and serum transferrin receptor. Other parameters show no significant different ($p > 0.05$) throughout the treatment.

Table 4.5: Haemoglobin parameters and iron profile for **control group** at baseline, week 4, week 8 and week 12 of treatment (n=11). The data are shown as mean ± SD and median ± IQR, n=57.

Parameters	**Baseline**		**Week 4**		**Week 8**		**Week 12**	
	Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Reticulocyte count (%)	0.46 ± 0.12	0.4 (0.2)	0.36 ± 0.20	0.3 (0.2)	0.62 ± 0.14	0.6 (0.2) *	0.71 ± 0.61	0.5 (0.8)
RBC (x10^12/L)	4.57 ± 0.44	4.6 (0.5)	4.64 ± 0.36	4.6 (0.5)	4.68 ± 0.39	4.6 (0.6)	4.80 ± 0.38	4.8 (0.5) *
Hb (g/dl)	11.94 ± 0.45	11.8 (0.7)	11.87 ± 0.39	11.9 (0.4)	12.05 ± 0.53	12.0 (0.4)	12.06 ± 0.58	12.0 (0.2)
PCV (%)	39.09 ± 2.73	39 (5)	39.36 ± 2.83	40 (4)	38.91 ± 2.91	39 (5)	39.64 ± 2.73	40 (6)
MCV (FL)	85.64 ± 4.31	85 (6)	86.18 ± 4.89	87 (5)	84.45 ± 5.31	85 (7)	83.64 ± 4.92	86 (9)
MCH (PG)	27.82 ± 1.53	28 (2)	27.82 ± 1.88	29 (2)	27.73 ± 2.10	29 (2)	27.64 ± 1.74	28 (2)
MCHC (g/dl)	32.45 ± 0.93	32 (1)	32.27 ± 0.65	32 (1)	32.82 ± 0.87	33 (2)	32.82 ± 0.75	33 (1)
Serum Iron(umol/L)	7.57 ± 2.84	6.3 (3.6)	7.6 ± 2.92	6.7 (4.4)	6.96 ± 2.42	6 (3.8)	6.67 ± 3.41	5.5 (4.7)
TIBC (umol/L)	61.91 ± 7.56	64 (11)	62.82 ± 7.53	64 (10)	62.00 ± 7.41	61 (11)	63.82 ± 8.37	62 (11)
Transferrin saturation (%)	12.45 ± 4.84	12 (6)	11.91 ± 5.24	9 (6)	12.55 ± 7.97	10 (7)	11.18 ± 6.33	9 (12)
Ferritin (ng/ml)	21.27 ± 6.06	21 (9)	22.91 ± 10.11	24 (16)	20.18 ± 8.42	21 (15)	20.55 ± 8.65	22 (13)
Transferrin (g/L)4	2.41 ± 0.51	2.5 (0.4)	2.55 ± 0.55	2.5 (1.1)	2.73 ± 0.52	3.0 (0.5) *	2.68 ± 0.61	2.5 (1.0)
Serum transferrin receptor (ng/ml)	25.71 ± 2.47	25.9 (5.48)	30.5 ± 3.22	30.2 (10.13)	32.84 ± 10.14	28.5(11.04)	33.06 ± 10.84	36.4(17.61) *

* Significant different ($P < 0.05$) compared with baseline using Wilcoxon signed rank test

The haematological parameters were monitored throughout the study period as shown in **Figure 4.12**. Reticulocyte count shows a slight decreased at week 4 of treatment andsignificantly increased at week 8 of treatment. Then it keeps on increasing until week 12 of treatment as shown in **Figure 4.12 (a).** The baseline mean value 0.46 ± 0.12 decreased to 0.36 ± 0.20 in week 4 and increased to 0.62 ± 0.14 in week 8 and continued increasing up to 0.71 ± 0.61 in week 12.

Red blood cell count in control group shows a significant increase at week 12 of treatment as shown in **Figure 4.12 (b)** but no significant change at week 4 and week 8 of treatment. The baseline mean value was 4.57 ± 0.44 and only exhibited a slight increase to 4.64 ± 0.36 in week 4 and 4.68 ± 0.39 in week 8. It has significant increase in mean value of 4.80 ± 0.38 at week 12. There is no significant change for haemoglobin, packed cell volume, mean cell volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration as observed in **figure 4.12 (c), (d), (e), (f)** and **(g).**

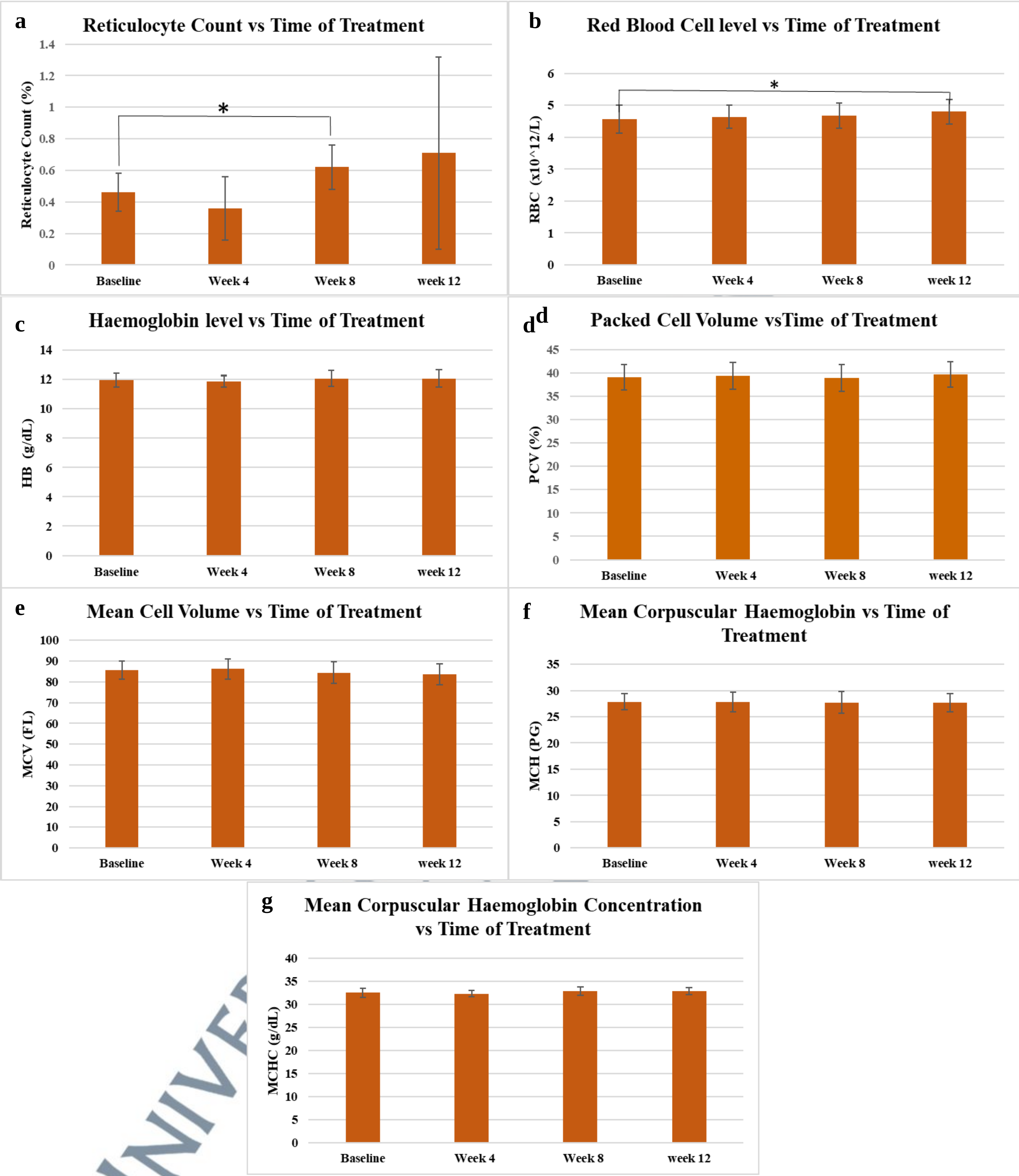
a
Reticulocyte Count vs Time of Treatment
Reticulocyte Count (%)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
Baseline
Week 4
Week 8
week 12
b
Red Blood Cell level vs Time of Treatment
RBC (x10^12/L)
6
5
4
3
2
1
0
*
Baseline
Week 4
Week 8
week 12
c
Haemoglobin level vs Time of Treatment
HB (g/dL)
14
12
10
8
6
4
2
0
Baseline
Week 4
Week 8
week 12
dd
Packed Cell Volume vsTime of Treatment
PCV (%)
45
40
35
30
25
20
15
10
5
0
Baseline
Week 4
Week 8
week 12
e
Mean Cell Volume vs Time of Treatment
MCV (FL)
100
90
80
70
60
50
40
30
20
10
0
Baseline
Week 4
Week 8
week 12
f
Mean Corpuscular Haemoglobin vs Time of Treatment
MCH (PG)
35
30
25
20
15
10
5
0
Baseline
Week 4
Week 8
week 12
g
Mean Corpuscular Haemoglobin Concentration vs Time of Treatment
MCHC (g/dL)
40
35
30
25
20
15
10
5
0
Baseline
Week 4
Week 8
week 12

Figure 4.12: Haematological parameters changes of Normal control. (**a**) Reticulocyte count (**b**) Red blood cell (**c**) Haemoglobin (**d**) Packed cell volume (**e**)Mean cell volume (**f**) Mean corpuscular Haemoglobin (**g**) Mean corpuscular Haemoglobin concentration The data are shown as mean ± SD. *Significant at $p<0.05$, n=57

The iron profile of the Normal Control subjects were measured during the study period as shown in **Figure 4.13**. Serum iron level shows a decreasing trend from week 4 until week 12 of treatment (**Figure 4.13 (a)).** The baseline means value of serum iron level decreases from 7.57 ± 2.84 to 6.96 ± 2.42 in week 8 and continued to decrease to 6.67 ± 3.41 in week 12. The total iron binding capacity in **Figure 4.13 (b)** shows slight increase from week 4 until week 12 or treatment. The mean value increased from 61.91 ± 7.56 to 62.82 ± 7.53 in week 4 and continued increasing till 63.82 ± 8.37 in week 12. For transferrin saturation (**Figure 4.13 (c))**, the value decreased at week 4 but at week 8 transferrin saturation was increase then decrease at week 12 of treatment. The baseline mean value decreased from 12.45 ± 4.84 to 11.91 ± 5.24 and increased again to 12.55 ± 7.97 in week 8 and continued to drop to 11.18 ± 6.33 in week 12.

