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Bran Bread: Chemical Composition, Fungal Load, Biological Impacts and Intervention

Sherif EA Badr*, Hanan MA, El Ghandour, Eman M Ragheb and AS Abdel Rahman

Regional Center for Food and Feed (RCFF), Agricultural Researches Center, Giza, Egypt.

ABSTRACT

Bran breads were purchased and collected from different markets and bakeries of different suburbs fourteen Egyptian governorates. Chemical compositions as moisture content, crude protein, fiber, fat, ash, minerals "Fe, Zn and Cu" were estimated to the collected bran breads and carbohydrate content was calculated by difference, furthermore; phytates as antinutritional content and total aflatoxin and ochratoxin were estimated. According to the iron and zinc contents the collected bran breads were reduce to two subjected samples of high level (84.0 ppm Fe + 33.5 ppm Zn) and low level (48.5 ppm Fe + 26.0 ppm Zn) respectively. Fortified bran breads with EDTA and Ascorbate salts of the same two Fe and Zn levels were baked and 24- participants shared in pannel test of the sensory evaluation and identification trials of the fortified 4- wheat flour samples showed that; the isolated fungi belong to 5 genera and 14.0 species about 303 isolates from all samples, it should be free raw material used from fungi and specially mycotoxins fungi such as *A. flavus* to reduce the risks for consumers' health to used bran flour. Experiments were initially designed to evaluate the biological impacts for collected and baked bran breads in their two different iron and zinc concentrations *in vivo* as designed and to evaluate the copper levels in terms of cholesterol levels. The duration of these experiments was 90- days divided into 2- intervals "Depletion & Repletion"; 45- days for each. All bran bread, whether collected from the Egyptian markets or baked not acted upon the body weight of the subjected rats as compared with controller during the depletion period ($p > 0.05$). The collected bran breads contributed to Fe, Zn and Cu deficiencies, decline in hemoglobin, RBC's, WBC's, HDL- C and caused histopathological changes on hearts and livers of rats. The baked bran breads whether fortified with two EDTA salts or two ascorbate salts were intervention for amelioration for CBC of the unsound rats.

Keywords: Bran breads, Phytate, CBC, Cholesterol, *In vivo*, Baking, Pannel test.

Corresponding author

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INTRODUCTION

According to (GSO, 2010) bran bread is a food product prepared by mixing of wheat flour and apostasy "soft bran layer" yeast may added also one or more from the inhibitor materials for fungus growth and the other basic materials which is mixed and fermented well and baked in suitable conditions. The requirements of bread with a special use (bran bread) used for the diet and similar to it, but do not include bread for low- calorie as a guide for diabetics. Kadan and Phillippy, 2007 reported that; increasing the amount of bran decreased the phytate degradation from 42% at the lowest level of bran to10% at the highest, and the amount of yeast had no significant effect. The bran contributed substantial amounts of magnesium, iron, and zinc. Breads with the lowest level of bran had phytate-to-zinc molar ratios between 5 and 10, which suggest medium zinc bioavailability. The chemical name of phytic acid is inositol hexaphosphoric acid, cyclohexanehexyl hexaphosphate, its molecular formula is $C_6H_{18}O_{24}P_6$ and has a molecular weight 660.08, its appearance is colorless to slightly yellow viscous liquid, it contains 12 acid groups which are able to complex with metals to form white insoluble mineral salts e.g. 1g phytic acid is able to complex with 500mg iron ions. When zinc forms a complex between a low-molecular-weight ligand or chelator and that complex can be absorbed, it is likely that the net effect on zinc absorption will be positive, because the solubility of zinc is increased. Ligands / chelators [e.g., EDTA], amino acids [e.g., histidine, methionine] and organic acids [e.g., citrate] have therefore been used in efforts to enhance zinc bioavailability (Bolonnerdal, 2000). Phytic acid is a strong inhibitor of iron absorption from fortified foods; in adults, this inhibitory effect can be overcome by adding ascorbic acid with the iron fortificant or by using a "protected" iron compound such as NaFeEDTA. In addition, the use of NaFeEDTA as an iron fortificant has been reported to increase zinc absorption in adult women (Lena Davidsson *et al.,* 2005). Compared with the untreated bread, citric acid alone and the combination of citric acid and phytase enhanced total iron dialyzability by 12- and 15-fold, respectively, while the combination of phytase, citric acid, and ascorbic acid improved total iron dialyzability in the mixture by 24-fold (Porres *et al.,* 2001).

From the rheological studies; Wheat flours are characterized by the flour extraction, which is the proportion of flour by weight, derived by milling from a known quantity of wheat (Slavin *et al*., 2000). White flour is refined form having extraction rate of 60-75 %. Brown flour has a higher extraction rate (85%), containing more bran and this may impart a darker color, strong flavor and aroma to the products. However, the minerals content is reduced to 30% than the whole wheat flour and also the concentration of essential nutrients is decreased with lowering the extraction rate (Pederson and Eggum, 1983), the popularity of these traditional breads is growing due to ethnic population. The flour extraction rate affects the protein content, farinographic water absorption and gluten strength (Orth and Mander, 1975). Wheat grains that is susceptible to these fungi infections through its growth, harvest, transport and storage, is the most staple food in our country.AFB1 is the mycotoxin with the greatest impact in Africa and together with hepatitis B1 viral infections possibly responsible for the high incidence of primary liver cancer in certain parts of Africa, such as Mozambique (Pitt, 2000). *Aspergillus* strains producing aflatoxin are *A. nominus, A. tamarii* (Goto *et al.,* 1997) and *A. pseudotamarii* (Ito *et al.,* 2001). Aflatoxin B1 (AFB1), the most potent aflatoxin, is extremely

toxic, mutagenic, carcinogenic and teratogenic to both humans and livestock (Mishra and Das, 2003). Aflatoxin B_1 can be reduce or prevented to be produced by *A.flavus* by applying lactic acid fermentation in fermented maize meal products.

