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Biological Evaluation of Cupcake Made with Wheat Flour Enriched with Chickpea, Purslane, Doum and Carob Flours as Functional Food for Patients with Anemia

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Abstract

Iron deficiency and specifically iron deficiency anemia remains one of the most severe and important nutritional deficiencies in the world today. In this study chickpea ,purslane, doum and carob flours were used as natural sources for production of the functional food (cupcake) for anemic patients. Cupcake supplemented with 80% wheat flour and 20% chickpea) replacement) had the highest score sensory evaluation. Supplementation of cupcake made from 80% wheat flour, 20% chickpea flour partially replacement of wheat flour with purslane leaves r, doum and carob powders at 7.5, 7.5 and 15%, respectively which had the highest score sensory evaluation. In double-fortified experiment were tested for their efficacy in managing iron deficiency anemia (IDA) in rats. Seven groups of female Wistar rats (n = 6) were used. The study was divided into two phases, viz., the development of anemia in the first phase by providing five groups with diet free of iron for 21 days and the rest two groups provided with normal basal diets, then second phase the random division of anemic rats into five groups feeding the experimental tested diets for 56 days The hemoglobin (Hb) concentration value, serum iron (SI), body weights, blood parameters and weights of all rats groups were measured. The biological examination revealed that the (G4) , (G5 (G6) and carob(G7) induced the greatest improvement effect on body weight gain in anemic rats as compared to (G2) which contained 92.69, 106.48, 101.18 and 102.05% in, G4, G5, G6 and G7, respectively compared with 37.32% in G2. The hematological and biochemical analyses showed that the changes in blood picture, serum iron, serum proteins levels were in favor of supplementation chickpea flour with purslane, doum and carob products when compared to (G2).The RBCs, Hb, Hct, MCV and MCH increased significantly. Moreover results indicated that administration of the supplemented diets was associated with an improvement in the levels of alanine aminotransferase ,aspartate aminotransferase, urea, creatinine and uric acid compared with the IDA model group and a significant decrease the levels of albumin and total protein were recorded. Lipid profile indicated that a significant decrease in triglycerides, total cholesterol, LDL-cholesterol and VLDL-cholesterol, which HDL-cholesterol increased significantly in G4, G5, G6 and G7 compared with those in G2. It can be concluded that chickpea flour with purslane, doum and carob products have good nutritive value and positive response on blood picture and serum biochemical parameters in anemic rats. Therefore, this study recommend that intake of chickpea, purslane, doum and carob powders may be beneficial for patients who suffer from iron deficiency anemia due to their nutritional and restorative properties.

Key words: Chickpea flour – Purslane – Doum – Carob - Iron deficiency anemia - Biochemical analysis - Hematological examination.

Introduction

Iron deficiency (ID) and specifically iron deficiency anemia (IDA) remains one of the most severe and important nutritional deficiencies in the world today. The economic implications of iron deficiency and the various intervention strategies to combat it suggest that food-based approaches and targeted supplementation are particularly cost-effective. The highest benefit to cost ratio is attained with food fortification **(Hong He *et al*., 2019).** Iron is an indispensable element for life, as it is an important component of human hemoglobin, cytochrome enzymes and many reductases **(Khatami *et al*., 2013).** Iron plays important roles in oxygen transportation, deoxyribonucleic acid (DNA) synthesis (**Hassan *et al*., 2016).**

However, iron deficiency is very common in all age groups and can lead to iron deficiency anemia (IDA) and bodily dysfunction **(Alquaiz *et al*., 2012).** Anemia occurs in one-third of the world's population and is mostly caused by iron deficiency **(Auerbach and Adamson, 2016).** IDA is the commonest nutritional deficiency in the world and can affect

mental and physical development (**Hassan *et al*., 2016)**. In Egypt the prevalence of anemia among the adolescent girls was 39.9%, the prevalence of iron deficiency anemia was 30.2% and that of iron deficiency without anemia was 11.4%. Despite Egypt's Adolescent Anemia Prevention Program, ID and IDA are still health problems that need to be addressed to improve adolescent girls' health. This is of public health importance as it gives an opportunity for school-based interventions to improve adolescent girls' health **(Mousa *et al*., 2016)**. Anaemia prevalence among women 15-49 years increased from 41% in 2016 to 53% in 2022 **(Kumar *et al*., 2022)**.

Also, legumes have been considered a rich source of protein throughout the world and contain approximately three times more proteins than cereals. Chickpea (*Cicer arietinum* L.) is the third most important pulse crop worldwide, with a cultivated area of 14.84 million hectares, a production of 15.08 million tons and an average yield of 1.01 t/ha in 2020 (**FAO, 2021**) . Also, chickpea flour is a good source of proteins, fibers, minerals and other bioactive components and it could be an ideal ingredient for improving the nutritional value of bread and bakery products **(Man *et al*., 2015)**. Legume consumption has health positive effects (2) and it decreases risks of heart diseases, cancers, diabetes, vulnerability, blood hypertension and intestinal disorders and decreases of low cholesterol level (**Anderson and Major, 2002)**.

Purslane **(***Portulaca oleracea* L.), commonly known as purslane, is using as medicinal plant due to its high contents of bioactive components (**Gabr *et al*., 2021**). Purslane is a weed belonging to the family portulacaceae, widespread throughout the world and it is considered one of the important plants because it contains a high percentage of omega-3 fatty acids, in addition to containing the essential minerals, ascorbic and carotenoids, research has shown the effectiveness of leaf extract as antioxidants **(Al-Dallee *et al*., 2022).** Purslane leaves had a high content of Micro elements like, zinc, iron, manganese and chromium which were 4.1, 315, 18.2 and 110 mg/100 g, respectively, also some Macro elements like calcium, potassium and magnesium contents were higher compared with wheat flour **(El-Gindy, 2017**).

Doum (*Hyphaene thebaica* L.) is a desert tree endemic to Egypt, Sub-Saharan Africa, and West India. The fruit's coating is eatable and may be crushed into a powder or sliced into pieces; the powder is generally dried and used as a flavoring ingredient in foods. Doum flour has a vast quantity of important minerals like potassium, sodium, calcium, magnesium, as well as phosphorus, according to different research. Doum flour also provides vitamin B complex, carbs, and fibers, all of which are beneficial to one's health. Several investigations have found that doum flour extracts are rich in phenolic and flavonoid compounds, and have substantial antioxidant and antibacterial properties (**Seleem, 2015**).

Carob is rich in sugars, tannins, amino acids, minerals like K, Ca, Zn, Na, P and Fe and vitamins like D, E, C, B6, Niacin and folic acid which has important functions in our health **(Youssef *et al*., 2013)**. Carob is an excellent source of dietary fiber and has been utilized successfully in the preparation of gluten-free products like cake **(Berk *et al*., 2017)**. Carob also has many polyphenolic components which could decrease several diseases as cancer, diabetes, lactose intolerance, hyperlipidemia and heart problems result to its antioxidant activity **(Gregoriou *et al*., 2021)**.

Materials and Methods

1. Materials:

1.1. Wheat flour (W.F.):

Wheat flour (72% ext.) was obtained from North Cairo Flour Mills and Bakeries Company (Cairo Governorate, Egypt).

1.2. Chickpea

Chickpea (*Cicer arietinum* L.) dehulled and roasted were obtained from local market in Benha Kalyuobia, Governorat, Egypt.

1.3. Purslane Leaves:

Fresh purslane (*Portulaca oleracea* L.) were harvested from a private fields in Benha, Kalyuobia Governorate, Egypt) prior to flowering period during August 2020

1.4. Doum fruit flakes : Flakes of doum fruit**(*Hyphaene thebaica* L)** were obtained from Ragab El-Attar market store located in Cairo Governorate, Egypt.

1.5. Carob pods: Carob pods (***Ceratonia siliqua***) were obtained from Ragab El-Attar market store located in Cairo governorate, Egypt.

1.6. Baking ingredients:

Fresh egg, vanilla, salts, baking powder, shortening, skim milk powder and sugar materials were obtained from local market at Benha, Kalyuobia, Governorate, Egypt.

1.7. Experimental animals: 42 Female experimental Wistar rats (with average weight 90±5 g 45 days old) were obtained from animal house of the Research Institute of Ophthalmology.

2. Methods:

2.1.a Preparation of chickpea, doum and carob powders ;Chickpea ,doum and carob powders were prepared by grinding by (Moulinex A59, France). sieved by using sieve at 60 mesh.

2.1.b Preparation of purslane leaves powder: Plants were cleaned from dust and from forgein matters, washed with tap water and then were dried in a hot air oven maintained at 55ºC, milled and kept in polyethylene bags until used.