The ferritin level increased to 22.91 ± 10.11 at week 4 of treatment from the baseline mean value of 21.27 ± 6.06 but suddenly decrease at week 8 to 20.18 ± 8.42 as shown in **Figure 4.13 (d).** Transferrin level kept increasing until week 8 of treatment up to 2.73 ± 0.52 then suddenly decrease to 2.68 ± 0.61 at week 12 as shown in **Figure 4.13 (e)**. The value of serum transferrin receptor keeps on increasing until week 12 of treatment. At week 12, it shows a significant increase when compared to baseline level as stated in **Figure 4.13 (f).**

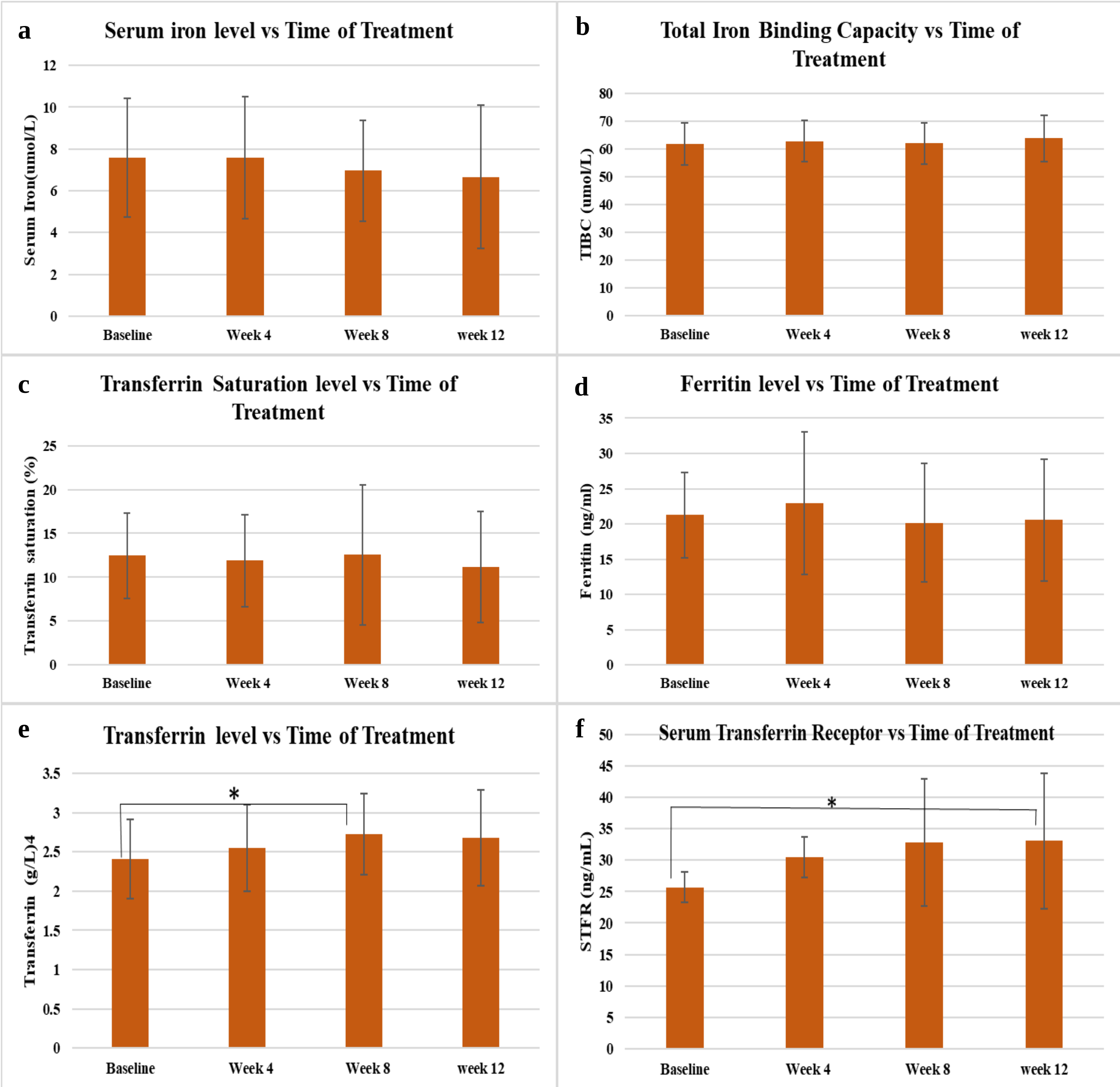

Figure 4.13: Comparison of iron profile in different time intervals. (**a**) Serum iron (**b**) Total iron binding capacity (**c**) Transferrin saturation (**d**) Ferritin (**e**)Transferrin (**f**) Serum transferrin receptor. The data are shown as mean ± SD. *Significant at $p<0.05$, n=57

4.4.2 Date palm treated subject with mild iron deficiency anaemia

Subjects with mild iron deficiency anaemia were given date palm treatment throughout the experiment. Blood samples was taken on day 1 of study and repeated at week 4, week 8 andweek 12 of study. The haemoglobin parameter and iron profile data from subject was used forstatistical analysis to compare median before and after given a treatment by using Wilcoxon Signed Rank test as described in **Section 3.5**

Result of statistical analysis and descriptive statistics was shown in **Table 4.6.** The results show a mean (SD), median (IQR) and P value for haemoglobin parameters and ironprofile. It demonstrated the p-value less than 0.05 ($p < 0.05$) for haemoglobin, packed cell volume, and total iron binding capacity at week 4 of experiment. At week 8, there is significant increase in haemoglobin and transferrin level while at week 12, there is significant increase in reticulocyte count, haemoglobin, packed cell volume and ferritin level. Transferrin saturation shows below the normal range (20%-55%) for all week of treatment. Other parameters show no significant different ($p > 0.05$), but the value is still within the normal range.

Table 4.6: Haematological parameters and iron profile for **date palm** group at baseline, week 4, week 8 and week 12 of treatment (n=12). The data are shown as mean ± SD and median ± IQR

Parameters	Baseline		Week 4		Week 8		Week 12	
	Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Reticulocyte count (%)	0.51 ± 0.20	0.5 (0.35)	0.59 ±0.38	0.4 (0.45)	0.63 ± 0.14	0.65 (0.17)	0.78 ± 0.36	0.8 (0.53) *
RBC (x10^12/L)	4.53 ± 0.40	4.5 (0.55)	4.69 ± 0.46	4.65 (0.45)	4.61 ± 0.44	4.55 (0.48)	4.72 ± 0.31	4.8 (0.65)
Hb (g/dl)	11.68 ± 0.38	11.7 (0.65)	12.86 ± 0.69	13.05 (1.18)*	12.56 ± 0.65	12.55 (1.03)*	13.2 ± 0.61	13.2 (0.95)*
PCV (%)	38 ± 1.85	38 (2.75)	39.83 ± 1.99	40.5 (3.75)*	38.58 ± 1.62	38.5 (2.5)	40.08 ± 1.37	40 (1.75) *
MCV (FL)	84.75 ± 7.07	86 (8.5)	85 ± 7.38	85.5 (9.75)	84.17 ± 7.00	85 (7.5)	85.67 ± 3.44	86 (4.75)
MCH (PG)	27.5 ± 2.74	28 (2.75)	27.58 ± 2.84	28 (2)	27.5 ± 2.93	28 (2)	28.33 ± 1.55	28.5 (1.75)
MCHC (g/dl)	32.42 ± 1.56	32 (1.75)	32.58 ± 1.24	32.5 (1)	32.92 ± 1.88	33 (2)	33 ± 0.95	33 (0.0)
Serum Iron(umol/L)	8.58 ± 3.63	7.85 (4.92)	8.33 ± 3.24	7.2 (4.53)	8.75 ± 4.00	8.55 (5.92)	7.55 ± 2.42	5.7 (4.73)
TIBC (umol/L)	63.58 ± 9.59	63.5 (16.25)	67 ± 10.20	66 (19.25)*	65 ± 9.96	67 (15.25)	64.08 ± 6.94	62 (13.25)
Transferrin saturation (%)	14 ± 6.61	13.5 (10.25)	13.17 ± 6.29	11 (9.75)	14.08 ± 6.99	13 (12.25)	12.08 ± 4.44	11.5 (10.25)
Ferritin (ng/ml)	15.5 ± 8.37	13.5 (16.5)	17.58 ± 10.68	13 (16.5)	19.25 ± 11.42	14 (19.5)	21.83 ± 10.13	20.5 (17.75)*
Transferrin (g/L)4	2.55 ± 0.48	2.6 (0.7)	2.73 ± 0.49	2.6 (0.85)	2.98 ± 0.43	3.1 (0.67)*	2.88 ± 0.45	2.8 (0.9)
Serum transferrin receptor (ng/ml)	23.34 ± 4.63	23.9 (7.27)	28.13 ± 13.40	28.6 (19.73)	25.84 ± 5.76	19.9 (32.19)	29.89 ± 3.72	28.9 (24.73)

* Significant different ($P < 0.05$) compared with baseline using Wilcoxon signed rank test

The haematological parameters during the treatment were shown in **Figure 4.14**. Reticulocyte count shows an increasing trend from baseline until week 12 of treatment. The mean value significantly increased to 0.78 ± 0.36; the percentage increase is approximately 52.94% at week 12 compared to 0.51 ± 0.20 at the baseline as shown in **Figure 4.14 (a)**. There was an increasing trend for red blood cell count as observed in **Figure 4.14 (b)** but was not statistically significant. The mean value increased to 4.72 ± 0.31 (4.19%) from 4.53 ± 0.40 at the baseline. However, there was a decrease in red blood cell count at week 8 of treatment. The haemoglobin level shows an increasing trend from baseline until week 12 of treatment as shown in **Figure 4.14 (c).** In week 12, the average haemoglobin level rose to 13.2 ± 0.61, reflecting a percentage increase by 13.01% from the baseline value (11.68 ± 0.38). Although there is a slight decrease at week 8 of treatment, but it still shows a significant increase when compared to baseline value. Week 4 and week 12 of treatment also shows a significant increase**.**

Packed cell volume as observed in **Figure 4.14 (d)** also shows the same pattern as haemoglobin. During the 12th week of treatment, the value increased to 40.08 ± 1.37, reflecting a growth of 5.47% compared to baseline but shows a slight decrease of 38.58 ± 1.62 at week 8 of treatment**.** At week 4 and week 12, packed cell volume shows a significant different when compared to baseline, 39.83 ± 1.99 and 40.08 ± 1.37 respectively. There is not much change for mean cell volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration as observed in **figure 4.14 (e), (f)** and **(g).**