The main objectives of the current study were to assess the chemical composition of Egyptian marketable bran breads, to evaluate their biological impacts *in vivo*, to study the convenient intervention moreover to isolate fungal growth from bran before used in biological analysis *in vitro* and to determine the effect of extraction rates on the quality of sourdough to product bran bread.

MATERIALS AND METHODS

Sampling

Bran breads were purchased and collected from different markets and bakeries of different suburbs in fourteen governorates; Cairo, Giza, Helwan and Kalyobia "Great Cairo", Alexandaria, Menofia, Gharbia, Dakkahlia, Al- Menia, Beni-Swif, Fayoum, Suis, Quena and Sohag.

Chemical analysis

Ingredients analysis for the collected bran breads

Moisture content; crude protein; fiber and fat according to AOAC, 2000; ash obtained by AOAC, 1995 were estimated to collected bran breads. According to Sara *et al.,* 2008 carbohydrate content was estimated by difference. Phytates were estimated to collected breads according to Wheeler and Ferrel, 1971. Estimation of minerals {Fe, Zn and Cu} in collected bread by inductive coupled plasma ICP "optima 2000" according to AOAC, 2002 and Iva *et al.,* 2003.

Determination of Afla- and Okratoxins of the collected bran breads

Total Aflatoxin and ochratoxin A standards were purchased from Sigma (St. Louis, MO, USA. Stock solutions of each mycotoxins were prepared by dissolving solid commercial toxin in the appropriate solvent at concentration of 1 mg/mL. AFs in toluene/acetonitrile 99:1 and OTA in toluene/acetic acid 99:1.

Extraction procedure of aflatoxins:

Extraction of aflatoxins from collected breads and the cleanup procedures were performed according to the AOAC method [1988]. Ground breads sample (50 g) were mixed with 2.5 g NaCl, 200mLof 80% (v/v) solution of methanol in water, 100mLof *n*-hexane and blended in a blender for 5 min. 100mL of the slurry solution was then centrifuged to remove the fat content extracted into the upper phase. After filtration, 25mL of the lower phase was shaken for 1 min in 25mL of chloroform in a separatory funnel. Finally, the chloroform phase

was separated and evaporated to dryness at 50°C. The extracted aflatoxins in samples were then redissolved in 200 µL of methanol. Finally, 5µL of this solution was introduced into the injection port of the HPLC.

Identification and detection:

In order to verify the presences or absences of aflatoxins and ochratoxins in the breads samples, immune affinity column and HPLC technique (Agillent 1200) series U.S.A were used according to (AOAC, 2006). For aflatoxins determination column C_{18}, Lichrospher 100 RP-18, 5µm × 25cm were used. The mobile phase consisted with water: methanol: acetonitrite (54:29:17, v/v/v) at flow rate of 1ml/min. The excitation and emission wave lengths for all aflatoxins were 362 and 460 nm (Flourences detector), respectively according to AOAC, (2006). Ochratoxin were determined using column Nova- Pak C_{18} 4µm, 3.9 × 150mm. The mobile phase consisted with acetonitril: acetic acid:water (495 : 10 : 495 v/v/v) at flow rate of 0.8ml/min. The excitation and emission wave lengths for Ochratoxins were 333 and 477 nm (Flourences detector), respectively according to AOAC, (2006).

Stenography of the collected bran breads:

Ingredient analysis of the 14- samples which shown in table (1) and illustrates that; protein, fat, ash, fiber, moisture contents for these samples as well as, the anti-nutritional content of phytic acid were approximately equal to each other but their iron and zinc contents were inconsistent. Consequently; the fourteen collected bran breads were divided and reduced into two combined samples according to iron and zinc levels of the collected 14- samples. First combined sample was included collected bran breads samples of No. (1, 3, 6, 7, 9, 11, 12, 13 and 14) of the table (1) and represent a positive control of low iron and zinc concentrations. Second combined sample was included collected bran breads samples of No. (2, 4, 5, 8 and 10) of the table (1) and represent a positive control of high iron and zinc concentrations. The two combined samples were analyzed for ingredient estimations as done above.

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Table (1):- Ingredient and phytate analysis and toxins estimations for the collected bran breads:

Sample No.	Gover.	Total Moist. %	Protein %	Fiber %	Fat %	Ash %	Moist. %	Minerals "ppm"			Phytate %	Toxins	
								Fe	Zn	Cu		Afla-	Okra-
1	Cairo	7.7	11.35	1.40	8.63	2.90	4.0	42.7	29.5	2.02	1.18	Free	Free
2	Giza	8.0	11.80	2.89	1.71	2.91	4.5	95.0	23.3	1.59	1.21	Free	Free
3	Helwan	7.4	11.25	1.36	10.35	2.21	3.5	39.8	26.2	1.66	1.09	Free	Free
4	Kalyobia	3.1	11.85	2.32	6.18	1.79	3.6	56.7	39.6	0.90	1.29	Free	Free
5	Alexandria	5.6	11.50	2.21	4.17	2.49	3.5	64.4	35.7	1.88	1.31	Free	Free
6	Menofia	5.7	11.15	2.14	8.82	2.06	3.8	51.7	27.6	1.34	1.28	Free	Free
7	Gharbia	5.5	12.40	2.04	3.51	2.81	3.9	46.0	25.3	1.09	1.31	Free	Free
8	Dakhlia	4.0	11.50	1.91	6.17	1.83	4.2	71.9	32.9	2.18	1.22	Free	Free
9	El- Menia	10.4	13.30	2.10	6.98	2.31	7.1	48.2	19.3	0.89	1.19	Free	Free
10	BeniSweif	3.8	12.70	2.22	6.07	2.65	5.5	123.0	25.1	1.01	1.33	Free	Free
11	Fayoum	5.1	14.55	2.62	4.24	1.67	4.8	47.5	28.4	1.13	1.37	Free	Free
12	Swis	4.9	13.40	2.17	4.64	1.97	6.9	49.0	20.1	2.09	1.28	Free	Free
13	Quena	5.5	13.60	2.41	6.20	1.91	7.0	51.9	23.8	1.58	1.30	Free	Free
14	Sohag	5.2	13.55	2.14	7.75	1.91	5.7	52.1	20.1	0.98	1.26	Free	Free

Baking of bran bread:

Baking Process of bran bread:

The five flours of the subjected bran breads{Control, fortified with (48.5ppm Fe + 26.0ppm Zn "low concentrations") EDTA, fortified with (84.0ppm Fe + 33.5ppm Zn "high concentrations") EDTA, fortified with (48.5ppm Fe + 26.0ppm Zn"low concentrations") Ascorbate and finally, fortified with (84.0ppm Fe + 33.5ppm Zn "high concentrations") Ascorbate} were baked according to Sosulski and Wu' (1988) in Food Technology Institute, Agriculture Research Center "ARC".

Quality characteristics of baked bran breads:

Bran breads were organoleptically evaluated for taste, odor, color, appearance, texture and palatapility after bakery process. The evaluation was carried out according to the method of (Faridi and Rubenthaler, 1984).

A study of biological impacts of bran breads and intervention

***In vivo* biological experiment**

Animals

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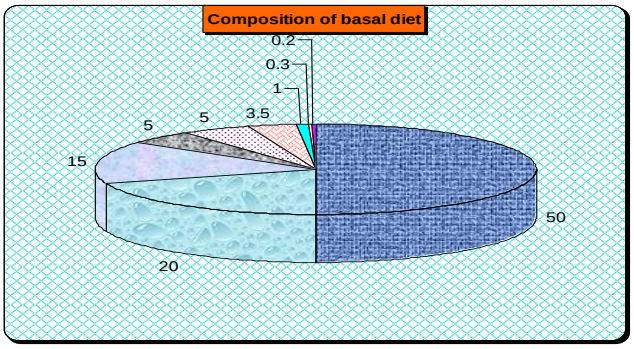
5-weeks-old male sprague- Dawley outbred Albino rats were divided into 7- groups, caged individually in stainless steel cages and maintained at 22-24° C and 45-55% relative humidity. Acid-washed glass food jars and polyethylene bottles with polyethylene stoppers were used. Diets and water were provided fresh daily *ad libitum* unless otherwise specified. All the utensils used in providing the diets were either stainless steel or acid washed glass Stahr, (1977).

Diets

Diet for the control- subjected experimental animal groups were previously prepared according to the composition of basal diet which is detailed in Pang *et al.,* (1992) and NRC, (1995) as illustrated in histogram (1).



Histogram (1) illustrates composition of basal diet in pie form as consider that the total size of the pie is 100; sucrose is 50, casein is 20, corn starch is 15, corn oil is 5, fiber is 5, mineral mixture is 3.5, vitamin mixture is 1, DL- methionine is 0.3 and finally, choline bitartarate is 0.2.

The dried and milled 2- collected or 4- baked bran breads for the 6- subjected experimental animal diet groups were included their formula in 50% of the composition of their basal diets instead of (35% sucrose, 10% starch and 5% cellulose). All nutrients were added according to the recommended dietary allowance [RDA] for each of the rat and according to NRC, (1995) except each of iron and zinc; were self supported from baked breads.

Experimental designs

Experiments were initially designed to evaluate the biological impacts for collected and fortified baked bran breads in their two different iron and zinc concentrations *in vivo* as designed by Saka (2012) and to evaluate the copper levels in terms of cholesterol levels. The duration of these experiments was 90- days divided into 2- intervals "Depletion & Repletion"; 45- days for each. Rats in these experiments were housed individually in stainless steel cages were previously randomly distributed on 7- subjected experimental groups and were randomly assigned to one of the following experimental groups of 14- rats each. All groups were eaten barley for 3- days before starting the experimental period in purpose of to be adapted. Two animals from each group were randomly withdrawn, representing zero time samples for all groups; then, each group contained 12- male rats.

Depletion interval:- In the 1St 45- days, negative control rats group (A) were fed control basal diet *ad libitum*, positive control rats group (B) were fed the (48.5ppm Fe + 26.0ppm Zn "low concentrations") collected bran bread diet *ad libitum*, positive control rats group (C) were fed (84.0ppm Fe + 33.5ppm Zn "high concentrations") collected bran bread diet *ad libitum*, subjected rats group (D) were fed the (48.5 ppm FeEDTA and 26.0 ppm ZnEDTA) baked bran bread, subjected rats group (E) were fed the (84.0 ppm FeEDTA and 33.5 ppm ZnEDTA) baked bran bread, subjected rats group (F) were fed the (48.5 ppm Fe Ascorbate and 26.0 ppm Zn Ascorbate) baked bran bread and finally, subjected rats group (G) were fed the (84.0 ppm Fe Ascorbate and 35.5 ppm Zn Ascorbate) baked bran bread.