After that,all matreials storged at 5°C until used**.**

2.2. Preparation of cupcake in the laboratory: The cupcake processing method was taken according to **Dubat (2010)** with some modification as follows:

Shortening was melted thoroughly, then sugar and salt were added with vigorous mixing. Whole egg was mixed with vanilla and whipped until got puff and smooth like-cream texture. Additionally, substituted WF with CPF at 10, 20 and 30% were individually mixed with baking powder and skimmed milk powder then gradually added to whipped egg mixture. This mixture was mixed gently until got homogenous dough using a mixing machine (Braun M1000). After getting appropriate texture the dough was poured into paper cups and backed at 180±5°C for 20-25 min. The baked cupcakes were cooled down at room temperature, about one hour before sensory evaluation and packaged in polyethylene bags and cold storage stored at 5°C.

2.3. Biological evaluation experiments:

2.3.1. Experimental animals:

Female 42 albino rats of 80-100 g weight (Webster breed) which were kept under normal laboratory conditions temperature (25±2°C) for one week (adaptation period) before the initiation of the experiment. During this period, the animals were fed individually adlib where they were allowed to free access of water and basal diet (**A.O.A.C., 2012)**.

2.3.2. Salt and vitamin mixtures:

Salt mixtures (normal with iron and iron-free) and vitamin mixtures were prepared according to the formula mentioned in (**A.O.A.C., 2012).**

2.4. Design for the biological experiments:

2.4.1. Method of procedure for biological experiments:

The basal diet was comprised of casein (15%), wood cellulose (5%), vitamin mixture (1.0%), salt mixture (4.0%) and corn oil (5%). The basal diet was completed to 100 g with corn starch. Food consumption and body weight were monitored daily and individually for each animal.

After a period of adaptation (one week) on basal diet, rats were divided into seven groups (6 rats/group with average weight 90±5 g) where every animal was placed in single cage and fed adlib. Experimental and control diets were prepared by incorporating the test diets and casein into the basal diet to achieve an isonitrogenous diet at 10% protein level. Two groups continued to feed on basal diet with normal iron-containing mineral mixture (negative control diet groups) and the rest 5 groups were iron-depleted to induce anemia upon feeding on experimental iron-free mineral mixture basal diet. Blood samples were drawn from every single animal every week of the feeding days until results of serum iron. Hematocrit and hemoglobin indicated iron deficiency state (after about 21 days feeding iron-free basal diet). Individual anemic rat groups were then fed the experimental cupcake diets either control cupcake or control cupcake supplementation with purslane, doum and carob powders. The rats were given the formulated diets and water ad-libitum for 56 days. Animals were weighed weekly and food consumption was determined. Following 8 weeks of dietary treatment, after 12 hours of food deprivation, the rats were anesthetized with diethyl ether and blood samples were collected from the the orbital plexus of veins in the inner canthus of eye.. Portion was collected into heparinized tubes to determine hemoglobin, hematocrit. Another portion was collected into tubes without anticoagulant to obtain serum and stored at -20°C until further analyses.

The vital organs (livers, kidneys, hearts and spleens) of rats were immediately removed, perfused by cold 0.9% sodium chloride solution and blotted on filter paper, then weighted and kept frozen at -20°C.

2.4.2. Experimental rat diets:

Rat groups were fed the following experimental diets:

As described in the following table:

Group NO.	Group description
1	Negative normal control group fed with basal diet.
2	Anemic rats fed with basal diet free of iron; (negative control diet group)
3	Normal control group fed with cupcake diet supplemented with chickpea seeds powder only replacement of 20% of wheat flour (blank control diet).
4	Anemic rats fed with cupcake diet supplemented with chickpea flour only replacement of 20% of wheat flour.
5	Anemic rats fed with cupcake diet supplemented with chickpea flour enriched with 7.5% purslane leaves powder .
6	Anemic rats fed with cupcake diet supplemented with chickpea flour enriched with 7.5% doum powder.
7	Anemic rats fed with cupcake diet supplemented with chickpea flour enriched with 15% carob powder

2.4.3. Biological evaluation parameters:

Biological evaluations at the different days were carried out by the determination of body weight gain (BWG.) according to **Chapman *et al*. (1959)**.

BWG = [(Final weight – Initial weight)/Initial weight]×100

2.2.4. Biochemical blood analysis:

- Hematological examination included the determination of hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were performed at the Biochemical Laboratory of Faculty of Veterinary Medicine, Cairo Universality according to the methods of **Jain (2000)**.
- Alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined according to the method recorded by **Reitman and Frankel (1957)** Alkaline phosphate (ALP) enzyme activity was determined according to the method recorded by **Tietz *et al*. (1983).**
- Serum total protein, serum albumin and serum globulin were determined according to **Doumas *et al*. (1971).**
- Serum total bilirubin was determined according to the method described by **Young (1995)**
- Serum triglycerides were determined according to the method recorded by **Fossati and Prencipe (1982).**
- Serum total cholesterol was determined using the enzymatic method as described by **Finely (1978).**
- Serum high density lipoprotein cholesterol (HDL-cho) was carried out according to the method described by **Lopes-Virella *et al*. (1977).**
- Serum low density lipoprotein cholesterol (LDL-cho) was calculated using the formula described by **Friedewald (1972).**

LDL-cholesterol (mg/dl) =TC – [HDL cholesterol + (Triglycerides/5)]

- Serum very low density lipoproteins can be calculated using formula described by **Bauer (1982):**

VLDL-cholesterol (mg/dl) = Triglycerides/5

- Creatinine reacts with picric acid in alkaline medium to form a red-orange color which measured at 628 nm according to the method of **Henery *et al.* (1974).**
- The enzymatic colorimetric method for urea assay in blood was measured according to the method described by **Tabacco *et al*. (1979).**
- Uric acid was determined by enzymatic colorimetric test using kits according to **Barham and Trinder (1972).**

2.3. Statistical analysis:

The statistical analysis was carried out using two-way ANOVA using SPSS, ver. 25 **(IBM Corp. Released 2013).** Data were treated as a complete randomization design according to **Steel *et al.* (1997).** Multiple comparisons were carried out applying Duncun test The significance level was set at < 0.05**.**

Results and Discussion

In an attempt to investigate the effect of supplementation with chickpea flour, purslane, doum and carob powders as natural sources for iron to wheat flour (72% ext.) for producing an affective product suitable for iron deficiency anemia patients. The biological parameters for experimental rats assays accompanied by measuring growth response due to diet feeding and changes in their blood chemistry indices, weight of vital organs and histopathological characteristics were showed in Tables (1 to 7).

1. Comparison the biological growth parameters for normal and anemic rats fed supplemented cupcake:

Data in Table (1) remarkable significant decrease in body weight was noted in G2 for the first 28 days compared to G1, then a significant increase was observed in body weight in G2 for the last 28 days. A progressive increase in body weight was noted in all anemic rat groups (fed on supplemented cupcake) which continued on feeding such diet for 56 days. Higher body weights were significantly apparent in G1 and G3, which contained 136.79 and 114.89% and a significant slight increase was observed in body weight in G5 ,G6 and G7the highest level recorded in positive total body weight was in G5 (106.48%) flowed G7 (102.05%) then G6 (101.18%) and finally G4 (92.69%). The obtained data were in line with those obtained BY **Hemeda *et al*. (2018)** who reported that the body weight gain in experimental rats fed with diet containing flour and protein isolate of two types of legumes (chickpea and soybean) was significantly higher for rats fed on soybean flour - chickpea protein isolate diet (56.00±4.27), followed by those fed on diets supplemented with chickpea flour - soy protein isolate (54.00±3.75) as compared to that of group fed on basal diet These results are in agreement with those obtained by **El-Serwy and Abd El-Hameid (2012)** who reported that the addition of purslane at 2 and 4% to the hight fat diet decreased the body weight. The obtained data were in line with those by **Shehata (2021)** who reported that a significant improve in body weight gain of diabetic rats consumed doum biscuits as compared with diabetic rats fed on control biscuits. This result is consistent with that obtained by **Rtibi *et al*. (2021)** who studied the influence of the carob powder and sweet whey powder inclusion into weaning feed in rabbits and reported that rabbits fed diets enriched with CP and WhP in combination improved final body weight**.**

Table 1. Weekly body weight gain (%) of rats feeding on normal, free-iron basal and cupcake replacement with 7.5% purslane leaves powder, 7.5% doum powder and 15% carob powder (mean±SE).