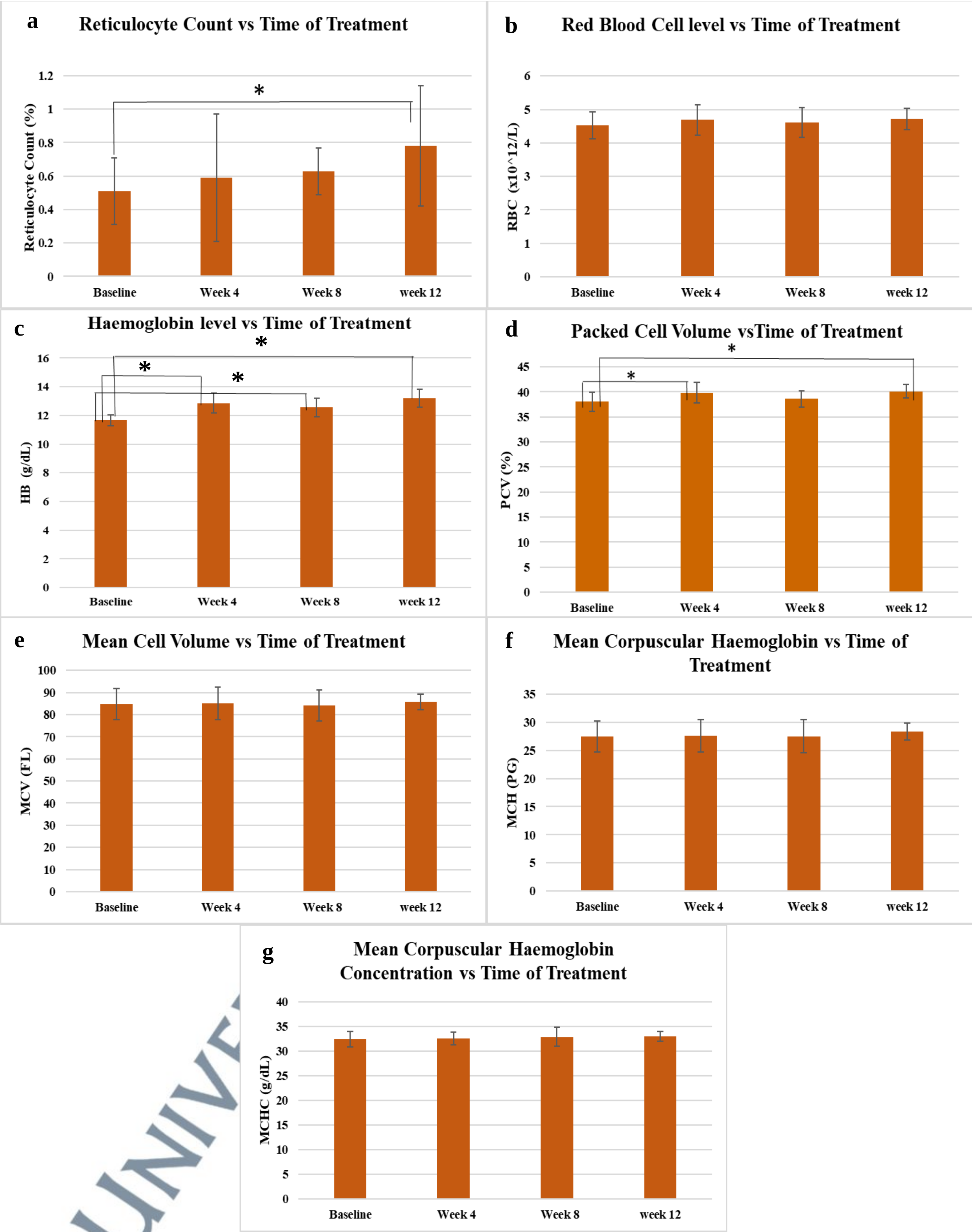
a
Reticulocyte Count vs Time of Treatment
Reticulocyte Count (%)
1.2
1
0.8
0.6
0.4
0.2
0
*
Baseline
Week 4
Week 8
week 12
b
Red Blood Cell level vs Time of Treatment
RBC (x10^12/L)
6
5
4
3
2
1
0
Baseline
Week 4
Week 8
week 12
c
Haemoglobin level vs Time of Treatment
HB (g/dL)
16
14
12
10
8
6
4
2
0
*
*
*
Baseline
Week 4
Week 8
week 12
d
Packed Cell Volume vsTime of Treatment
PCV (%)
45
40
35
30
25
20
15
10
5
0
*
*
Baseline
Week 4
Week 8
week 12
e
Mean Cell Volume vs Time of Treatment
MCV (FL)
100
90
80
70
60
50
40
30
20
10
0
Baseline
Week 4
Week 8
week 12
f
Mean Corpuscular Haemoglobin vs Time of Treatment
MCH (PG)
35
30
25
20
15
10
5
0
Baseline
Week 4
Week 8
week 12
g
Mean Corpuscular Haemoglobin Concentration vs Time of Treatment
MCHC (g/dL)
40
35
30
25
20
15
10
5
0
Baseline
Week 4
Week 8
week 12

Figure 4.14: Comparison of haematological parameter in different time intervals of treatment. (**a**) Reticulocyte count (**b**) Red blood cell (**c**) Haemoglobin (**d**) Packed cell volume (**e**)Mean cell volume (**f**) Mean corpuscular Haemoglobin (**g**) Mean corpuscular Haemoglobin concentration. The data are shown as mean ± SD. *Significant at $p<0.05$, n=57

The trend of iron profile throughout the treatment period was as shown in **Figure 4.15**. The serum iron level demonstrates a declining pattern from the fourth week up to the twelfth week of treatment. However, there is a notable improvement observed during the eighth week of treatment, with a 2% increase. The baseline mean value was 8.58± 3.63, it decreased to 8.33 ± 3.24 in week 4 and increased again to 8.75 ± 4.00 in week 8, only to decrease again at week 12 as low as 7.55 ± 2.42.

Total iron binding capacity in **Figure 4.15 (b)** was significantly increased at week 4 of treatment but starting week 8 it starts to decrease until week 12 of treatment. The mean value at week 12 had reduced to 64.08 ± 6.94 compared to the mean value of week 4 and week 8. For transferrin saturation (**Figure 4.15 (c))**, the value was decreased at week 4 but at week 8 transferrin saturation was increased then decrease at week 12 of treatment.

The ferritin level shows an increasing trend from baseline until week 12 of treatment as shown in **Figure 4.15 (d)** and at week 12 of treatment ferritin shows a significant increased up to 40.83% when compared to baseline which is 15.5 ± 8.37. Transferrin level kept on increasing until week 8 of treatment then suddenly decrease at week 12 as shown in **Figure 4.15 (e)**. During eighth week of treatment, there is a substantial rise in transferrin levels when compared to the initial baseline, marking a noteworthy increase of 16.86%. The mean value of the transferrin at baseline was 2.55 ± 0.48 increased up to 2.88 ± 0.45 at week 12. The value of serum transferrin receptor keeps on increasing until week 12 of treatment although it has slightly decrease at week 8 of treatment as observed in **Figure 4.15 (f).** The final mean value of serum transferrin at week 12 was 29.89 ± 3.72, which increased up to 21.91% compared to the baseline mean value.

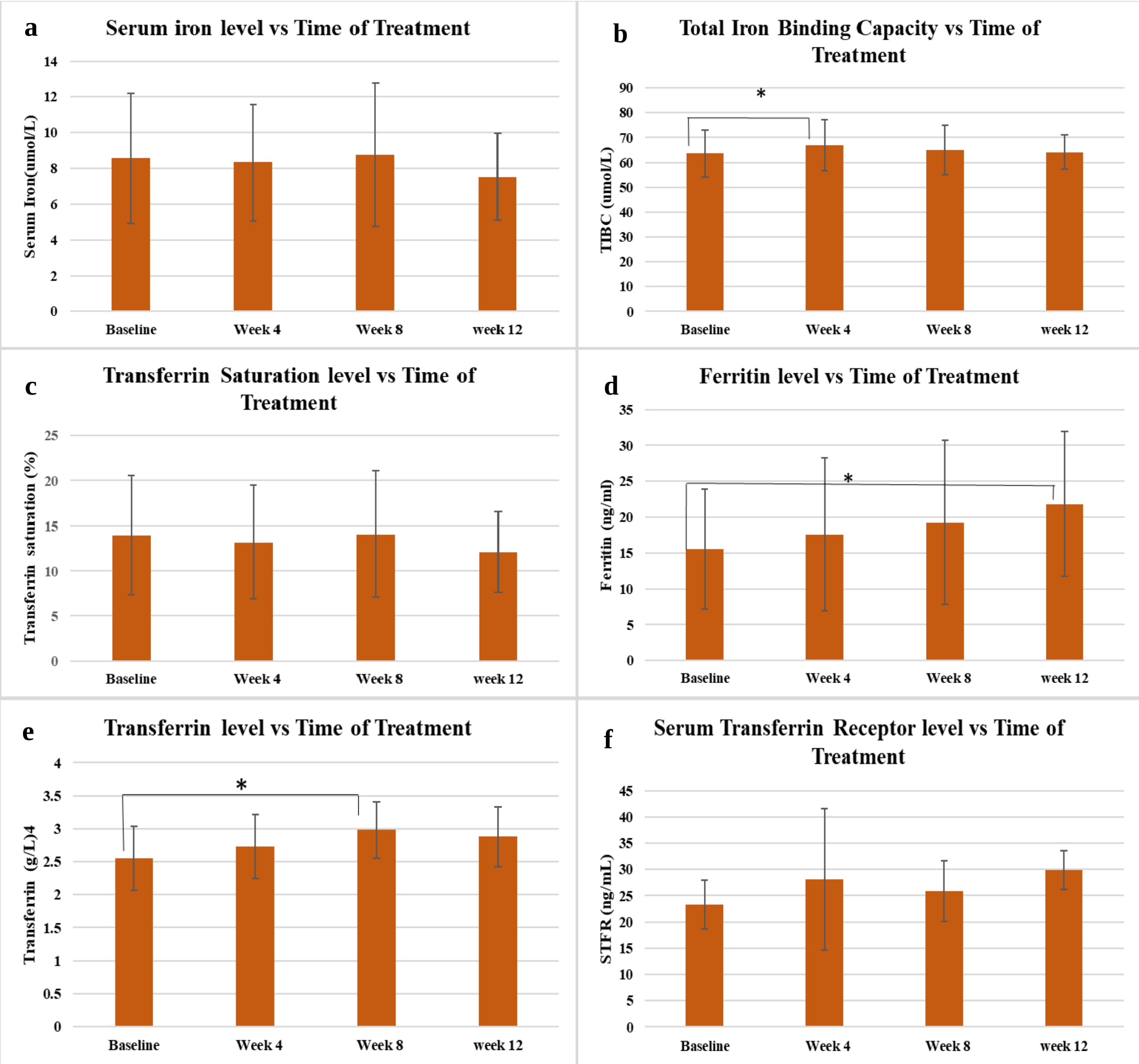

Figure 4.15: Comparison of iron profile between in different time intervals of treatment. (**a**) Serum iron (**b**) Total iron binding capacity (**c**) Transferrin saturation (**d**) Ferritin (**e**)Transferrin (**f**) Serum transferrin receptor. The data are shown as mean ±SD. *Significant at p<0.05, n=57