Repletion interval:- In the 2nd 45- days, subjected rats group (B) were fed the (48.5 ppm FeEDTA and 26.0 ppm ZnEDTA) baked bran bread *ad libitum*, subjected rats group (C) were fed (84.0 ppm FeEDTA and 33.5 ppm ZnEDTA) baked bran bread, diet *ad libitum*, subjected rats group (D) were fed the (48.5 ppm Fe and 26.0 ppm Zn) collected bran bread diet *ad libitum*, subjected rats group (E) were fed the (84.0 ppm Fe and 33.5 ppm Zn) collected bran bread diet *ad libitum*.

All other nutrients were added according to the recommended dietary allowance [RDA] for each of the rat and according to NRC, (1995). According to Saka (2012), rats of both zero time and each interval "45- days" were anaesthetized with CO_2, blood samples were collected *via* the retro-orbital plexus. Serum was obtained by centrifuging at 3000 rpm for 15 min as reported in Akhigbe *et al* (2008). Then; rats were scarified and each kidney, liver and heart were harvested from rats of each group at zero time, end of first 45- days and end of second 45- days were weighed and rapidly kept in formalin solution 10% and room temperature for histopathology examination. Body weights of rats of subjected groups were accurately weighted at zero time, end of each week along the experimental period.

Analysis & Investigation

All the following estimations were carried out in the Regional Center for Food and Feed. These estimations were taken place at the 3- interval periods "zero time, the end of the first "depletion" interval and at the end of the second "repletion" interval, except diet analysis which were conducted only after complete homogenization of their ingredients.

1) According to AOAC, (2002) estimations of minerals as iron, zinc and copper in both prepared diets and rats blood serum by inductive coupled plasma ICP "optima 2000" by zeman spectrometer "4100".
2) Diets analysis for determination of moisture content; crude protein; fiber and fat according to AOAC, (2000); ash obtained by AOAC, (1995).
3) Blood serum analysis for estimation of urea by Newman and Price, (1999) and creatinine by Friedman and Young, (1997). Also, "GOT" and "GPT" were estimated according to Friedman and Young, (1997); While Alkaline phosphatase level was determined by Moss and Henderson, (1999). In addition to assess of HDL- and LDL- cholesterol according to Guder and Zawta, (2001).

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4) Complete Blood Count "CBC" according to ABC *Vet* (1996); where, the Animal Blood Counter Veterinary is a fully automated (Microprocessor controlled) hematology analyzer used for the *in vitro* diagnostic testing of whole blood specimens. WBC "White blood cell count", RBC "Red blood cell count", HGB "Hemoglobin" and PLT "Platelet count" are 4- Parameter hematology systems were estimated.
5) Histopathological examination for the collected organs "liver, kidneys, and heart" through the 3- interval periods according to the **(Bancroft *et. al.,* 1996)**.

Statistical analysis

Analysis of variance for a completely randomized design was done according to Gomez and Gomez (1984) by using SPSS software program.

RESULTS AND DISCUSSION

Baking of bran bread

Isolation and identification of fungi associated with bran flour-

The five samples of fortified wheat flour which previously prepared to bake the subjected bran breads were used for isolation and identification of the associated fungi. Results of occurrence and percentage of the associated fungi are presented in table (2).

Table (2): Isolates fungi isolated from bran flour by using potato dextrose agar (PDA).

Isolated Fungai	Frequency of occurrence %				
	Control	EDTA High Conc.	EDTA Low Conc.	Ascorbate High Conc.	Ascorbate Low Conc.
Aspergillus niger.	8.0	6.0	10.0	0.0	3.0
A. flavus.	5.0	8.0	7.0	0.0	0.0
A.fumigatus.	12.0	8.0	4.0	2.0	3.0
A. Terreus.	5.0	10.0	8.0	0.0	2.0
A.ssp.	5.0	9.0	6.0	0.0	0.0
Fusarium solani	3.0	0.0	0.0	0.0	2.0
F. oxysporum.	4.0	0.0	0.0	0.0	0.0
F.semitectum	6.0	9.0	4.0	0.0	0.0
Fusarium ssp.	7.0	10.0	7.0	2.0	5.0
Penicillium citrinum	10.0	14.0	10.0	0.0	0.0
Penicillium citreonigrum	3.0	5.0	5.0	0.0	3.0
Penicillium spp.	8.0	5.0	5.0	0.0	0.0
Alternaria spp.	19.0	19.0	11.0	0.0	0.0
Rhizopus sp.	3.0	7.0	5.0	0.0	0.0
Total individual .	**98.0**	**101.0**	**82.0**	**4.0**	**18.0**
Total			**303**		

Identification trials showed that, the isolated fungi belong to 5 genera and 14.0 species about 303 isolates from all samples. Fungi were identified as:*Aspergillus niger A*. *flavus*, *A*. *fumigatus. A. Terreus.Fusarium solani,F. oxysporum, F*. *semitectum F*.pp*., Penicillium citrinum P. citreonigrum., P. spp.*, *A.alternaria* and *Rhizopus* sp. Among the so-called "Field fungi",