Group	Weight gain (%) (week)							
	1	2	3	4	5	6	7	8
G1	**10.01 ±0.21^{aH}**	**23.74 ±0.68^{bG}**	**38.96 ±1.08^{aF}**	**55.07 ±1.21^{aE}**	**73.15 ±1.58^{aD}**	**92.25 ±1.70^{aC}**	**113.97 ±1.99^{aB}**	136.79 ±1.90^{aA}
G2	**-3.65 ±0.13^{dH}**	**-7.05 ±0.36^{eG}**	**-9.43 ±0.43^{eF}**	**-6.58 ±0.44^{fE}**	**1.60 ±0.55^{gD}**	**11.65 ±0.61^{gC}**	**23.93 ±0.78^{gB}**	37.32 ±0.95^{fA}
G3	**10.90 ±0.44^{aH}**	**25.88 ±0.50^{aG}**	**36.96 ±0.58^{bF}**	**47.26 ±0.79^{bE}**	**61.10 ±0.96^{bD}**	**75.70 ±0.98^{bC}**	**95.26 ±1.77^{bB}**	114.89 ±1.45^{bA}
G4	**5.38 ±0.20^{cH}**	**10.96 ±0.27^{dG}**	**17.83 ±0.47^{dF}**	**28.98 ±0.65^{eE}**	**41.60 ±1.16^{fD}**	**55.77 ±1.32^{fC}**	**73.97 ±1.69^{fB}**	92.69 ±2.04^{eA}
G5	**4.85 ±0.22^{cH}**	**11.63 ±0.33^{dG}**	**20.19 ±0.53^{cF}**	**33.18 ±1.07^{cE}**	**51.48 ±1.70^{cD}**	**69.20 ±2.93^{cC}**	**87.49 ±1.63^{cB}**	106.48 ±1.85^{cA}
G6	**4.99 ±0.25^{cH}**	**10.79 ±0.37^{dG}**	**19.35 ±0.64^{cF}**	**31.58 ±0.71^{dE}**	**44.92 ±0.77^{eD}**	**61.43 ±1.97^{eC}**	**81.52 ±2.43^{eB}**	101.18 ±2.68^{dA}
G7	6.57 ±0.10^{bH}	12.62 ±0.21^{cG}	20.72 ±0.46^{cF}	33.42 ±0.51^{cE}	46.79 ±0.44^{dD}	62.69 ±0.80^{dC}	82.88 ±0.83^{dB}	102.05 ±0.91^{dA}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

G1: Normal rats group feeding on normal basal diet. (Negative control)
G2: Anemic rats group feeding on free-iron basal diet. (Positive control)
G3: Normal rats group feeding on normal control cupcake (80%wheat flour +20%chickpe flour).
G4: Anemic rats group feeding on normal control cupcake.
G5: Anemic rats group feeding on cupcake replacement 7.5% purslane leaves powder.
G6: Anemic rats group feeding on cupcake replacement 7.5% doum powder.
G7: Anemic rats group feeding on cupcake replacement 15% carob powder.

2. Chemical rats blood indices Evaluation for supplemented cupcake diets:

The blood analysis for experimental normal and anemic rats fed on normal basal, anemic basal (iron-free diet) and supplemented cupcake diets, at zero time of the experiment, after feeding anemic rats for 21 days then 35 days and finally after feeding for 56 days were done.

2.1. Hemoglobin (Hb) and hematocrite (Hct) examination in rats blood:

Examination of Hb in rat blood plasma is an important parameter to indicate symptoms of anemic case (anemia) or iron–deficiency. After feeding 21 days the lower values for Hb and Hct in plasma compared to levels registered at zero time, were noted in blood of five anemic induced rat groups. The Hb and Hct levels for rats in both normal groups (fed basal and control cupcake increased to the normal level after feeding rat's for 56 days as indicated in Table (2) which increased significantly from 8.35, 8.75 8.77 and 8.78 (g/dL) for Hb at zero time to 13.23, 14.13, 13.53 and 13.42 (g/dL) at 56 days, while Hct level increased significantly from 24.15, 25.08, 25.18 and 25.50% at zero time to 37.33, 38.97, 38.00 and 38.13%) at 56 days. However, the levels of Hb and Hct decreased significantly in plasma of positive control rats group after feeding for 35 and 56 days compared to the levels of rats fed on both normal basal and supplemented cupcake diets. The obtained data in negative control rats are in agreement with data estimated by **Thakur *et al.* (2019)** who found that rats fed on Fe-deficient diet for 35 days indicated a significant decrease ($p<0.05$) in Hb, RBC, HCT, MCV, MCH and MCHC values comparing with normal control rats. A reduce in hemoglobin level probably is due to iron, which has a large role in hemoglobin production. Results clarified that anemic rats consumed diets containing control cupcake show significant differences ($p<0.05$) in hematological parameters values compared to anemic rats. In contrast, anemic rats fed with cupcake diets showed significant increase in Hb and Hct levels with the of feeding period up to 56 days.

Hematological parameters significant increase in red blood cells (RBCs), hemoglobin content (Hb), hematocrite (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) in rats blood plasma which feeding (G1), (G3), (G4), (G5), (G6) and (G7).

Rbcs count increased significantly from ($5.53x10^6$/µL) in (G2) to ($6.62x10^6$/µL), ($6.67x10^6$/µL), ($6.54x10^6$/µL) and ($6.62x10^6$/µL) in G4, G5, G6 and G7, respectively. Moreover, another increase in Hb and Hct levels was attained which reached to the normal level in the rest anemic rats groups fed supplemented cupcake diets. Highest levels of Hb and Hct recorded in anemic rat group fed on cupcake supplemented with purslane..

2.2. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC):

The same trend observed in rat blood RBCs, Hb and Hct upon feeding rats the different tested diets was also followed with that found values for MCV, MCH and MCHC as demonstrated in **Table(2)** Statistical analysis revealed that there is a lightly significant increase in MCV, which increased from 37.50, 39.31, 4 0.80 and 40.93 (fl) at zero time to 56.42, 58.42, 85.11 and 57.39 (fl) in G4, G5, G6 and G7, respectively at 56 day. MCH in blood plasma of rats increased significantly from 12.97, 13.71, 14.19 and 14.11(pg) at zero time to 20.00, 21.19, 20.69 and 20.28 (fl) at 56 day in G4, G5, G6 and G7, respectively. MCHC increased significantly from 34.59, 34.93 and 34.58 (g/dL) at zero time to 35.46, 35.63 and 35.20 (g/dL) at 56 days in G4, G6 and G7, respectively, while it increased significantly from 35.00 to 36.29 in G5.

Anemic rats fed on supplemented cupcake diets caused significantly elevated ($p<0.05$) in hematological parameters values Hb, HCT, RBC, MCV, MCH and MCHC levels were also increased. compared with anemic rats These results probably are due to supplemented cupcake diets contained higher values of iron revealed higher contents of hematological parameters, which were close to the normal values of normal control rats.

These results are agreement with those reported by **Huang *et al.* (2014)** whom investigated the improving effect of chickpea seeds on iron deficiency anemia in female Wistar rats induced by oral administration of iron-deficient diet and founded ameliorations in hematological parameters The similar results were demonstrated by **Mokhtarifar *et al.* (2017)** who investigate the effects of purslane seeds on Hb levels in adolescent girls with IDA, and to compare the effects versus ferrous sulfate. In this study, significant improvements were observed in the mean Hb, Hct, and MCV levels with purslane.. These results are in agreement with those obtained by **Bayad (2016)** who reported that daily administration of the higher levels of the decoction of doum fruits (2 g/kg) for 2 months induced a significant increase ein RBCs, PCV and Hb in rats. This result is consistent with that obtained by **Rtibi *et al.* (2021)** who studied the influence of the carob powder (CP) and sweet whey powder (WhP) inclusion into weaning feed in rabbits and reported that there were significant differences in the RBC, Hb, HCT and MCVwere increased in group fed basal diet + CP/WhP

Table 2. Hematological and biochemical measurements in blood plasma of rats feeding on normal, free-iron basal and cupcake replacement with 7.5% purslane leaves powder, 7.5% doum powder and 15% carob powder (mean±SE).