4.4.3 Gold milk treatment in subject with mild iron deficiency anaemia

Subjects with mild iron deficiency anaemia were given goat milk treatment throughout the experiment. Blood samples was taken on day 1 of study and repeated at 4 week, 8 week and12 week of study. The haematological parameters and iron profile data from subject was used forstatistical analysis to compare median before and after the treatment by using Wilcoxon Signed Rank test as described in **Section 3.5**

Result of statistical analysis and descriptive statistics was shown in **Table 4.7**. The results shown as mean (SD), median (IQR) and P value for haematological parameters and ironprofile. It demonstrated the p-value less than 0.05 ($p < 0.05$) for haemoglobin, red blood celland total iron binding capacity at week 4 of experiment. At week 8, there is significant increase in reticulocyte count and transferrin while at week 12, there is significant increase in reticulocyte count, red blood cell, haemoglobin, total iron binding capacity, ferritin andtransferrin. Transferrin saturation in goat milk treatment group is below the normal range (20%-55%). Mean corpuscular haemoglobin value is also below the normal range (27 pg- 32 pg) at all weeks. Serum iron is increase to 9.16 umol/L at week 4 of treatment but suddenly decrease at week 12 to 5.99 umol/L (normal range < 6.6 umol/L – 26 umol/L). Even though there is no significant different at each point of assessment for other parameter, yet the value is still within the normal range.

Table 4.7: Haematological parameters and iron profile for **goat milk** group at baseline, week 4, week 8 and week 12 of treatment (n=11). The data are shown as mean ± SD and median ± IQR

Parameters	Baseline		Week 4		Week 8		Week 12	
	Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Reticulocyte count (%)	0.38 ± 0.14	0.4 (0.2)	0.49 ± 0.20	0.5 (0.3)	0.65 ± 0.34	0.5 (0.2) *	0.85 ± 0.43	0.8 (0.5) *
RBC (x10^12/L)	4.75 ± 0.34	4.7 (0.2)	4.91 ± 0.29	4.8 (0.5) *	4.87 ± 0.26	4.8 (0.4)	4.94 ± 0.35	5.1 (0.6) *
Hb (g/dl)	11.51 ± 0.77	11.7 (2.5)	12.13 ± 1.43	12.5 (2.1) *	12.06 ± 1.38	12.6 (2)	12.23 ± 1.56	12.6 (2) *
PCV (%)	37.45 ± 3.23	38 (7)	38.64 ± 3.47	39 (4)	38.36 ± 3.80	39 (4)	38.55 ± 3.91	39 (4)
MCV (FL)	78.73 ± 7.01	80 (15)	79.09 ± 7.56	78 (14)	78.73 ± 8.25	77 (15)	78.09 ± 7.87	76 (15)
MCH (PG)	24.82 ± 3.02	25 (7)	24.82 ± 3.21	25 (7)	25.09 ± 3.14	25 (6)	25 ± 3.52	25 (7)
MCHC (g/dl)	31.55 ± 1.29	32 (2)	31.18 ± 1.32	32 (2)	31.82 ± 1.40	32 (3)	31.73 ± 1.48	32 (3)
Serum Iron(umol/L)	8.56 ± 5.93	6.2 (9.5)	9.16 ± 6.51	7.9 (11.7)	8.02 ± 5.17	6.5 (8.4)	5.99 ± 3.25	5.9 (3.5)
TIBC (umol/L)	65 ± 7.93	66 (12)	70 ± 9.44	69 (13) *	68.45 ± 11.80	65 (21)	68.36 ± 8.90	68 (11) *
Transferrin saturation (%)	13.55 ± 9.42	12 (16)	13.45 ± 9.84	11 (17)	12.09 ± 8.03	11 (9)	9.27 ± 5.53	10 (7)
Ferritin (ng/ml)	14.91 ± 8.87	10 (15)	17.09 ± 8.55	16 (16)	18.36 ± 10.57	16 (16)	18.55 ± 11.36	15 (21) *
Transferrin (g/L)4	2.67 ± 0.46	2.8 (0.5)	2.81 ± 0.44	2.8 (0.8)	3.18 ± 0.48	3.2 (1) *	3.11 ± 0.63	2.9 (0.9) *
Serum transferrin receptor (ng/ml)	20.68 ± 1.63	18.6 (9.35)	23.19 ± 2.46	21.4 (13.93)	16.88 ± 3.31	16.97 (17.22)	27.52 ± 4.35	30.92 (25.32)

* Significant different (P < 0.05) compared with baseline using Wilcoxon signed rank test

Comparison of haematological parameter at different time of treatment was shown in **Figure 4.16**. Reticulocyte counts shows an increasing trend from baseline until week 12 of treatment. At week 12 it shows a significant increase up to123.68% (0.85 ± 0.43) when compared to baseline value, 0.38 ± 0.14 as shown in **Figure 4.16 (a)**. There was an increasing trend for red blood cell count in goat milk treatment as observed in **Figure 4.16 (b)**. The mean value of 4.75 ± 0.1 at baseline increased up to 4% by week 12 although it showed a slight decrease at week 8, where the mean value recorded was 4.87 ± 0.26. The figure also shows sudden decrease in red blood cell count at week 8 of treatment. Haemoglobin level shows an increasing trend from baseline mean value of 11.51 ± 0.77 up to 12.23 ± 1.56 in week 12 of treatment as shown in **Figure 4.16 (c).** While there was a minor decrease observed at the eighth week of treatment (reducing to 12.06 ± 1.38), the overall trend indicates a noteworthy increase of 4.78% compared to the baseline value, which remains statistically significant. Assessment at week 4 and week 12 of treatment also shows a significant increase**.** Packed cell volume as observed in **Figure 4.16 (d)** also shows the same pattern as haemoglobin. It increased up to 38.55 ± 3.91 at week 12 of treatment by 3% but sudden decrease occurred at week 8 of treatment**.** At week 4 and week 12, packed cell volume shows a significant different when compared to baseline. The mean value at week 4 was 38.64 ± 3.47 and week 12 was 38.55 ± 3.91 whereas the mean value at baseline was 37.45 ± 0.98. There is not much change for mean cell volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration as observed in **figure 4.16 (e), (f)** and **(g).**

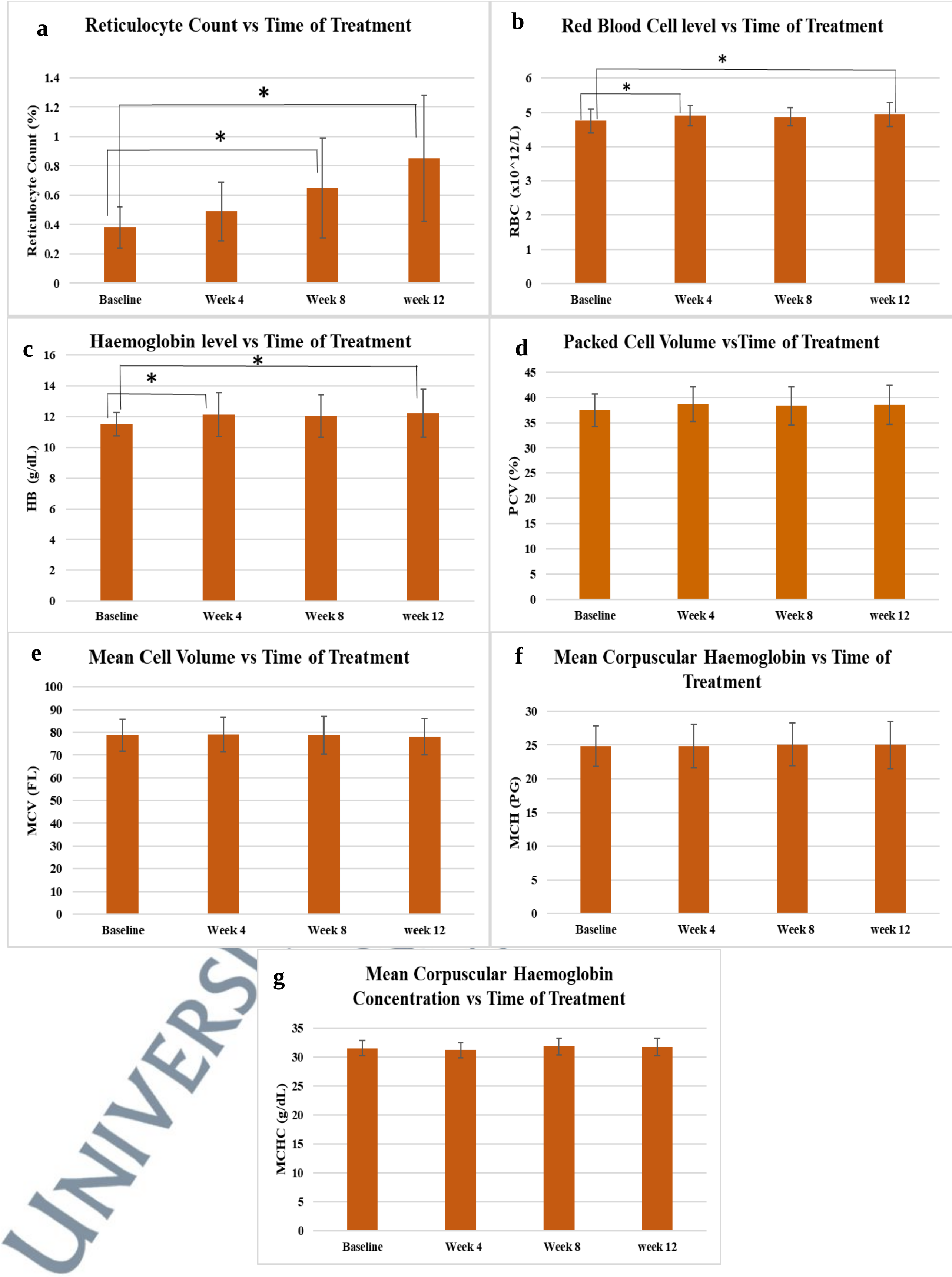
a
Reticulocyte Count vs Time of Treatment
Reticulocyte Count (%)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
Baseline
Week 4
Week 8
week 12
b
Red Blood Cell level vs Time of Treatment
RBC (x10^12/L)
6
5
4
3
2
1
0
*
*
Baseline
Week 4
Week 8
week 12
c
Haemoglobin level vs Time of Treatment
HB (g/dL)
16
14
12
10
8
6
4
2
0
*
*
Baseline
Week 4
Week 8
week 12
d
Packed Cell Volume vsTime of Treatment
PCV (%)
45
40
35
30
25
20
15
10
5
0
Baseline
Week 4
Week 8
week 12
e
Mean Cell Volume vs Time of Treatment
MCV (FL)
100
90
80
70
60
50
40
30
20
10
0
Baseline
Week 4
Week 8
week 12
f
Mean Corpuscular Haemoglobin vs Time of Treatment
MCH (PG)
30
25
20
15
10
5
0
Baseline
Week 4
Week 8
week 12
g
Mean Corpuscular Haemoglobin Concentration vs Time of Treatment
MCHC (g/dL)
35
30
25
20
15
10
5
0
Baseline
Week 4
Week 8
week 12