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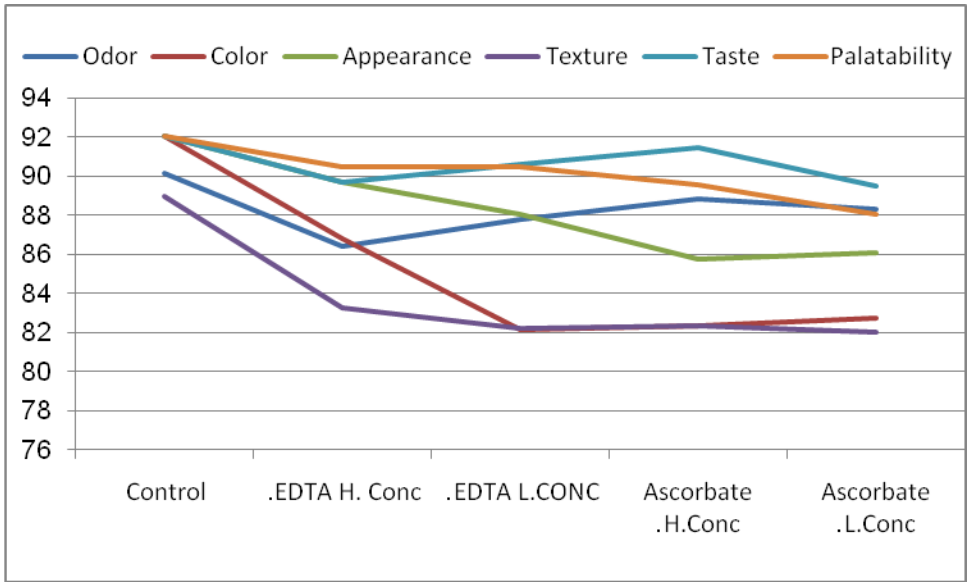
Alternaria spp. were found in higher occurrence in all samples (control and four samples**)** followed by *A.fumigatus, A. flavus* as "storage fungi". The lowest frequency in sample (Fe+Zn Ascorbate high concentration bran bread) these results are harmony with (Legan, 1993). On the other hand, *Aspergillus flavus* was found in higher occurrence in sample (Fe+Zn EDTA high concentration bran bread) "storage fungus". It should be free raw material used from fungi and especially mycotoxins fungi such as *A. flavus* to reduce the risks for consumers' health to used bran flour.

Sensory characteristics of fortified bran breads with EDTA and ascorbates of iron and zinc in different levels:

From the pannel of the bran breads samples, about twenty four participants were shared in this evaluation and gave in the end the evaluation sheets which statically analyzed and reported in histogram (2). The principles of this evaluation based on, each participant gives a degree for each sensory character for the individual four types of baked bran breads as compared with control baked sample, theses degrees are corresponded to a definite grades e.g. {100-85 excellent, <85-75 very good, <75-65 good, and < 65-50 fair and < 50 is very bad}, the participants considerations were control sample was outstanding. The mean of these degrees and putted in the histogram (2).



Histogram (2): sensory characteristics of baked fortified bran breads with EDTA and ascorbates of iron and zinc in different levels.

Histogram (2) indicates that, control sample gained the highest degrees in the appearance, and texture properties. No significance differences ($p > 0.05$) between odor, texture and taste of the control baked bran breads and the 4- fortified baked bran breads. The participants to agree unanimously on the color of all fortified baked bran breads were lower than that of the control sample with high significance differences ($p < 0.01$). The appearances of the baked control sample gained higher score ($p < 0.05$) than the two levels of iron and zinc fortified bran

breads with EDTA salts and ($p < 0.01$) than that of the fortified bran breads with ascorbate salts. Finally; palatability is a sum of all properties and it is a property by which can evaluate the baked fortified bran breads in general, no significance differences ($p > 0.05$) between the palatability of the control bran breads and the subjected fortified bran breads samples as shown in histogram (2).

Biological impacts of bran breads and evaluation the intervention.

Successful fortification of a food depends on the fortificant acting in a relatively benign manner in the food. Potential changes in physicochemical and functional properties as well as changes in nutrient content during the required shelf life of fortified foods need to be evaluated before any firm recommendation in dose and source of minerals is made (Jorge, 2003). Iron and zinc can be added to many foods including milk, cereals, flours, fruit juices, sugar and water. When the objective of food fortification is to increase the intake of specific nutrients that are known to be deficient in a population, the selection of an adequate vehicle is crucial for program success. The following characteristics should be met when choosing a vehicle for such purposes (Rosado, 2001): *1*) Food chosen as a vehicle should be ingested by the target population in sufficient quantities and with a small variability in the amount ingested. *2*) The fortified food should be stable and physicochemical properties such as appearance, texture and flavor should not change when the nutrient is added. *3*) The added nutrient should be relatively bio available and well tolerated. *4*) Fortification of the food should not significantly increase its price. *5*) Fortification should be carried out with available ingredients and technology and preferably at low cost.

Because minerals are added to foods to increase nutritive value, the major factor to be considered in choosing the appropriate compound to be added to a particular food is bioavailability. However, using an exceedingly bioavailable compound does not make sense if the food is rendered unpalatable or unacceptable as a result of physicochemical changes. Minerals are chemically reactive compounds and their bioavailability will be greatly affected by interactions with food components when added or during processing and storage. Therefore, bioavailability of a mineral in food is not strictly a function of the bioavailability of the compound chosen (Jorge, 2003).

Body weight Gain "BWG".

All bran bread, whether collected from the Egyptian markets or baked not acted upon the body weight of the subjected rats as compared with controller during the depletion period ($p > 0.05$) as shown in histogram (3). In repletion period, the two rats groups were ate "Fe Zn EDTA" fortified baked breads and completed with collected bran breads were exposed to weight loss with significance deference ($p<0.05$) than the three rat controllers and with high significance deference ($p< 0.01$), rats were fed baked bran breads in high concentration of Fe Zn ascorbate form gave no significance difference toward negative controller and increasing in body weight with high significance difference than other groups ($p< 0.01$) as illustrate in histogram (3).