Parameters	Groups	Zero time (after feed on basal diet)	Feeding on free-iron diet for (week)			
			0	3	5	8
RBCs (10^6/µL)	G1	6.52 ± 0.04^{cdB}	6.56 ± 0.03^{aB}	6.64 ± 0.03^{aA}	6.71 ± 0.05^{aA}	6.69 ± 0.01^{aA}
	G2	6.65 ± 0.03^{aA}	6.34 ± 0.06^{dB}	5.97 ± 0.03^{eC}	5.79 ± 0.07^{dD}	5.53 ± 0.27^{cE}
	G3	6.49 ± 0.01^{cdC}	6.52 ± 0.01^{abC}	6.56 ± 0.01^{abBC}	6.61 ± 0.00^{bAB}	6.64 ± 0.02^{aA}
	G4	6.65 ± 0.08^{aA}	6.44 ± 0.05^{bcC}	6.49 ± 0.04^{bcBC}	6.54 ± 0.06^{bcB}	6.62 ± 0.08^{aA}
	G5	6.62 ± 0.06^{abAB}	6.38 ± 0.02^{cdD}	6.46 ± 0.03^{cC}	6.59 ± 0.03^{bB}	6.67 ± 0.02^{aA}
	G6	6.46 ± 0.03^{dB}	6.18 ± 0.05^{eD}	6.32 ± 0.05^{dC}	6.46 ± 0.04^{cB}	6.54 ± 0.03^{bA}
	G7	6.55 ± 0.06^{bcAB}	6.23 ± 0.06^{eD}	6.44 ± 0.06^{cC}	6.53 ± 0.04^{bB}	6.62 ± 0.03^{abA}
Hb (g/dL)	G1	14.32 ± 0.26^{aB}	14.28 ± 0.30^{aB}	14.05 ± 0.09^{aC}	14.35 ± 0.18^{aB}	14.62 ± 0.12^{aA}
	G2	14.27 ± 0.20^{aA}	8.88 ± 0.06^{cB}	8.17 ± 0.10^{fC}	7.67 ± 0.16^{fD}	6.92 ± 0.19^{eE}
	G3	13.97 ± 0.17^{bAB}	13.73 ± 0.11^{bC}	13.72 ± 0.09^{bC}	13.93 ± 0.03^{bB}	14.13 ± 0.10^{bA}
	G4	13.97 ± 0.13^{bA}	8.35 ± 0.08^{dE}	10.33 ± 0.16^{eD}	12.40 ± 0.16^{dC}	13.23 ± 0.16^{dB}
	G5	14.12 ± 0.27^{abA}	8.75 ± 0.12^{cD}	11.03 ± 0.09^{cC}	12.88 ± 0.07^{cB}	14.13 ± 0.15^{bA}
	G6	14.12 ± 0.16^{abA}	8.77 ± 0.19^{cE}	10.55 ± 0.28^{dD}	12.02 ± 0.10^{eC}	13.53 ± 0.18^{cB}
	G7	14.13 ± 0.24^{abA}	8.78 ± 0.16^{cE}	10.18 ± 0.12^{eD}	12.20 ± 0.24^{deC}	13.42 ± 0.07^{cB}
Hct %	G1	38.00 ± 0.06^{bA}	38.15 ± 0.10^{aA}	38.28 ± 0.09^{aA}	38.55 ± 0.23^{aA}	38.55 ± 0.43^{abA}
	G2	38.73 ± 0.27^{aA}	30.38 ± 0.65^{bB}	25.67 ± 1.24^{dC}	22.95 ± 0.78^{dD}	21.22 ± 0.66^{dE}
	G3	38.75 ± 0.13^{aA}	38.83 ± 0.13^{aA}	39.05 ± 0.15^{aA}	39.15 ± 0.20^{aA}	39.25 ± 0.18^{aA}
	G4	38.93 ± 0.59^{aA}	24.15 ± 0.29^{dE}	27.91 ± 0.66^{cD}	32.05 ± 0.69^{cC}	37.33 ± 0.64^{cB}
	G5	38.45 ± 0.29^{aA}	25.08 ± 0.97^{cD}	28.68 ± 0.89^{bC}	33.57 ± 0.67^{bB}	38.97 ± 0.51^{aA}
	G6	38.38 ± 0.25^{aA}	25.18 ± 0.85^{cD}	28.62 ± 0.48^{bcC}	32.80 ± 0.63^{bcB}	38.00 ± 0.33^{bcA}
	G7	38.48 ± 0.32^{aA}	25.50 ± 1.18^{cD}	29.32 ± 1.33^{bcC}	33.27 ± 1.03^{bB}	38.13 ± 0.68^{bcA}
MCV(fl)	G1	58.29 ± 0.36^{bA}	58.13 ± 0.37^{bA}	57.65 ± 0.25^{bA}	57.45 ± 0.20^{bA}	57.65 ± 0.64^{bcA}
	G2	58.25 ± 0.63^{bA}	47.97 ± 1.34^{cB}	43.01 ± 2.01^{dC}	39.60 ± 0.98^{eD}	38.55 ± 2.41^{dE}
	G3	59.71 ± 0.21^{aA}	59.56 ± 0.16^{aA}	59.53 ± 0.28^{aA}	59.26 ± 0.29^{aA}	59.08 ± 0.33^{aA}
	G4	58.56 ± 1.17^{abA}	37.50 ± 0.33^{fE}	43.01 ± 0.96^{dD}	48.99 ± 1.07^{dC}	56.42 ± 0.41^{cB}
	G5	58.09 ± 0.64^{bA}	39.31 ± 1.50^{eD}	44.38 ± 1.44^{cC}	50.95 ± 1.23^{cB}	58.42 ± 0.83^{abA}

	G6	59.42±0.14^{abA}	40.80±1.72^{dE}	45.30±1.08^{cD}	50.81±1.21^{cC}	58.11±0.79^{abB}
	G7	58.75±0.23^{abA}	40.93±1.59^{dD}	45.48±1.85^{cC}	50.92±1.54^{cB}	57.63±0.99^{bcA}
MCH (pg)	G1	21.96±0.52^{aA}	21.77±0.56^{aA}	21.17±0.23^{aB}	21.39±0.39^{aB}	21.86±0.20^{aA}
	G2	21.45±0.28^{bcA}	14.02±0.20^{cdB}	13.69±0.12^{dB}	13.24±0.33^{dC}	12.57±0.76^{eD}
	G3	21.52±0.30^{abcA}	21.06±0.18^{bBC}	20.91±0.16^{aC}	21.09±0.05^{aBC}	21.27±0.17^{bAB}
	G4	21.01±0.42^{dA}	12.97±0.17^{eE}	15.93±0.33^{cD}	18.96±0.38^{cC}	20.00±0.10^{dB}
	G5	21.32±0.32^{cdA}	13.71±0.22^{dD}	17.07±0.21^{bC}	19.55±0.16^{bB}	21.19±0.17^{bA}
	G6	21.85±0.15^{abA}	14.19±0.21^{cE}	16.69±0.33^{bD}	18.61±0.07^{cC}	20.69±0.18^{cB}
	G7	21.59±0.52^{abcA}	14.11±0.32^{cdE}	15.81±0.28^{cD}	18.68±0.45^{cC}	20.28±0.15^{dB}
	G1	37.68±0.68^{aAB}	37.44±0.73^{aAB}	36.70±0.27^{bB}	37.23±0.56^{bAB}	37.93±0.59^{aA}
	G2	36.84±0.75^{abA}	29.26±0.42^{cE}	31.94±1.23^{eD}	33.45±0.91^{dC}	32.61±0.12^{cB}
	G3	36.05±0.50^{bA}	35.37±0.35^{bA}	35.13±0.18^{cdA}	35.59±0.23^{cA}	36.01±0.42^{bA}
MCHC (g/dL)	G4	35.90±0.76^{bB}	34.59±0.72^{bC}	37.08±1.40^{bcA}	38.74±1.28^{aA}	35.46±0.43^{bBC}
	G5	36.71±0.49^{abB}	35.00±1.56^{bC}	38.54±1.24^{aA}	38.42±0.87^{aA}	36.29±0.70^{bB}
	G6	36.77±0.21^{abA}	34.93±1.83^{bB}	36.92±1.56^{bcA}	36.68±1.02^{bcA}	35.63±0.76^{bB}
	G7	36.73±0.8^{abA}	34.58±1.57^{bB}	34.87±1.57^{dB}	36.72±0.98^{bcA}	35.20±0.45^{bB}

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A, B & C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

G1: Normal rats group feeding on normal basal diet (Negative control).
G2: Anemic rats group feeding on free-iron basal diet (Positive control).
G3: Normal rats group feeding on normal control cupcake.
G4: Anemic rats group feeding on normal control cupcake.
G5: Anemic rats group feeding on cupcake enriched with 7.5% purslane leaves powder replacement.
G6: Anemic rats group feeding on cupcake enriched with 7.5%doum powder replacement.
G7: Anemic rats group feeding on cupcake enriched with15% carob powder replacement.