Figure 4.16: Comparison of haematological parameter in different time intervals of treatment. (**a**) Reticulocyte count (**b**) Red blood cell (**c**) Haemoglobin (**d**) Packed cell volume (**e**)Mean cell volume (**f**) Mean corpuscular Haemoglobin (**g**) Mean corpuscular Haemoglobin concentration. The data are shown as mean ± SD. *Significant at p<0.05, n=57

Measurement of iron profile at different point of time during the treatment was shown in **Figure 4.17**. Figure **4.157(a)** illustrates that during the treatment period with goat milk, the serum iron level demonstrates a decline starting from week 4 until week 12. However, there is a positive shift observed at week 4, indicating an improvement of 7% in the serum iron level. The final mean value was 5.99 ± 3.25 at week 12. The baseline mean value was 8.56 ± 5.93 and it peaked up to 9.16 ± 6.51 in week 4. Total iron binding capacity in **Figure 4.17 (b)** was significantly increased at week 4 of treatment (mean value = 70 ± 9.44) but it starts to decrease at week 8 until week 12 of treatment. The mean value decreased to 68.36 ± 8.90 in week 12 from 68.45 ± 11.80 in week 8. For transferrin saturation (**Figure 4.17(c)**), the value decreased to 13.45 ± 9.84 and continued to decrease to 9.27 ± 5.53 at week 12 of treatment.

Figure 4.17 (d) illustrates a rising pattern in ferritin levels, starting from the baseline to twelfth week of treatment. The increment amount is up to 24.41%**.** At week 12 transferrin level increased to 3.18 ± 0.48 at week 8 of treatment and suddenly decreased to 3.11 ± 0.63 at week 12 as shown in **Figure 4.17 (e).** At week 8, transferrin shows a significant increase compared to baseline. The serum transferrin receptor value continues to increase until it is 27.52 ± 4.35 at week 12 of treatment, although it shows a slight decrease of 16.88 ± 3.31 at week 8 of treatment, as shown in **Figure 4.17 (f)**.

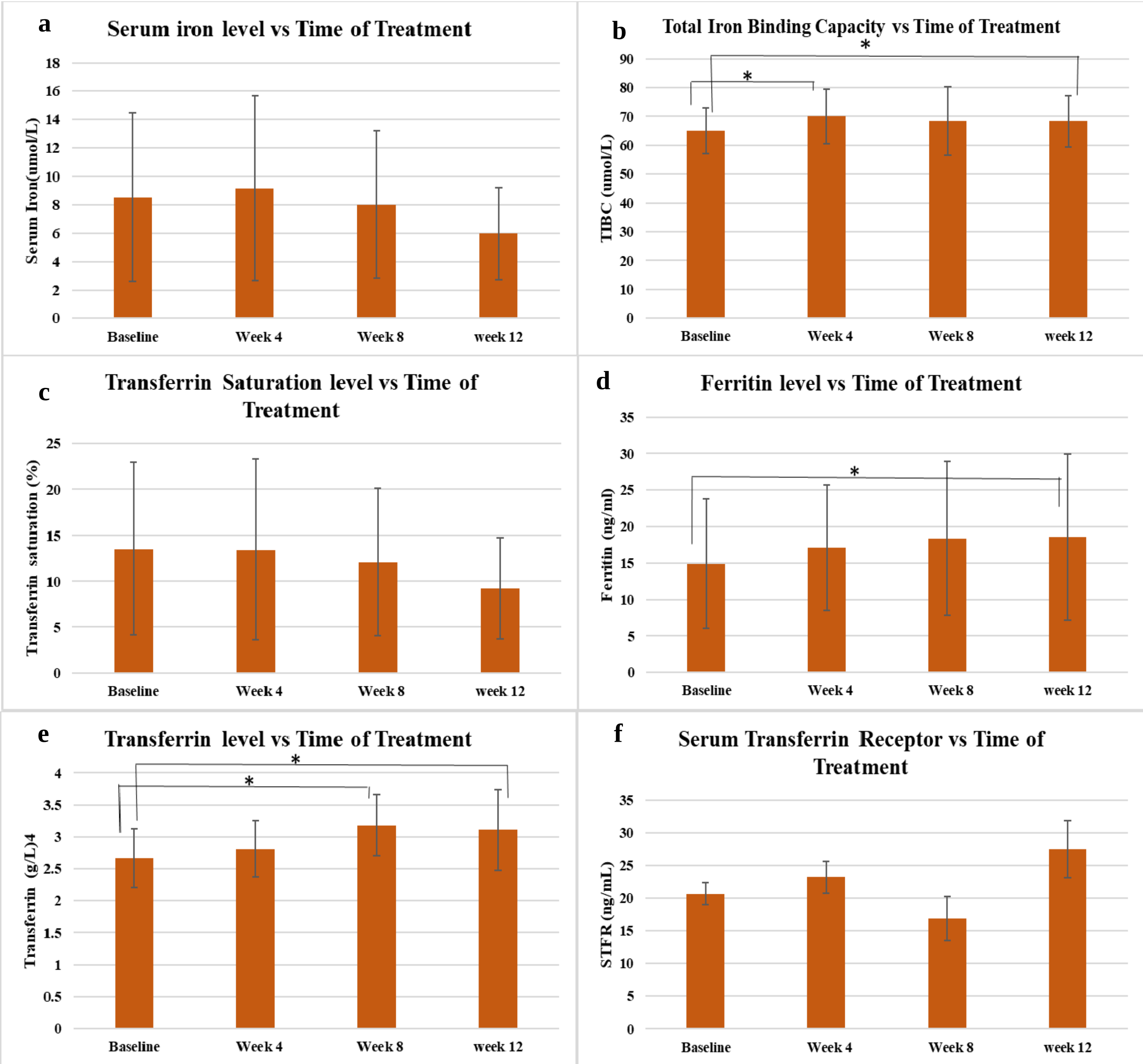

Figure 4.17: Comparison of iron profile between times of treatment. (**a**) Serum iron (**b**) Total iron binding capacity (**c**) Transferrin saturation (**d**) Ferritin (**e**)Transferrin (**f**) Serum transferrin receptor The data are shown as mean ±SD. *Significant at $p<0.05$, n=57

4.4.4 Date palm and goat milk treated subjects with mild iron deficiency anaemia

Subjects were given date palm together with goat milk treatment throughout the experiment. Blood samples was taken on day 1 of study and repeated at week 4, week 8 and week 12 of study. The haematological parameter and iron profile data from subject was used for statistical analysis to compare median before and after given a treatment by using Wilcoxon Signed Rank test as described in **Section 3.5**

Result of statistical analysis and descriptive statistics was shown in **Table 4.8.** The results were shown as mean (SD), median (IQR) and P value for haematological parameters and iron profile. The *p*-value recorded is less than 0.05 ($p < 0.05$) for haemoglobin, total iron binding capacity, ferritin and transferrin at week 4 of experiment. At week 8, there is significant difference in haemoglobin and transferrin while at week 12, there is significant difference in reticulocyte count, haemoglobin, total iron binding capacity, transferrin saturation, ferritin and transferrin. Transferrin saturation (normal range: 20%-55%) and mean corpuscular haemoglobin (normal range: 27pg- 32pg) were below the normal range for throughout the experiment. Serum iron is decreased below a normal range (<6.6 umol/L) at week 12 of treatment. Other parameters show no significant different within the time of treatment, but the value is still within the normal range.

Table 4.8: Haematological parameters and iron profile for **date palm + goat milk** group at baseline, week 4, week 8 and week 12 of treatment (n=12). The data are shown as mean ± SD and median ± IQR