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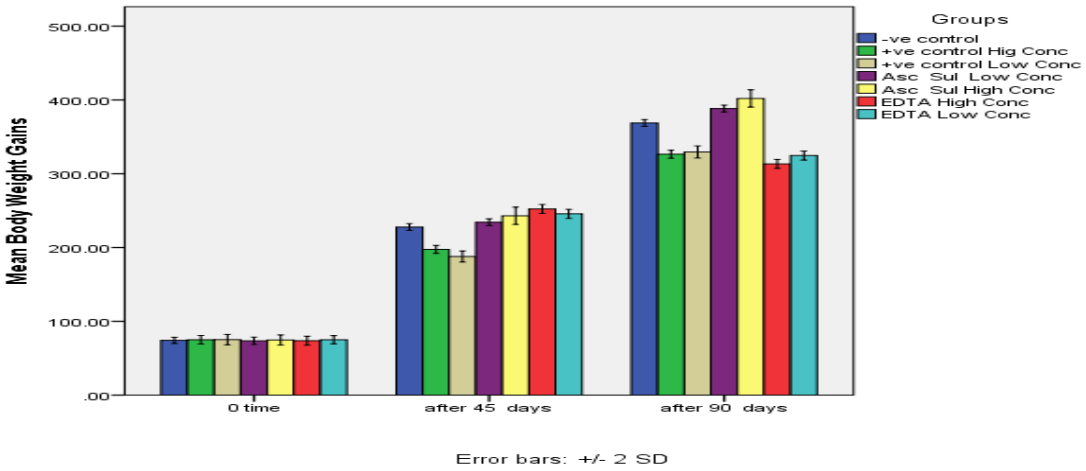

Histogram (3): Effect of collected & fortified bran breads on BWG. Complete Blood Counter "CBC":- WBC's "$10^3/mm^3$" and RBC's "$10^6/mm^3$"

According to LaFleur (2008); white blood cells, or leukocytes are cells of the immune system involved in defending the body against both infectious disease and foreign materials, five different and diverse types of leukocytes exist, but they are all produced and derived from a multi potent cell in the bone marrow known as a hematopoietic stem cell. They live for about three to four days in the average human body, leukocytes are found throughout the body, including the blood and lymphatic system, Maton *et al.,* (2008). The number of leukocytes in the blood is often an indicator of disease, they make up approximately 1% of the total blood volume in a healthy adult, an increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia, the physical properties of leukocytes, such as volume, conductivity, and granularity, may change due to activation, the presence of immature cells, or the presence of malignant leukocytes in leukemia; Alberts (2005).

According to Pierigè *et al.,* (2008); red blood cells, or erythrocytes, are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O_2) to the body tissues via the blood flow through the circulatory system, they take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries, these cells' cytoplasm is rich in haemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the blood's red color. In humans, mature red blood cells are oval and flexible biconcave disks, they lack a cell nucleus and most organelles to accommodate maximum space for haemoglobin, 2.4 million new erythrocytes are produced per second, the cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages, each circulation takes about 20 seconds, approximately a quarter of the cells in the human body are red blood cells Pierigè *et al.,* (2008).

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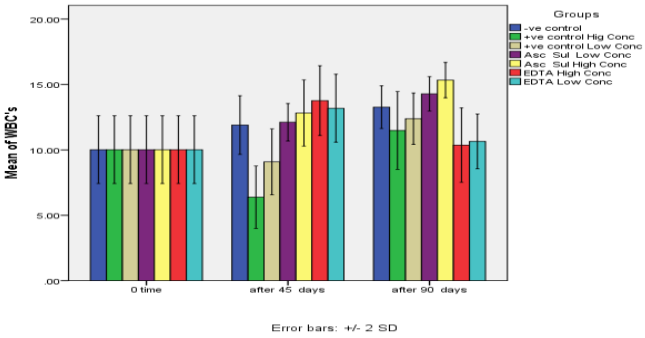
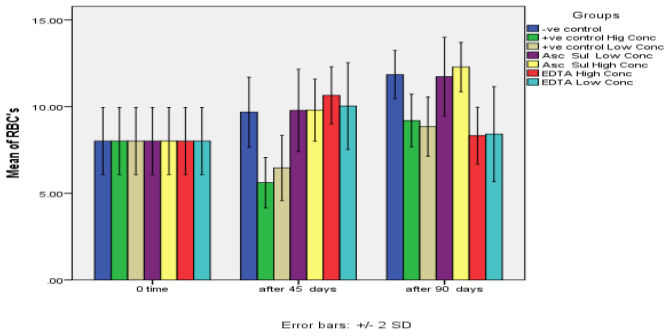


(a)



(b)

Histograms (4):- Effects of collected & baked bran breads on (a) WBC's and (b) RBC's.

In depletion period "after 45- days"; WBC's and RBC's of two rat groups were fed the collected bran breads "positive controllers" decreased with high significance differences ($p< 0.01$) than that fed four fortified bran breads groups, in the same time; WBC's and RBC's of two rat groups were fed the collected bran breads decreased with significance differences ($p< 0.05$) than that negative control. On the other hand; WBC's and RBC's for rats group fed fortified bran breads with EDTA of high concentration iron and zinc recorded highest value than that obtained in the other three fortified groups as shown in histograms (4a), (4b).