2.3. Vital organs functions of experimental rats:

Analyzed vital organs function of rat analyzed comprised of two organs functions (liver and kidney). Liver functions included alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) enzymes activity. Serum total protein, Serum albumin, Serum globulin, and Serum bilirubin. Kidney functions tests included the determination of serum urea, uric acid and total creatinine. Results of tests for organ functions pointed out those normal values were attained similar to the normal values for laboratory animals. Furthermore, significant differences (P<0.05) existed in all analyzed indices for these between blood of all groups of rats fed on all experimental diets when analyzed after 21, 35 and 56 days or until the end of feeding experiment

2.3.1. Plasma liver enzymes:

The results of plasma liver enzymes of healthy and anemic rats are tabulated in Table (3).Concerning (ALT), (AST) and alkaline phosphatase (ALP) enzymes activity, it could be observed that (G1) had a significant lower values than that of (G2) for ALT, AST and ALP which contained 94.63, 28.10 and 98.75 (U/L) in G1 compared with 146.07, 53.93 and 140.70 (U/L), respectively in G2 at 56 days. Statistical analysis did not appear any significant differences for ALT between (G1), (G3), (G4), (G5), (G6) and (G7), which contained 94.63, 95.33, 100.53, 98.00, 100.47 and 100.77 U/L, respectively.

From the obtained results it could be noticed that the substitute of wheat flour with chickpea, purslane, doum and carob powders in cupcake processing led to significantly decrease of ALT, AST and ALP as a liver enzymes.

G2 showed a significant (P≤0.05) increase in plasma AST, ALP, and ALT compared with (G1). On the other hand, (G3) showed a decrease in plasma concentration of ALT, AST and ALP. The significant decrease in plasma biomarker enzymes (AST, ALT and ALP). In anemic groups treating animals especially (G5)gave the best results for all the enzymes compared to positive control group. These results are in agreement with those obtained by **Nagib (2021)** who reported that feeding fatty liver rats diets with 10% (chickpea, lupin and their combination) improved the parameters of liver enzymes (AST, ALT and ALP), as compared to the group which treated with high fat diet only after 8 weeks of feeding. These results are in concurrence with the finding obtained by by **El-Serwwy and Abd El-Hamid (2012**) who reported that the addition of purslane leaves powder at 2 and 4% to the height fat diet led to significantly reduce serum of AST and ALT activity after 4 weeks of feeding. The obtained data were in line with those of **Shehata (2021)**) who reported that a significant decrease in values of alanine and aspartate aminotransferase (ALT and AST) as well as alkaline phosphatase (ALP) of diabetic rats consumed biscuits fortified with doum flour .The same results were obtained by **El Rabey *et al.* (2017)** who reported that the treatment with 20%

carob legume methanol extract in hypercholesterolemic male rats for 8 weeks significantly improved the liver functions (AST, ALT and ALP) by decreasing the liver enzymes activity.

Table 3. Liver enzymes activity in serum blood of rats feeding on normal, free-iron basal and cupcake replacement with 7.5% purslane leaves powder, 7.5% doum powder and 15% carob powder (mean±SE).

Parameters	Groups	Zero time (after adaptation for 7 days on basal diet)	After 3weks	After 5 weeks	After 8weeks
ALT (U / L)	G1	92.17±1.28^{aA}	93.53±1.04^{bA}	94.00±1.16^{bA}	94.63±0.99^{bA}
	G2	91.03±1.68^{aD}	107.57±1.11^{aC}	125.10±2.17^{aB}	146.07±3.89^{aA}
	G3	90.13±2.60^{aA}	91.83±2.84^{bA}	93.73±2.45^{bA}	95.33±1.91^{bA}
	G4	90.30±1.86^{aB}	104.43±1.13^{aA}	102.20±1.23^{bA}	100.53±1.47^{bA}
	G5	90.43±1.68^{aC}	106.00±2.55^{aA}	103.20±1.35^{bAB}	98.00±0.79^{bB}
	G6	89.03±1.75^{aB}	68.17±34.08^{cC}	101.70±1.29^{bA}	100.47±1.22^{bA}
	G7	89.13±2.09^{aB}	104.43±2.09^{aA}	102.57±1.87^{bA}	100.77±1.74^{bA}
AST (U / L)	G1	24.83±1.39^{aC}	26.43±1.65^{cB}	27.53±1.48^{eAB}	28.10±1.42^{eA}
	G2	24.27±1.29^{aD}	38.17±1.41^{bC}	45.33±1.40^{aB}	53.93±1.73^{aA}
	G3	24.07±1.45^{abC}	26.23±1.11^{cB}	27.30±1.46^{eAB}	28.80±1.30^{eA}
	G4	22.50±0.92^{bDA}	37.43±1.25^{bA}	35.27±0.84^{cB}	33.87±0.69^{cB}
	G5	23.07±1.09^{abC}	37.53±1.24^{bA}	32.07±1.41^{dB}	30.60±0.90^{dB}
	G6	23.47±1.07^{aC}	38.10±1.48^{abA}	34.83±1.52^{cB}	33.50±1.31^{cB}
	G7	24.43±0.93^{aC}	39.57±0.49^{aA}	37.17±0.43^{bB}	35.80±0.44^{bB}
ALP (U / L)	G1	99.56±0.97^{aA}	99.98±0.56^{dA}	97.32±8.68^{cA}	98.75±0.35^{eA}
	G2	99.07±0.26^{aD}	113.84±0.96^{cC}	128.25±1.38^{aB}	140.70±1.02^{aA}
	G3	98.82±0.92^{aA}	98.98±1.08^{dA}	99.42±0.96^{cA}	99.59±0.57^{deA}
	G4	97.88±1.05^{aD}	120.07±1.60^{bA}	110.11±0.89^{bB}	100.19±0.76^{deC}
	G5	98.98±0.96^{aD}	123.20±1.32^{aA}	111.91±0.75^{bB}	101.76±0.79^{cdC}
	G6	99.26±0.74^{aD}	122.29±1.38^{abA}	112.63±1.08^{bB}	103.20±1.40^{bcC}
	G7	98.09±0.78^{aD}	121.40±0.96^{abA}	113.47±0.63^{bB}	105.86±0.76^{bC}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

A, B & C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.

G1: Normal rats group feeding on normal basal diet (Negative control).
G2: Anemic rats group feeding on free-iron basal diet (Positive control).
G3: Normal rats group feeding on normal control cupcake.
G4: Anemic rats group feeding on normal control cupcake.
G5: Anemic rats group feeding on cupcake enriched with 7.5% purslane leaves powder replacement.
G6: Anemic rats group feeding on cupcake enriched with 7.5%doum powder replacement.
G7: Anemic rats group feeding on cupcake enriched with15% carob powder replacement.

2.3.2. Liver functions:

Data presented in Table (4) showed liver functions of experimental rats. The levels in the serum showed that (G1) had significant higher values from total protein, albumin and globulin than that (G2), which contained 6.77, 4.83 and 1.94 g/dL in (G1) compared to 4.80, 3.26 and 1.53 g/dL in G2 at 56 days. Also, the serum in rats of (G3), (G4), G5), (G6) and (G7) had significant higher values from total protein, albumin and gobulin than that of (G2) at the end of feeding period. Increasing of feeding period of G4, G5, G6 and G7 from 21 days to 56 days led to significant increase in all parameters of liver functions. Total protein increased significantly from 5.68, 5.80, 5.34 and 5.67 g/dL at 21 days to 5.82, 6.21, 5.74 and 5.93 g/dL at 56 feeding days in G4, G5, G6 and G7, respectively. Albumin increased significantly from 3.81, 3.97, 3.64 and 3.81 g/dL at 21 days to 3.88,4.26,3.94 and 4.00 g/dL at 56 feeding days in G4, G5, G6 and G7, respectively. Globulin increased significantly from 1.87, 1.83, 1.70 and 1.86 g/dL at 21 days to 1.94, 1.95, 1.80 and 1.93 g/dL at 56 feeding days in G4, G5, G6 and G7, respectively. In contrast the levels in the serum showed that the rats in (G1) had significant lower values from bilirubin than that of (G2), which contained 0.75 mg/dL in G1 compared to 1.68 mg/dL in G2 at 56 days. Also, the serum of rats of (G3), (G4), (G5), (G6) and (G7) had significant lower values from bilirubin than that of control positive group (G2 at the end of feeding period . Increasing of feeding period of G4, G5, G6 and G7 from 21 days to 56 days led to significant decrease in bilirubin levels from 1.12, 1.11, 1.14 and 1.13 mg/dL at 21 days to 0.93, 0.89, 0.92 and 0.92 mg/dL at 56 feeding days in G4, G5, G6 and G7, respectively.

Table 4. Liver functions in blood plasma of rats feeding on normal, free-iron basal and cupcake replacement with 7.5% purslane leaves powder, 7.5% doum powder and 15% carob powder (mean±SE).