Parameters	**Baseline**		**Week 4**		**Week 8**		**Week 12**	
	Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Reticulocyte count (%)	0.48 ± 0.25	0.4 (0.4)	0.45 ± 0.20	0.45 (0.2)	0.54 ± 0.17	0.5 (0.2)	0.73 ± 0.45	0.5 (0.25) *
RBC (x10^12/L)	4.71 ± 0.26	4.75 (0.17)	4.8 ± 0.24	4.85 (0.4)	4.8 ± 0.34	4.85 (0.47)	4.86 ± 0.32	4.8 (0.6)
Hb (g/dl)	11.38 ± 0.66	11.55 (0.8)	12.15 ± 1.10	12.2 (0.73) *	12.15 ± 0.98	12.2 (1.23) *	12.31 ± 0.97	12.05 (1.38)*
PCV (%)	37.33 ± 2.67	38 (4.24)	38.17 ± 2.82	39 (2.75)	38.17 ± 3.32	38 (2.5)	38.5 ± 2.19	39 (2.75)
MCV (FL)	79.42 ± 5.51	80 (3.75)	79.83 ± 6.58	80.5 (4.75)	79.33 ± 5.95	79.5 (3.75)	79.5 ± 6.32	80 (5.5)
MCH (PG)	25.75 ± 2.37	26 (2)	25.58 ± 2.42	25.5 (2.75)	25.58 ± 2.53	25.5 (3)	25.67 ± 2.74	26 (2.75)
MCHC (g/dl)	31.83 ± 1.03	32 (1)	31.92 ± 0.79	32 (0)	32 ± 0.95	32 (1.75)	32.25 ± 1.05	32 (1.75)
Serum Iron(umol/L)	7.26 ± 3.13	5.8 (5.77)	6.35 ± 3.06	5.35 (4.65)	6.95 ± 3.73	5.75 (3.65)	6.02 ± 2.02	6.05 (2.4)
TIBC (umol/L)	65.42 ± 4.12	65 (4.5)	69.58 ± 3.89	70 (4) *	66.25 ± 4.00	65 (4)	69.42 ± 5.81	69 (6.5) *
Transferrin saturation (%)	11.25 ± 5.06	9 (8.75)	9.58 ± 4.68	8 (7.25)	10.67 ± 6.32	8.5 (5.5)	8.83 ± 3.32	9 (3.75) *
Ferritin (ng/ml)	10.75 ± 4.09	10 (5)	12.58 ± 4.80	12 (6.75) *	12.33 ± 6.95	9.5 (9.5)	14.75 ± 8.13	12.5 (14.25)*
Transferrin (g/L)4	2.62 ± 0.34	2.55 (0.45)	2.89 ± 0.33	2.95 (0.6) *	3.16 ± 0.25	3.2 (0.43) *	3.24 ± 0.26	3.25 (0.3) *
Serum transferrin receptor (ng/ml)	18.54 ± 5.90	18.1 (5.68)	21.63 ± 8.80	22.5 (16.08)	15.83 ± 11.43	12.5 (15.42)	25.11 ± 9.05	24.6 (16.19)

* Significant different (P < 0.05) compared with baseline using Wilcoxon signed rank test

Changes in haematological parameter at different point of treatment period was shown in **Figure 4.18.** From the baseline up to the 12th week of treatment, there is a consistent upward trend observed in the reticulocyte count. Notably, at week 12, there is a substantial and statistically significant rise of 52.08% compared to the initial baseline value of 0.48 ± 0.25, as depicted in **Figure 4.18 (a).**

There was an increasing trend for red blood cell count in group treated with date palm and goat milk treatment as observed in **Figure 4.18 (b).** The mean value of 4.71 ± 0.26 at baseline increased up to 3.18% by week 12. Haemoglobin level shows an increasing trend from baseline mean value of 11.38 ± 0.66 to 12.31 ± 0.97 in week 12 of treatment as shown in **Figure 4.18 (c).** Although it remained constant at week 4 and week 8 of treatment (mean value = 12.15 ± 0.98), but it still shows a significant increase when compared to baseline value.

There is not much change in packed cell volume, mean cell volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration as observed in **Figure 4.18 (d), (e), (f) and (g).**

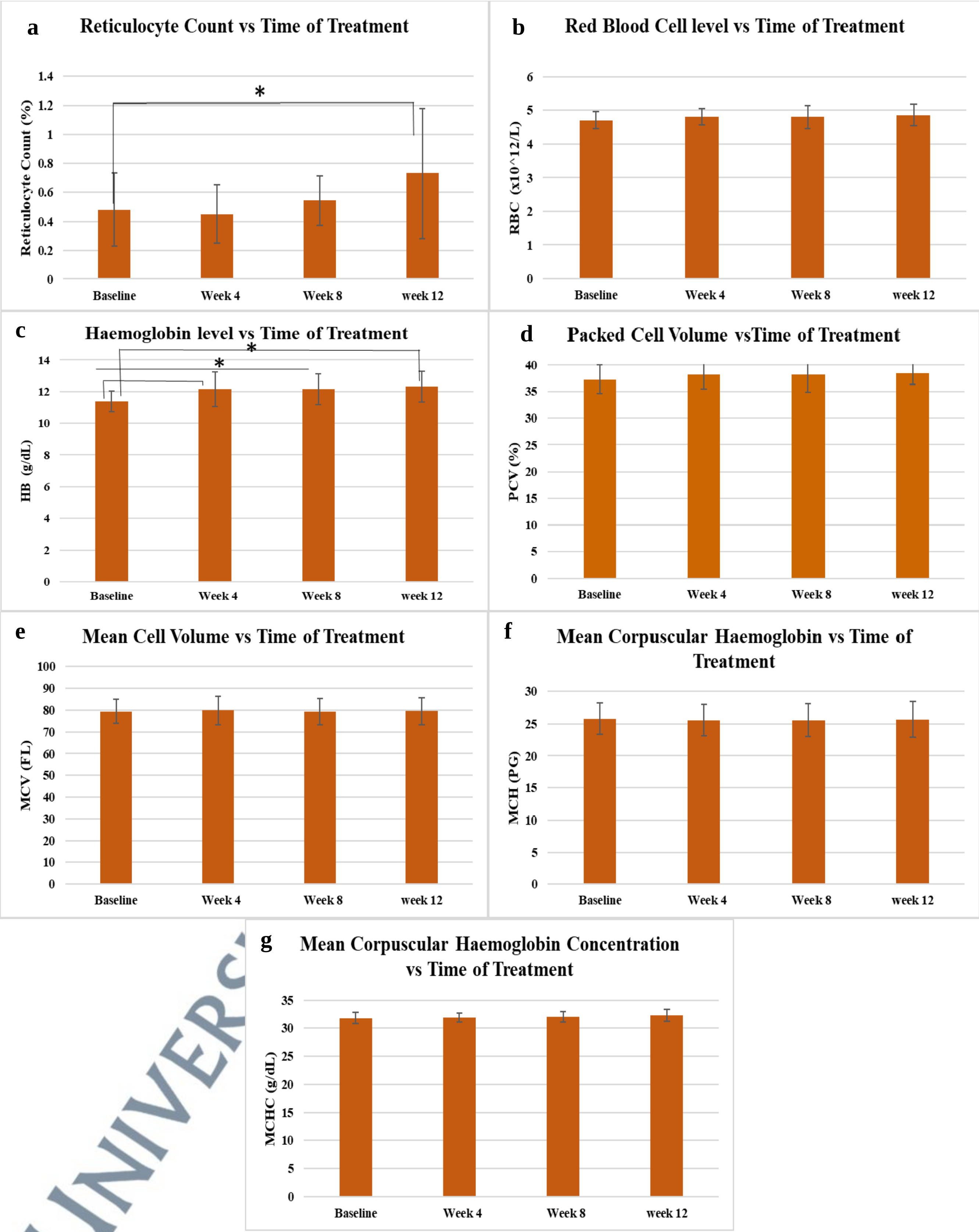
a
Reticulocyte Count vs Time of Treatment
Reticulocyte Count (%)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
*
Baseline
Week 4
Week 8
week 12
b
Red Blood Cell level vs Time of Treatment
RBC (x10^12/L)
0
1
2
3
4
5
6
Baseline
Week 4
Week 8
week 12
c
Haemoglobin level vs Time of Treatment
HB (g/dL)
0
2
4
6
8
10
12
14
*
*
Baseline
Week 4
Week 8
week 12
d
Packed Cell Volume vsTime of Treatment
PCV (%)
0
5
10
15
20
25
30
35
40
Baseline
Week 4
Week 8
week 12
e
Mean Cell Volume vs Time of Treatment
MCV (FL)
0
10
20
30
40
50
60
70
80
90
100
Baseline
Week 4
Week 8
week 12
f
Mean Corpuscular Haemoglobin vs Time of Treatment
MCH (PG)
0
5
10
15
20
25
30
Baseline
Week 4
Week 8
week 12
g
Mean Corpuscular Haemoglobin Concentration vs Time of Treatment
MCHC (g/dL)
0
5
10
15
20
25
30
35
Baseline
Week 4
Week 8
week 12

Figure 4.18: Comparison of haematological parameter in different time intervals of treatment. (**a)** Reticulocyte count (**b**) Red blood cell (**c**) Haemoglobin (**d**) Packed cell volume (**e**)Mean cell volume (**f**) Mean corpuscular Haemoglobin (**g**) Mean corpuscular Haemoglobin concentration The data are shown as mean ± SD.

*Significant at $p<0.05$, n=5

Changes in iron profile data at different point of treatment period was shown in **Figure 4.19**. Serum iron level shows a decreasing trend from week 4 until week 12 of treatment as shown in **Figure 4.19 (a)** but it shows an improvement at week 8 of treatment. The final mean value was 6.02 ± 2.02 at week 12. The baseline mean value was 7.26 ± 3.13 and it deacrease to 6.95 ± 3.73 in week 8. Total iron binding capacity in **Figure 4.19 (b)** was significantly increased at week 4 of treatment, up to 6.36% compared to the mean value of baseline. At week 8, it decreased to 66.25 ± 4.00 and increased again at week 12 of treatment to 69.42 ± 5.81.

For transferrin saturation **(Figure 4.19 (c)),** the value decreased to 9.58 ± 4.68 at week 4 compared to the mean value of 11.25 ± 5.06 at baseline. The mean value increased at week 8 to 10.67 ± 6.32 and continued decreasing till 8.83 ± 3.32 at week 12 of treatment.

In **Figure 4.19 (d)**, the ferritin levels exhibit a consistent increasing trend, starting from a mean value of 10.75 ± 4.09 at baseline and progressing to 14.75 ± 8.13 by the twelfth week of treatment. This signifies a notable increase of 37.21%. The mean value increased to 12.58 ± 4.80 at week 4 and decreased slightly at week 8. Transferrin level keep on increasing from 2.62 ± 0.34 at baseline to 3.24 ± 0.26 in week 12 as shown in **Figure 4.19 (e).** The mean value of serum transferrin receptor keeps on increasing up to 35.44% in week 12 of treatment although it decreased to 15.83 ± 11.43 mean value at week 8 of treatment as observed in **Figure 4.19 (f).**