In repletion period "after 90- days"; Fe Zn EDTA fortified bran breads were compensated the dropping in the WBC's and RBC's of the blood for two rats groups which fed collected bran breads in the depletion process. On the contrary; the two collected bran breads contributed to lowering in the WBC's and RBC's counts in the blood of rats were fed Fe Zn EDTA fortified diets with high significance difference ($p< 0.01$). In the same time, blood of rats were fed two concentrations of Fe Zn Ascorbate fortified bran breads diets recorded continuously increasing in WBC's and RBC's with high significance differences than that recorded in depleted groups for each period as shown in histograms (4a), (4b).

HGB "g/dl" and PLT's "$10^3/mm^3$":-

Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (with the exception of the fish family Channichthyidae) as well as the tissues of some invertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism, and collects the resultant carbon dioxide to bring it back to the respiratory organs to be dispensed from the organism, Sidell *et al.,* (2006). In mammals, the protein makes up about 97% of the red blood cells' dry content, and around 35% of the total content (including water), Hemoglobin has an oxygen binding capacity of 1.34 mL O_2 per gram of hemoglobin, which increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules, Costanzo and Linda, (2007).

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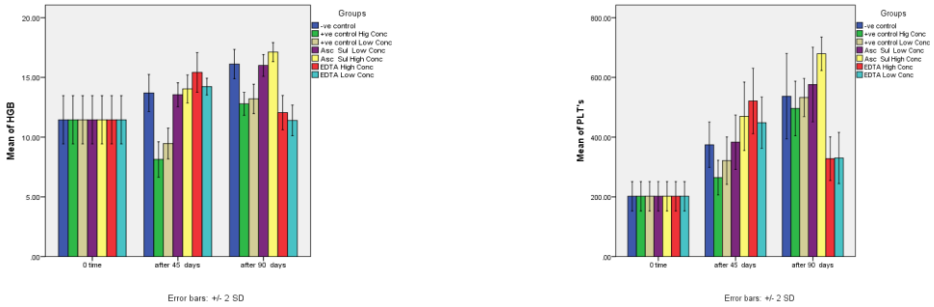
Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein.

According to Campbell and Neil (2008) study; platelets, or thrombocytes are small, irregularly shaped clear cell fragments (i.e. cells that do not have a nucleus), 2–3 µm in diameter which are derived from fragmentation of precursor megakaryocytes, the average lifespan of a platelet is normally just 5 to 9 days, platelets are a natural source of growth factors, they circulate in the blood of mammals and are involved in hemostasis, leading to the formation of blood clots. If the number of platelets is too low, excessive bleeding can occur.



(a) (b)

Histograms (5):- Effects of collected & baked bran breads on (a) HGB and (b) PLT's.

In depletion period "after 45- days"; hemoglobin and platelets levels in rats' blood were fed collected bran breads were recorded distinct diminution with highly significance differences ($p< 0.01$) than another groups and zero times in case of hemoglobin only while, hemoglobin and platelets in rats' blood were fed fortified bran breads with chelated iron and zinc EDTA and Ascorbic complexes were recorded distinct rising with highly significance differences ($p< 0.01$) than another groups and zero times. Hemoglobin and platelets in rats' blood were fed fortified bran breads with chelated iron and zinc EDTA complexes were higher with significance ($p< 0.05$) than that chelated iron and zinc Ascorbic complexes as shown in histograms (5a), (5b).

In repletion period "after 90- days"; chelated iron and zinc EDTA complexes were effective for regaining the dropping in hemoglobin and platelets levels in rats' blood were fed the collected bran breads with high significance levels ($p< 0.01$) whereas; the collected bran breads were destroyed the hemoglobin and platelets levels which raised in the previous interval significance levels ($p< 0.01$) as shown in histograms (4a), (4b). Hemoglobin and platelets in rats' blood were fed fortified bran breads with chelated iron and zinc ascorbate complexes were continued their increasing with significance levels ($p< 0.05$) and ($p< 0.01$) respectively, as illustrated in histograms (5a), (5b).

Histopathological investigation for the heart organs of the subjected rat groups showed that; normal cardiac myocytes of the heart of rat at zero time fig (1a). The collected bran breads to cause congestion of myocardial blood vessels fig (1b) and minute focal leucocytes

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aggregation fig (1c) for rat hearts which fed on the collected bran breads through depleted and repleted periods respectively. No histopathological changes on hearts of rat groups were fed on the baked bran breads whether fortified with two EDTA salts or two ascorbate salts as shown in fig (1d).

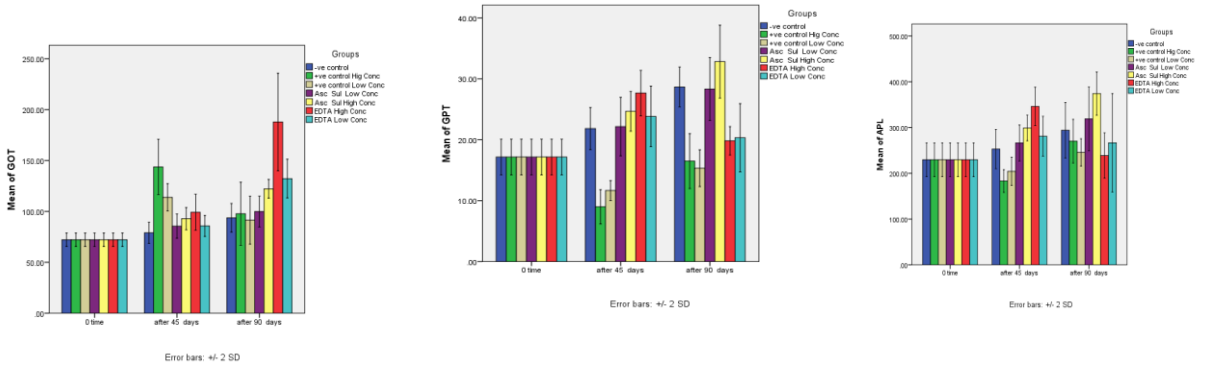
(a) (b) (c) (d)

Fig (1): Histopathological studies for Heart of the subjected rat groups (H and E × 400).