Parameters	Groups	Zero time (after adaptation for 7 days on basal diet)	After 3weeks	After 5weeks	After 8weeks
Total protein (g/dl)	G1	6.69±0.03^{aAB}	6.66±0.05^{aB}	6.71±0.03^{aAB}	6.77±0.03^{aA}
	G2	6.66±0.08^{abA}	5.57±0.05^{bB}	5.13±0.08^{fC}	4.80±0.05^{fD}
	G3	6.52±0.03^{cAB}	6.45±0.01^{bcB}	6.53±0.02^{bAB}	6.61±0.04^{bA}
	G4	6.54±0.13^{bcA}	5.48±0.13^{bcD}	5.68±0.11^{cdC}	5.82±0.10^{deB}
	G5	6.43±0.15^{cdA}	5.44±0.17^{cD}	5.80±0.17^{cC}	6.21±0.14^{cB}
	G6	6.39±0.09^{dA}	5.19±0.04^{dD}	5.34±0.04^{eC}	5.74±0.04^{eB}
	G7	6.52±0.14^{cA}	5.46±0.06^{bcD}	5.67±0.06^{dC}	5.93±0.01^{dB}
Albumin (g/dl)	G1	4.76±0.02^{aA}	4.81±0.04^{aA}	4.84±0.02^{aA}	4.83±0.01^{aA}
	G2	4.74±0.05^{aA}	3.84±0.04^{cB}	3.52±0.07^{fC}	3.26±0.03^{fD}
	G3	4.54±0.05^{bB}	4.56±0.03b^{AB}	4.61±0.03b^{AB}	4.62±0.03^{bA}
	G4	4.60±0.08^{bA}	3.69±0.07^{dC}	3.81±0.07^{dB}	3.88±0.06^{eB}
	G5	4.52±0.06^{bA}	3.69±0.09^{dD}	3.97±0.10^{cC}	4.26±0.06^{cB}
	G6	4.60±0.08^{bA}	3.57±0.10^{eD}	3.64±0.11^{eC}	3.94±0.05^{dB}
	G7	4.58±0.09^{bA}	3.68±0.02^{dD}	3.81±0.01^{dC}	4.00±0.03^{dB}
Globulin (g/dL)	G1	1.93±0.05^{aAB}	1.85±0.04^{abC}	1.87±0.02^{abBC}	1.94±0.03^{aA}
	G2	1.92±0.03^{aA}	1.73±0.02^{cB}	1.61±0.03^{dC}	1.53±0.03^{cD}
	G3	1.97±0.04^{aA}	1.89±0.03^{aB}	1.92±0.02^{aAB}	1.99±0.04^{aA}
	G4	1.94±0.07^{aA}	1.79±0.07^{bcC}	1.87±0.06^{abB}	1.94±0.05^{aA}
	G5	1.91±0.09^{aA}	1.75±0.08^{cC}	1.83±0.08^{bB}	1.95±0.08^{aA}
	G6	1.79±0.09^{bA}	1.62±0.09^{dC}	1.70±0.09^{cB}	1.80±0.08^{bA}
	G7	1.94±0.06^{aA}	1.78±0.05^{bcC}	1.86±0.05^{abB}	1.93±0.04^{aA}
Bilirubin (mg/dL)	G1	0.84±0.01^{aA}	0.75±0.03^{dB}	0.74±0.01^{eB}	0.75±0.03^{eB}
	G2	0.80±0.01^{bD}	1.15±0.03^{aC}	1.42±0.02^{aB}	1.68±0.03^{aA}
	G3	0.79±0.02^{bC}	0.82±0.01^{cB}	0.84±0.01^{dA}	0.79±0.01^{dC}
	G4	0.81±0.02^{abD}	1.12±0.02^{abA}	1.02±0.03^{bB}	0.93±0.02^{bC}
	G5	0.78±0.02^{bD}	1.11±0.03^{bA}	0.98±0.01^{cB}	0.89±0.02^{cC}
	G6	0.80±0.01^{bD}	1.14±0.01^{abA}	1.02±0.01^{bB}	0.92±0.01^{bcC}
	G7	0.80±0.02^{bD}	1.13±0.02^{abA}	1.02±0.02^{bB}	0.92±0.02^{bcC}

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G1: Normal rats group feeding on normal basal diet (Negative control).
G2: Anemic rats group feeding on free-iron basal diet (Positive control).
G3: Normal rats group feeding on normal control cupcake.
G4: Anemic rats group feeding on normal control cupcake.
G5: Anemic rats group feeding on cupcake enriched with 7.5% purslane leaves powder replacement.
G6: Anemic rats group feeding on cupcake enriched with 7.5%doum powder replacement.
G7: Anemic rats group feeding on cupcake enriched with15% carob powder replacement.

3.2.3.2. Kidney functions:

Data in Table (5) showed that kidney functions of experimental rats. The levels in the serum showed that the rats in (G1) had significant lower values from creatinine, urea and uric acid than that of (G2), which contained 0.64, 16.24 and 3.07 mg/dL in G1 compared to 0.78, 32.34 and 5.13 mg/dL in G2 at 56 days . Also, the serum in rats of (G3), (G4), G5), (G6) and (G7) had significant lower values from creatinine, urea and uric acid than that of (G2) at the end of feeding period .

Table 5. Kidney functions in blood plasma of rats feeding on on normal, free-iron basal and cupcake replacement with 7.5% purslane leaves powder, 7.5% doum powder and 15% carob powder (mean±SE).

Parameters	Groups	Zero time (after adaptation for 7 days on basal diet)	After 3weeks	After 5weeks	After 8weeks
Creatinine	G1	0.67±0.00^{cA}	0.67±0.00^{dA}	0.66±0.00^{eA}	0.64±0.00^{dB}
	G2	0.71±0.01^{aD}	0.99±0.01^{aA}	0.90±0.00^{aB}	0.78±0.01^{aC}
	G3	0.69±0.01^{bAB}	0.70±0.01^{cA}	0.68±0.01^{dB}	0.66±0.01^{cC}
	G4	0.67±0.01^{cD}	0.91±0.01^{bA}	0.78±0.01^{cB}	0.69±0.01^{bC}
	G5	0.68±0.01^{bcC}	0.92±0.01^{bA}	0.79±0.01^{bcB}	0.66±0.00^{cD}
	G6	0.67±0.01^{cC}	0.91±0.01^{bA}	0.80±0.00^{bB}	0.68±0.00^{bC}
	G7	0.67±0.01^{cD}	0.92±0.01^{bA}	0.80±0.01^{bB}	0.69±0.01^{bC}
Urea	G1	14.59±0.27^{bB}	16.29±0.60^{dA}	16.40±0.58^{eA}	16.24±0.96^{eA}
	G2	15.30±0.50^{abD}	22.48±0.65^{cC}	28.32±0.94^{aB}	32.34±0.44^{aA}
	G3	14.72±0.26^{abC}	15.30±0.46^{eC}	16.34±0.43^{eB}	17.23±0.68^{dA}
	G4	15.34±0.68^{abD}	23.72±0.36^{abA}	22.27±0.22^{bB}	19.63±0.63^{bC}
	G5	14.70±0.30^{abD}	23.06±0.44^{bcA}	20.50±0.36^{dB}	18.21±0.37^{cC}
	G6	15.30±0.51^{abD}	23.90±0.76^{aA}	21.58±0.67^{bcB}	18.65±0.26^{cC}
	G7	15.46±0.43^{aD}	23.65±0.54^{abA}	21.26±0.67^{cdB}	18.76±0.25^{cC}
Uric acid	G1	2.92±0.12^{aB}	3.13±0.07^{bA}	3.03±0.06^{cAB}	3.07±0.06^{cA}
	G2	3.02±0.13^{aD}	4.09±0.15^{aB}	4.88±0.10^{aB}	5.13±0.12^{aA}
	G3	3.05±0.05^{aA}	3.15±0.06^{bA}	3.12±0.05^{cA}	3.17±0.04^{cA}
	G4	2.95±0.18^{aD}	4.00±0.08^{A}	3.84±0.09^{bB}	3.65±0.07^{bC}
	G5	2.97±0.10^{aD}	4.20±0.14^{aA}	3.81±0.15^{bB}	3.53±0.14^{bC}
	G6	3.07±0.17^{aD}	4.25±0.16^{aA}	3.94±0.11^{bB}	3.70±0.10^{bC}
	G7	3.07±0.18^{aD}	4.22±0.20^{aA}	3.95±0.22^{bB}	3.69±0.21^{bC}

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G2: Anemic rats group feeding on free-iron basal diet (Positive control).
G3: Normal rats group feeding on normal control cupcake.
G4: Anemic rats group feeding on normal control cupcake.
G5: Anemic rats group feeding on cupcake enriched with 7.5% purslane leaves powder replacement.
G6: Anemic rats group feeding on cupcake enriched with 7.5%doum powder replacement.
G7: Anemic rats group feeding on cupcake enriched with15% carob powder replacement.