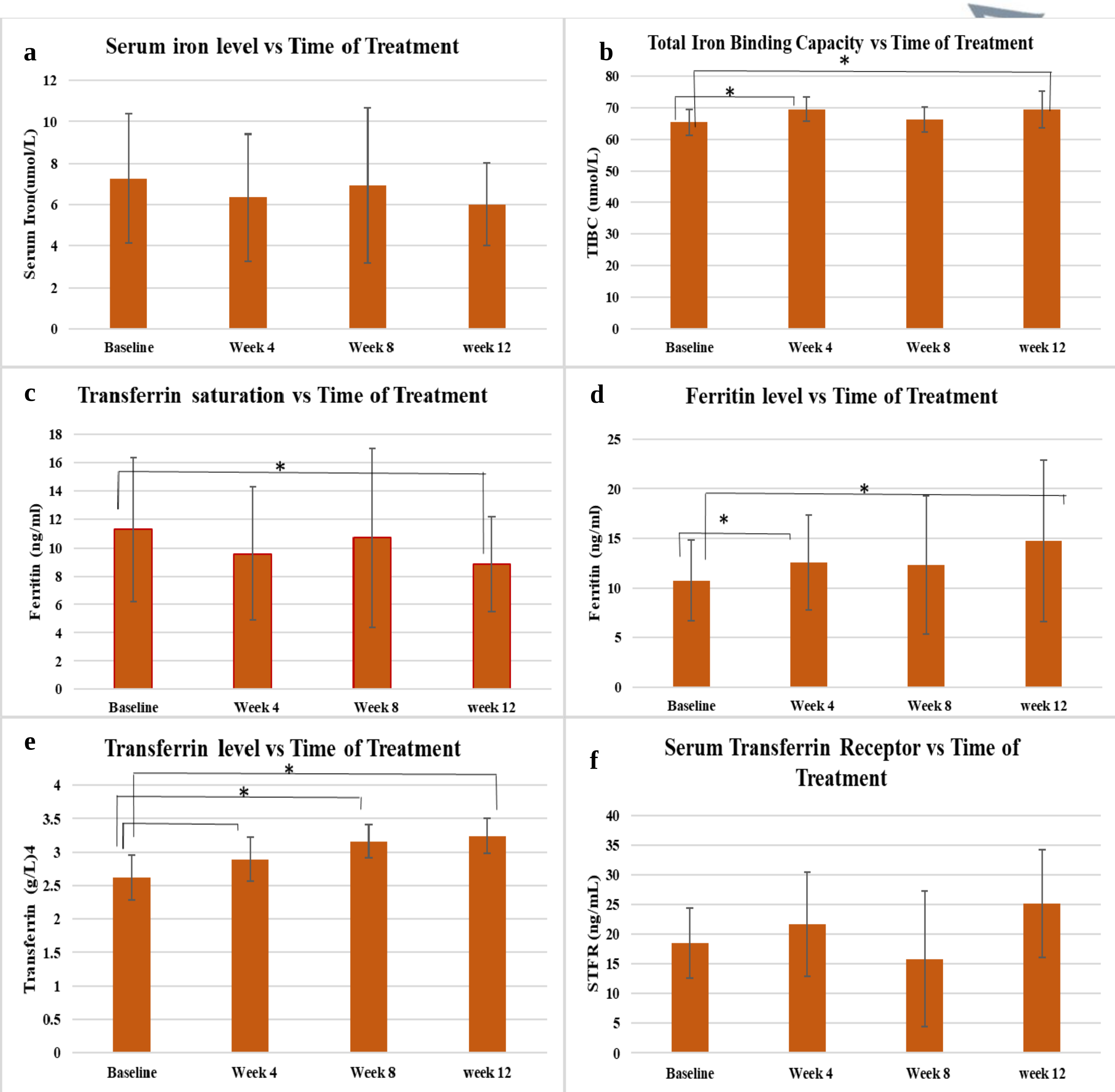

Figure 4.19: Comparison of iron profile in different time intervals of treatment. (**a**) Serum iron (**b**) Total iron binding capacity (**c**) Transferrin saturation (**d**) Ferritin (**e**)Transferrin (**f**) Serum transferrin receptor. The data are shown as mean ±SD. *Significant at $p<0.05$, n=5

4.4.5 Iron tablet treated subjects with mild iron deficiency anaemia

Subjects with mild iron deficiency anaemia were given iron tablet treatment throughout the experiment. Blood samples was taken on day 1 of study and repeated at week 4, week 8 and week 12 of study. The haematological parameters and iron profile data from subject was used for statistical analysis to compare median before and after given a treatment by using Wilcoxon Signed Rank test as described in **Section 3.5.**

Result of statistical analysis and descriptive statistics was shown in **Table 4.9.** The results shown as mean (SD), median (IQR) and P value for haematological parameters and iron profile. From this result, the p-value is less than 0.05 ($p < 0.05$) for all the parameters except reticulocyte count, mean corpuscular haemoglobin concentration and serum transferrin receptor at week 4 of experiment. At week 8, there is significant difference in all parameters except for serum iron, transferrin saturation, transferrin and Serum transferrin receptor.

At week 12, while there is significant difference in red blood cell, haemoglobin, packed cell volume, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total iron binding capacity, ferritin and serum transferrin receptor.

Table 4.9: Haematological parameters and iron profile for **iron tablet** group at baseline, week 4, week 8 and week 12 of treatment (n=11). The data are shown as mean ± SD and median ± IQR

Parameters	**Baseline**		**Week 4**		**Week 8**		**Week 12**	
	Mean (SD)	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**	**Mean (SD)**	**Median (IQR)**
Reticulocyte count (%)	0.43 ± 0.07	0.4 (0.1)	0.43 ± 0.22	0.4 (0.4)	0.65 ± 0.16	0.7(0.2) *	0.64 ± 0.48	0.5 (0.3)
RBC (x10^12/L)	4.61 ± 0.29	4.6 (0.4)	4.82 ± 0.38	4.7 (0.6) *	4.89 ± 0.41	4.9 (0.8) *	5.07 ± 0.37	5 (0.8) *
Hb (g/dl)	11.38 ± 1.68	11.5 (1.1)	12.65 ± 1.14	12.8 (1.3) *	13.41 ± 1.03	13.2 (1.2) *	14.2 ± 0.99	14 (0.7) *
PCV (%)	36.73 ± 3.77	37 (5)	40.00 ± 2.60	39 (2) *	41.36 ± 3.07	42 (5) *	42.82 ± 2.82	43 (4) *
MCV (FL)	79.82 ± 8.03	79 (9)	83.64 ± 6.03	84 (7) *	84.55 ± 4.74	83 (9) *	84.64 ± 3.23	84 (5) *
MCH (PG)	25.18 ± 3.84	25 (4)	26.73 ± 2.96	27 (4) *	27.91 ± 1.75	28 (2) *	28.45 ± 1.36	28 (3) *
MCHC (g/dl)	31.45 ± 2.16	32 (1)	31.73 ± 1.79	32 (2)	32.45 ± 1.03	33 (1) *	33.36 ± 1.02	33 (1) *
Serum Iron(umol/L)	7.54 ± 6.31	5 (5.3)	19.90 ± 8.56	17.7 (13.4) *	13.25 ± 7.18	11.7 (11.8)	9.61 ± 4.08	8.7 (5.3)
TIBC (umol/L)	70.45 ± 2.24	74 (12)	65.82 ± 1.97	65 (12) *	62.55 ± 2.02	63 (8) *	63.73 ± 2.51	62 (11) *
Transferrin saturation (%)	11.09 ± 10.03	6 (7)	30.09 ± 11.09	28 (16) *	21.36 ± 10.59	21 (19)	15 ± 6.06	15 (9)
Ferritin (ng/ml)	8.55 ± 7.42	7 (4)	26.64 ± 11.61	21 (16) *	25.36 ± 11.87	22 (12) *	28.73 ± 16.00	24 (26) *
Transferrin (g/L)4	2.89 ± 0.41	3 (0.5)	2.64 ± 0.27	2.6 (0.3) *	3.03 ± 0.39	2.9 (0.7)	2.85 ± 0.30	2.8 (0.3)
Serum transferrin receptor (ng/ml)	21.58 ± 6.11	20.36 (9.76)	23.75 ± 8.65	25.74 (13.34)	31.39 ± 13.43	28.64 (27.91)	35.19 ± 15.54	33.44 (22.55)*

* Significant different (P < 0.05) compared with baseline using Wilcoxon signed rank test

Changes in haematological parameters at different point of treatment period was shown in **Figure 4.20.** Reticulocyte count shows an increasing trend from baseline until week 12 of treatment. At week 12 it shows a significant increase up to 48.84% when compared to baseline value, 0.43 ± 0.07 as shown in **Figure 4.20 (a).**

There was an increasing trend for red blood cell count in iron tablet treatment group as observed in **Figure 4.20 (b).** The mean value of 4.61 ± 0.29 at baseline increased up to 9.98% by week 12. Haemoglobin level shows an increasing trend from baseline mean value of 11.38 ± 1.68 to 14.20 ± 0.99 in week 12 of treatment as shown in **Figure 4.20 (c).** The mean value increased up to 12.65 ± 1.14 week 4 and 13.41 ± 1.03 in week 8 of treatment. In week 12, there was an elevation in the mean packed cell volume, reaching 16.58%, as opposed to the baseline measurement of 36.73 ± 3.77. The mean value kept increasing in week 4 and week 8 as shown in **Figure 4.20 (d)**. There was an increasing trend for mean cell volume in iron tablet treatment group. The mean value of 79.82 ± 8.03 at baseline increased to 83.64 ± 6.03 at week 4 and continued increasing to 84.55 ± 4.74 in week 8 and reached 84.64 ± 3.23 at week 12 as shown in **Figure 4.20 (e).**

The mean corpuscular haemoglobin also increased from 25.18 ± 3.84 to 26.73 ± 2.96 in week 4 and 27.91 ± 1.75 in week 8. Similarly, mean corpuscular haemoglobin concentration also exhibited an increasing trend from the baseline mean value of 31.45 ± 2.16 to 31.73 ± 1.79 in week 4 and 32.45 ± 1.03 in week 8. At week 12, the mean value for mean corpuscular haemoglobin iron tablet treatment was 28.45 ± 1.36 and mean corpuscular haemoglobin concentration increased up to 33.36 ± 1.02 as observed in **Figure 4.20 (f) and (g).**