Increasing of feeding period of G4, G5, G6 and G7 from 21 to 56 days led to significant decrease in all parameters of kidney functions creatinine decreased significantly from 0.91,0.92,0.91 and 0.92 mg/dL at 21 days to 0.69, 0.66, 0.68 and 0.69 mg/dL at 56 feeding days in G4, G5, G6 and G7, respectively. Urea decreased significantly from 23.72, 23.05, 23.90 and 23.65 mg/dL at 21 days to 19.63, 18.21, 18.75 and 18.76 mg/dL at 56 days in G4, G5, G6 and G7, respectively. Uric acid decreased significantly from 4.00, 4.20, 4.25 and 4.22 mg/dL at 21 days to 3.65, 3.53, 3.70 and 3.69 mg/dL at 56 days in G4, G5, G6 and G7, respectively. The obtained data are in line with those of **Helal *et al.* (2018)** who reported that daily consumption of different concentrations (2.5 and 5%) of purslane leaves, seeds and mixture on diabetic rats for 28 days had significant decrease on Serum urea, uric acid and serum creatinine . These results are in agreement with those obtained by **Bayad (2016)** who reported that daily administration of the higher levels of the decoction of doum fruits by gavage once daily for 2 months (2 g/kg) for 2 months induced a significant decreas on serum urea and creatinine in rats.

2.3.3. Lipid profile of rats :

Serum lipid profile (triglycerides, total cholesterol, HDL- cholesterol, LDL-cholesterol and VLDL-cholesterol) in experimental rats are presented in Table (6). The levels in the serum showed that rats in (G1) had significant lower values of triglycerides, total cholesterol, LDL-cholesterol and VLDL-cholesterol than that of (G2),which contained 58.45, 75.83, 19.09 and 11.69 mg/dL in G1 compared to 112.11, 118.05, 67.38 and 22.42 mg/dL in G2 at 56 days . Also, the serum in rats of (G3), (G4), 5), (G6) and (G7) had significant lower values than that of control positive group (G2) at the end of feeding period .

Table 6. Lipid profile in blood plasma of rats feeding on normal, free-iron basal and cupcake replacement with 7.5% purslane leaves powder, 7.5% doum powder and 15% carob powder (mean±SE).

Parameters	Groups	Zero time (after adaptation for 7 days on basal diet)	After 3weeks	After 5weeks	After 8weeks
Triglycerides	G1	77.73±2.51^{abA}	68.74±3.36^{cB}	60.78±2.52^{cC}	58.45±2.35^{dD}
	G2	79.02±2.26^{aD}	91.05±3.55^{aC}	102.19±3.02^{aB}	112.11±4.17^{aA}
	G3	78.97±2.57^{abA}	69.35±2.02^{cB}	62.65±1.75^{cC}	58.90±1.06^{eD}
	G4	77.28±2.46^{bC}	88.00±1.18^{bA}	82.72±1.11^{bB}	70.04±1.49^{cD}
	G5	77.90±1.93^{abC}	92.19±2.91^{aA}	84.46±2.14^{bB}	68.69±1.64^{D}
	G6	78.07±2.14^{abC}	90.76±1.52^{aA}	82.59±1.28^{bB}	74.19±1.55^{bD}
	G7	78.42±2.53^{abC}	91.69±4.37^{aA}	84.07±2.61^{bB}	75.37±2.19^{bD}
Total cholesterol (mg/dL)	G1	81.33±1.15^{abA}	79.71±1.12^{cB}	76.80±0.82^{eC}	75.83±0.54^{dC}
	G2	81.92±1.04^{aD}	96.84±1.58^{aC}	110.68±1.04^{aB}	118.05±2.05^{aA}
	G3	81.97±1.47^{aA}	78.69±1.45^{dB}	75.70±1.21^{fC}	74.92±1.27^{eD}
	G4	81.72±1.12^{a}	97.04±1.89^{aA}	94.16±2.76^{bB}	86.58±1.66^{bC}
	G5	80.07±0.79^{bD}	96.50±2.01^{aA}	93.18±0.77^{bB}	85.37±0.61^{bC}
	G6	80.55±1.29^{abC}	93.95±1.02^{bA}	87.67±1.48^{dB}	81.40±2.02^{cB}
	G7	80.70±1.35^{abD}	96.25±0.92^{aA}	89.68±1.58^{cB}	82.68±2.46^{cC}
HDL-chol. (mg/dL)	G1	48.75±1.09^{cA}	45.69±1.67^{bB}	38.87±1.43^{aC}	45.05±1.29^{bD}
	G2	49.45±1.21^{bcA}	40.36±0.41^{dA}	30.22±0.26^{dB}	28.25±0.80^{fC}
	G3	49.42±1.65^{bcA}	46.10±1.34^{aB}	39.25±1.09^{aC}	46.50±1.19^{aD}
	G4	49.48±0.57^{acA}	40.94±1.13^{dA}	34.01±1.23^{bcB}	40.32±1.38^{dA}
	G5	50.42±1.04^{abA}	42.48±1.41^{cB}	34.90±1.38^{bC}	42.62±1.32^{cB}
	G6	50.60±1.06^{aA}	41.95±1.21^{cB}	34.35±1.04^{bD}	40.95±1.26^{dC}
	G7	49.02±1.02^{cA}	40.20±0.36^{dB}	33.03±0.24^{cD}	38.73±0.32^{eC}
LDL-chlo. (mg/dL)	G1	17.04±1.89^{aC}	20.27±2.04^{cB}	25.77±1.37^{eA}	19.09±1.18^{eB}
	G2	16.66±0.93^{aD}	38.27±0.97^{aC}	60.02±0.20^{aB}	67.38±1.28^{aA}
	G3	16.76±3.12^{aB}	18.72±2.58^{dB}	23.92±1.65^{fA}	16.64±1.64^{fC}
	G4	16.78±2.02^{aD}	38.50±3.16^{aB}	43.61±4.16^{bA}	32.25±3.23^{bC}
	G5	14.07±1.14^{bD}	35.58±2.71^{bB}	41.39±1.49^{bcA}	29.01±0.90^{cC}
	G6	14.34±2.33^{bD}	33.84±2.43^{bB}	36.80±2.05^{dA}	25.63±2.49^{dC}
	G7	16.00±0.79^{abD}	37.71±1.09^{aB}	39.83±1.79^{cA}	28.89±2.14^{cC}
VLDL-chol. (mg/dL)	G1	15.55±0.50^{aA}	13.75±0.67^{cB}	12.16±0.50^{cC}	11.69±0.47^{dD}
	G2	15.80±0.45^{aD}	18.21±0.71^{aC}	20.44±0.6^{aB}	22.42±0.83^{aA}
	G3	15.79±0.51^{aA}	13.87±0.41^{cB}	12.53±0.35^{dC}	11.78±0.21^{eD}
	G4	15.46±0.49^{aC}	17.60±0.24^{bA}	16.55±0.22^{bB}	14.01±0.30^{cD}
	G5	15.58±0.39^{aC}	18.44±0.58^{aA}	16.89±0.43^{bB}	13.74±0.33^{cD}
	G6	15.61±0.43^{aC}	18.15±0.30^{aA}	16.52±0.26^{bB}	14.84±0.31^{cD}
	G7	15.68±0.51^{aC}	18.34±0.87^{aA}	16.81±0.52^{bB}	15.05±0.44^{bD}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.
A, B & C: There is no significant difference (P>0.05) between any two means, within the same row have the same superscript letter.
G1: Normal rats group feeding on normal basal diet (Negative control).
G2: Anemic rats group feeding on free-iron basal diet (Positive control).
G3: Normal rats group feeding on normal control cupcake.
G4: Anemic rats group feeding on normal control cupcake.
G5: Anemic rats group feeding on cupcake enriched with 7.5% purslane leaves powder replacement.
G6: Anemic rats group feeding on cupcake enriched with 7.5%doum powder replacement.
G7: Anemic rats group feeding on cupcake enriched with15% carob powder replacement.