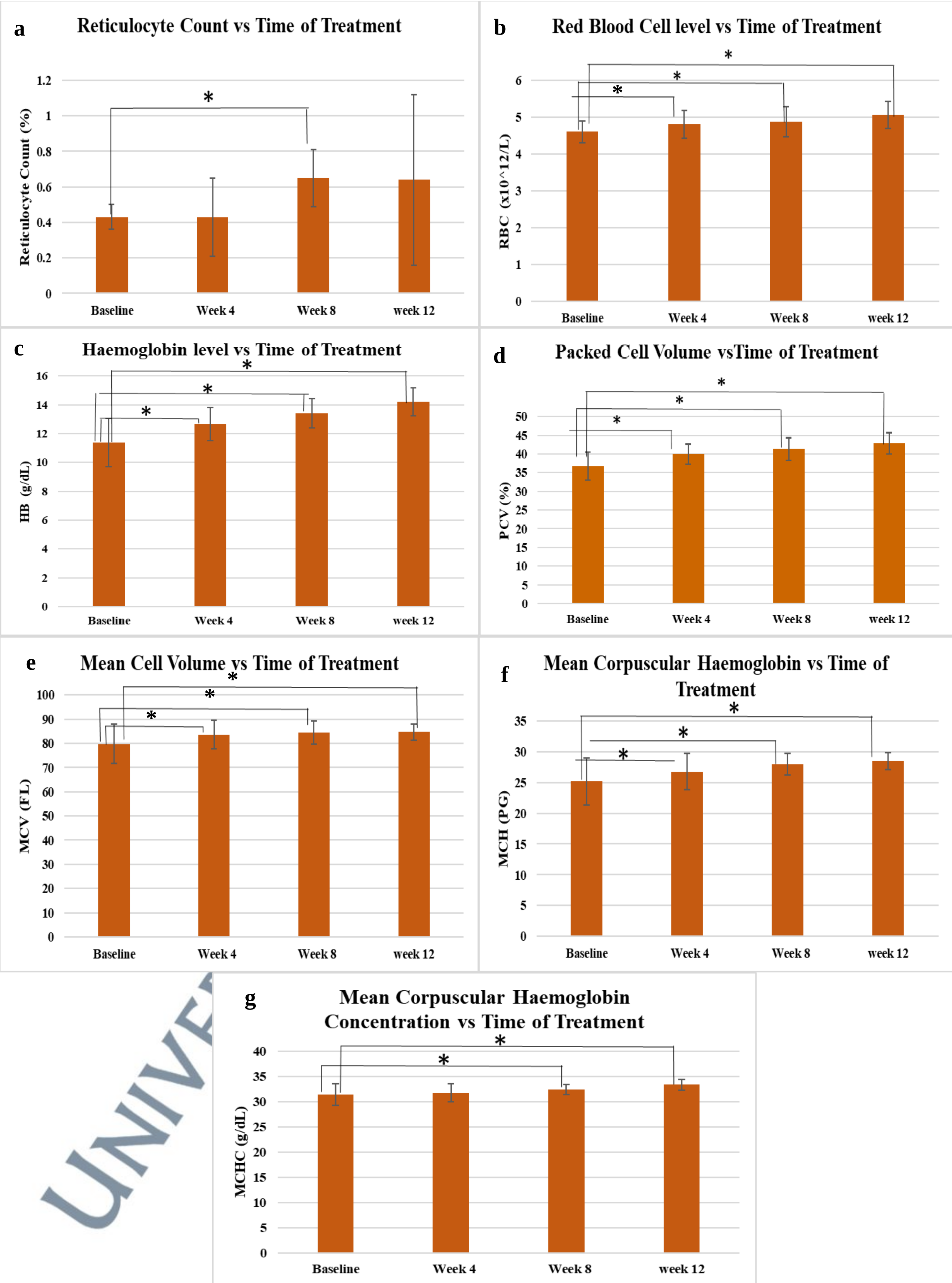
a
Reticulocyte Count vs Time of Treatment
Reticulocyte Count (%)
1.2
1
0.8
0.6
0.4
0.2
0
*
Baseline
Week 4
Week 8
week 12
b
Red Blood Cell level vs Time of Treatment
RBC (x10^12/L)
6
5
4
3
2
1
0
*
*
*
Baseline
Week 4
Week 8
week 12
c
Haemoglobin level vs Time of Treatment
HB (g/dL)
16
14
12
10
8
6
4
2
0
*
*
*
Baseline
Week 4
Week 8
week 12
d
Packed Cell Volume vsTime of Treatment
PCV (%)
50
45
40
35
30
25
20
15
10
5
0
*
*
*
Baseline
Week 4
Week 8
week 12
e
Mean Cell Volume vs Time of Treatment
MCV (FL)
100
90
80
70
60
50
40
30
20
10
0
*
*
*
Baseline
Week 4
Week 8
week 12
f
Mean Corpuscular Haemoglobin vs Time of Treatment
MCH (PG)
35
30
25
20
15
10
5
0
*
*
*
Baseline
Week 4
Week 8
week 12
g
Mean Corpuscular Haemoglobin Concentration vs Time of Treatment
MCHC (g/dL)
40
35
30
25
20
15
10
5
0
*
*
Baseline
Week 4
Week 8
week 12

Figure 4.20: Comparison of haematological parameter in different time intervals of treatment. (**a**) Reticulocyte count (**b**) Red blood cell (**c**) Haemoglobin (**d**) Packed cell volume (**e**)Mean cell volume (**f**) Mean corpuscular Haemoglobin (**g**) Mean corpuscular Haemoglobin concentration. The data are shown as mean ± SD. *Significant at $p<0.05$, n=5

Changes in iron profile data at different point of treatment period was shown in **Figure 4.21**. Serum iron level shows a decreasing trend from week 4 until week 12 of treatment as shown in **Figure 4.21 (a)** but it shows an increase at week 4 of treatment comparing to the baseline. The final mean value was 9.61 ± 4.08 at week 12. The baseline mean value was 7.54 ± 6.31 and it peaked up to 19.9 ± 8.56 in week 4. Total iron binding capacity in **Figure 4.21 (b)** was significantly decreased to 65.82 ± 1.97 at week 4 of treatment compared to the mean value of baseline, 70.45 ± 2.24. At the start of week 8, it decreased to 62.55 ± 2.02 and increased slightly to week 12 of treatment up to 63.73 ± 2.51.

For transferrin saturation **(Figure 4.21 (c)),** the value increased sharply to 30.09 ± 11.09 at week 4 compared to the mean value of 11.09 ± 10.03 at baseline. However, the mean value decreased at week 8 to 21.36 ± 10.59 and continued decreasing till 15 ± 6.06 at week 12 of treatment.

The ferritin level shows an increasing trend from mean value of 8.55 ± 7.42 at baseline up to 28.73 ± 16.00 in week 12 of treatment as shown in **Figure 4.21 (d)**. The mean value increased to 26.64 ± 11.61 at week 4 and decreased slightly to 25.36 ± 11.87 at week 8. Transferrin level reduced from 2.89 ± 0.41 at baseline to 2.64 ± 0.27 in week 4. The mean value for transferrin level increased to 3.03 ± 0.39 in week 8 and decreased to 2.85 ± 0.30 as shown in **Figure 4.21 (e).** The mean value of serum transferrin receptor keeps on increasing from 21.58 ± 6.11 at baseline to 23.75 ± 8.65 at week 4, 31.39 ± 13.43 at week 8, up to 35.19 ± 15.54 in week 12 as observed in **Figure 4.21 (f).**

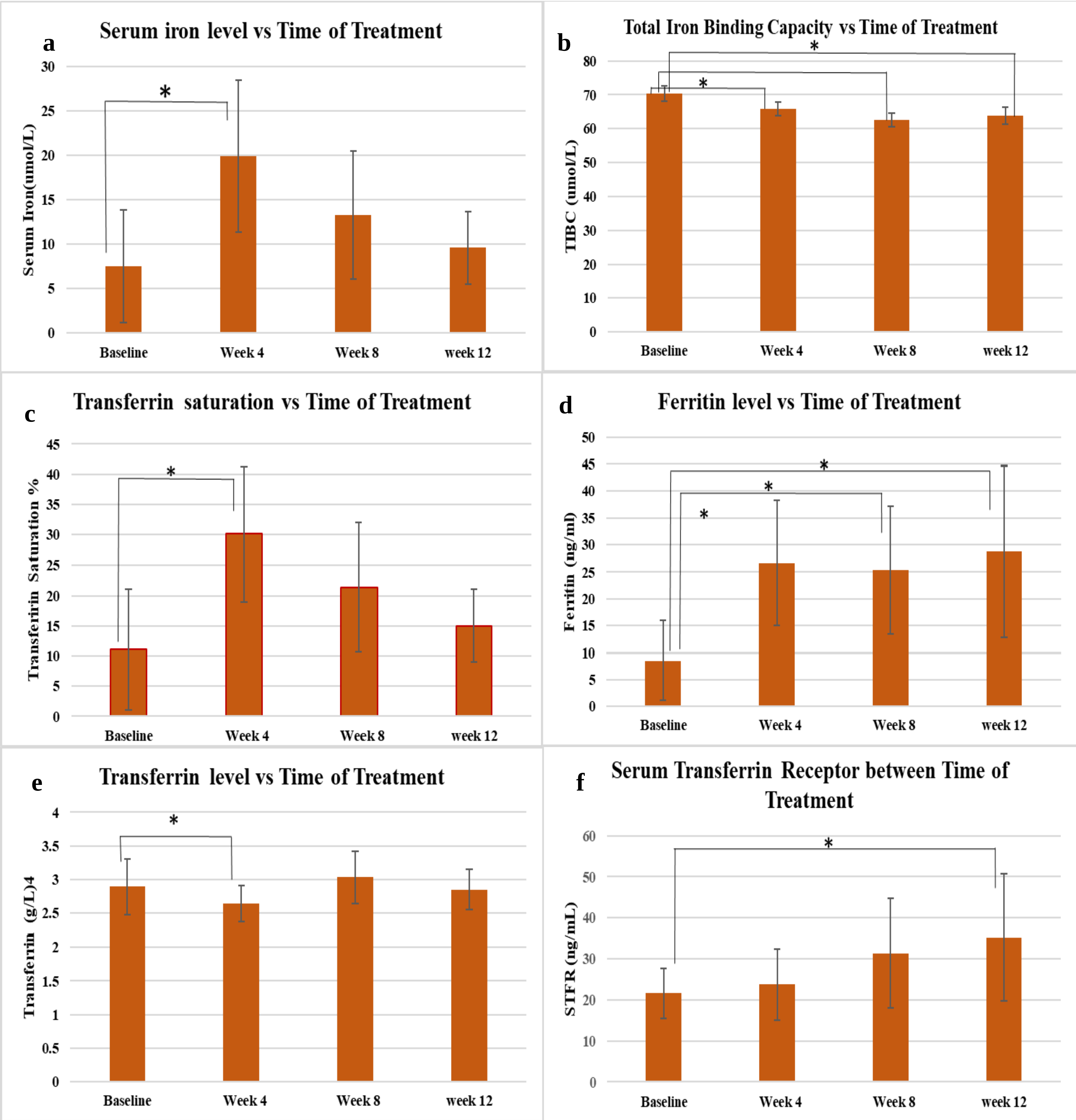

Figure 4.21: Comparison of iron profile in different time intervals of treatment. (**a**) Serum iron (**b**) Total iron binding capacity (**c**) Transferrin saturation (**d**) Ferritin (**e**)Transferrin (**f**) Serum transferrin receptor. The data are shown as mean ±SD. *Significant at p<0.05, n=57.