Increasing of feeding period of G4, G5, G6 and G7 from 21 to 56 days led to significant decrease in triglycerides, total cholesterol, LDL-cholesterol and VLDL-cholesterol. Triglycerides decreased significantly from 88.00, 92.19, 90.76 and 91.69 mg/dL at 21 days to 70.04, 68.69, 74.19 and 75.37 mg/dL at 56 days in G4, G5, G6 and G7, respectively. Total cholesterol decreased significantly from 97.04, 96.50, 93.95 and 96.25 mg/dL at 21 days of feeding to 86.58, 85.37, 81.40 and 82.68 mg/dL at 56 days of feeding in G4, G5, G6 and G7, respectively. LDL-cholesterol decreased significantly from 38.50, 35.58, 33.84 and 37.71 mg/dL at 21 days to 32.25, 29.01, 25.63, and 28.89 mg/dL at 56 days in G4, G5, G6 and G7, respectively. Finally VLDL-cholesterol decreased from 17.60, 18.44, 18.15 and 18.34 mg/dL at 21 days to 14.01, 13.74, 14.84, and 15.05 mg/dL at 56 days in G4, G5, G6 and G7, respectively. The\ obtained data were in line with those of **Yahia *et al. (2017)*** who reported that chickpea protein hydrolysate (CPH) significantly decreased the serum and liver TC and increased HDL, VLDL and LDL-cholesterol ratio in hypercholesterolemic rats. The same trend was

reported by **Nagib (2021)** who reported that feeding fatty liver rats diets with 10% (chickpea, lupin and their combination) caused significant decrease in serum cholesterol, triglycerides, LDL-c and VLDL-c, while HDL-c recorded significant increase, as compared to the positive control groups after 8 weeks of feeding. These results was in harmony with those of **Mousa *et al.* (2023)** who studied the potential role of fresh purslane as a hypolipidemic agent and stated that there was a significant decrease in lipid profile (total cholesterol, triglyceride, and low-density lipoprotein,. On the other hand, administration of fresh purslane (25%, 50%, and 75%) showed an increase in HDL compared to (HFD) group. These results was in harmony with those of **Shehata (2021)** who reported that diabetic rats fed on biscuits fortified with 30% doum flour recorded the best result for all measurements of lipid profile and values of parameters reached to nearly normal values. In the best treatment the levels of TG, TC, LDL-C and VLDL-C decreased by 52.8, 47.6, 68.6 and 52.8%, respectively, and values of HDL-C increased by 60.1% compared to diabetic\ rats. The same trend was reported by **El Rabey *et al.* (2017*)*** who reported that oral administration of 20% 20% carob legume methanol extract to hypercholesterolemic rats for 8 weeks significantly improved the serum lipid profile parameters by decreasing the total cholesterol, TG, LDLc, and VLDLc and increasing HDLc.

3. Organs (%) of rats:

Organs percentage of in experimental rats are presented in Table (7). There are significant differences in all treatments and all orangs. Liver percentage ranged from 2.20 to 3.79%, which was significantly lower in (G6), while it was higher in (G2). Kidney percentage ranged from 0.65 to 1.19%, which was significantly lower in (G1), while it was significantly higher in (G2). The same trend was found in heart and spleen percentages. Liver, kidney, heart and spleen percentages in (G4), (G5), (G6) (G7) had significantly lower percentage than that of (G2).

Statistical analysis did not appear any significant differences in percentage of liver, kidney, heart and spleen between anemic rats feeding on normal control cupcake (G4), (G5), (G6) and (G7).

Table 7. Organs (%) of rats feeding on normal, free-iron basal and cupcake replacement with 7.5% purslane leaves powder, 7.5% doum powder and 15% carob powder (mean±SE).

Groups	Liver	Kidney	Heart	Spleen
G1	**2.71±0.13^{b}**	**0.65±0.01^{c}**	**0.36±0.01^{d}**	0.21±0.00^{c}
G2	**3.79±0.12^{a}**	**1.19±0.02^{a}**	**0.56±0.02^{a}**	0.39±0.01^{a}
G3	**2.46±0.07^{c}**	**0.81±0.02^{b}**	**0.47±0.02^{b}**	0.40±0.01^{a}
G4	**2.38±0.08^{cd}**	**0.81±0.01^{b}**	**0.39±0.01^{cd}**	0.28±0.01^{b}
G5	**2.33±0.07^{cd}**	**0.82±0.01^{b}**	**0.40±0.01^{cd}**	0.28±0.01^{b}
G6	**2.20±0.02^{d}**	**0.80±0.01^{b}**	**0.39±0.00^{cd}**	0.28±0.00^{b}
G7	2.25±0.04^{cd}	0.84±0.01^{b}	0.41±0.01^{c}	0.29±0.01^{b}

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the
G1: Normal rats group feeding on normal basal diet (Negative control).
G2: Anemic rats group feeding on free-iron basal diet (Positive control).
G3: Normal rats group feeding on normal control cupcake.
G4: Anemic rats group feeding on normal control cupcake.
G5: Anemic rats group feeding on cupcake enriched with 7.5% purslane leaves powder replacement.
G6: Anemic rats group feeding on cupcake enriched with 7.5%doum powder replacement.
G7: Anemic rats group feeding on cupcake enriched with15% carob powder replacement.

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التقييم البيولوجي للكب كيك المصنوع من دقيق القمح المدعم بدقيق كل من الحمص, الرجلة ,والدوم والخروب كغذاء وظيفي لمرضى فقر الدم

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لا يزال نقص الحديد وخاصة فقر الدم الناجم عن نقص الحديد أحد أهم حالات نقص التغذية في العالم اليوم. تم في هذه الدراسة استخدام دقيق كل من الحمص, الرجلة , الدوم والخروب كمصدر لإنتاج غذاء وظيفي لمرضى فقر الدم (فى صورة كب كيك). وكانت أعلى درجات التقييم الحسي للكب كيك المعد من 80٪ دقيق قمح و 20٪ دقيق حمص مع الإستبدال الجزئى لدقيق القمح (80%) بكل من مسحوق أوراق الرجلة , الدوم والخروب تبين من التقييم الحسى أن أفضل نسب الإستبدال كانت 7.5، 7.5 و15٪ على التوالى.في التجربة المزدوجة التدعيم التى تم اختبار فعاليتها في التحكم بفقر الدم الناجم عن نقص الحديد وتم استخدام سبع مجموعات من إناث فئران ويستار (بكل مجموعة 6 فئران). تم قياس تركيز الهيموجلوبين (Hb) وحديد المصل (SI) وأوزان الجسم ومعلمات الدم وأوزان جميع مجموعات الجرذان على التوالي. أظهر الفحص البيولوجي أن الوجبات الغذائية االمدعمة بدقيق الحمص وحده (G4) أو الحمص المدعم بمسحوق كل من الرجلة, والدوم والخروب أحدثت أكبر تأثير في زيادة وزن الجسم في الفئران المصابة بفقر الدم. مقارنة بمجموعة الكنترول الموجبة. وأظهرت التحليلات الدموية والكيميائية الحيوية أن التغيرات في صورة الدم ومستويات الحديد في الدم وبروتينات المصل كانت لصالح مكملات دقيق الحمص مع مسحوق الرجلة ,الدوم والخروب مقارنة بمجموعة الكنترول الموجبة. علاوة على ذلك، أشارت النتائج إلى أن تناول الوجبات الغذائية المدعمة كان مرتبطًا بتحسن مستويات ألانين أمينوترانسفيراز (ALT)، وأسبارتات أمينوترانسفيراز (AST)، واليوريا، والكرياتينين وحمض اليوريك مقارنة بمجموعة الكنترول الموجبة وحدث انخفاض ملحوظ في مستويات الألبومين. كما تم تسجيل نسبة البروتين الكلي، حيث أشارت نسبة الدهون إلى انخفاض معنوي في الدهون الثلاثيةوالكوليسترول الكلي وكوليسترول البروتين الدهني منخفض الكثافة وكوليسترول البروتين االدهنى منخفض الكثافةجدا، حيث زاد الكوليسترول الحميد بشكل ملحوظ في المجموعة الرابعة ، الخامسة، السادسة والسابعة مقارنة بتلك الموجودة في مجموعة الكنترول الموجبة. ويمكن الاستنتاج أن كل من دقيق الحمص مع الرجلة ,الدوم والخروب له قيمة غذائية جيدة واستجابة إيجابية لصورة الدم والمعلمات البيوكيميائية في الدم للفئران المصابة بفقر الدم. لذلك، توصي هذه الدراسة بأن تناول منتجات الحمص، الرجلة، الدوم والخروب قد يكون مفيدًا للمرضى الذين يعانون من فقر الدم الناجم عن نقص الحديد.

الكلمات المفتاحية: دقيق الحمص – الرجلة – الدوم – الخروب – فقر الدم بعوز الحديد – التحليل البيوكيميائي – فحص الدم.