Assessment of some organs functions in rats blood serum:

Liver Functions (GOT "U/l", GPT "U/l" & APL "U/l"):-

Aspartate transaminase (AST) called serum glutamic oxaloacetic transaminase (SGOT) or aspartate aminotransferase (ASAT) is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells, and cardiac and skeletal muscle and is therefore not specific to the liver. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage, elevated AST levels are not specific for liver damage, and AST has also been used as a cardiac marker, Nyblom *et al.,* (2004), Nyblom *et al.,* (2006). Alanine transaminase (ALT), also called serum glutamic pyruvate transaminase (SGPT) or alanine aminotransferase (ALAT) is an enzyme present in hepatocytes (liver cells), Nyblom *et al.,* (2004), Nyblom *et al.,* (2006). Alkaline phosphatase level (APL) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called *dephosphorylation*. As the name suggests, alkaline phosphatases are most effective in an alkaline environment, Tamás *et al.,* (2002).



(a) (b) (c)

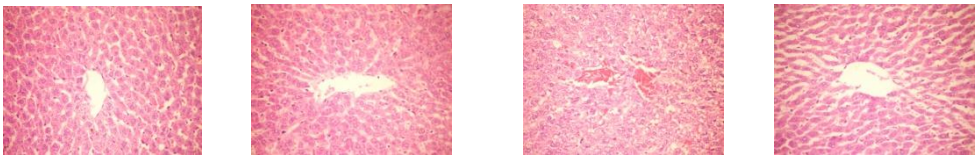
Histograms (6):- Influence of collected and baked bran bread on liver functions (a)- GOT, (b)- GPT & (c)- APL.

In depletion period "after 45- days"; the rats were fed collected bran breads of different levels of phytates particularly positive control of high concentrations recorded highest increment in

GOT's in their blood than controller and other subjected groups as well as zero time with high significance difference ($p< 0.01$) and recorded sharp decrement in GPT's in the same manner of GOT with significance difference ($p< 0.01$) whereas, the decrement in APL's in blood for these rats group were significance difference ($p< 0.05$). No significance difference ($p> 0.05$) in GOT's and GPT's levels between the rat groups were fed baked fortified bran breads whether with EDTA or ascorbic complexes while, APL's in rats group were fed fortified baked bran breads with high EDTA concentrations increased with ($p< 0.01$) and ($p< 0.05$) in case of rats group were fed fortified baked bran breads with high ascorbic concentrations as shown in histograms (6a), (6b) and (6c).

In repletion period "after 90- days"; the collected bran breads destroyed whatsoever; GOT's, GPT's and ALP's levels in rats blood as compared with that controller with highly significance differences ($p< 0.01$) in the same time; the fortified baked bran breads with the two EDTA concentrations compensated the decline in GOT's and ALP's levels in rats blood as compared with that controller with no significance differences ($p> 0.05$) but they couldn't compensate the GPT's level as compared with that in blood of the control rats group on the contrary, the GPT's level were decrease than that controller highly significance differences ($p< 0.01$). GOT's, GPT's and ALP's levels in blood of rats were fed the fortified baked bran breads with the two ascorbic concentrations were increase directly proportional to that control rats group with significance difference ($p< 0.05$) as illustrated in histograms (6a), (6b) and (6c).

Histopathological investigation for the liver organs of the subjected rat groups showed that; no histopathological changes of the liver of rat at zero time fig (2a). The collected bran breads to cause slight activation of kupffer cells as showing in fig (2b) and showing congestion of central vein and slight granularity of the cytoplasm of hepatocytes fig (2c) for rat livers which fed on the collected bran breads through depleted and repleted periods repectively. No histopathological changes on livers of rat groups were fed on the baked bran breads whether fortified with two EDTA salts or two ascorbate salts as shown in fig (2d).



(a) (b) (c) (d)

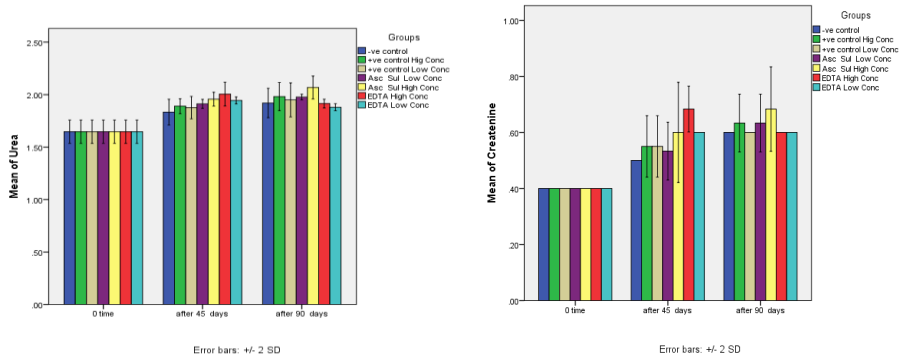
Fig (2): Histopathological studies for Liver of the subjected rat groups (H and E × 400).

Kidneys Functions (Urea "mg/dl" & Createnine "mg/dl"):-

A test can be done to measure the amount of urea nitrogen in the blood "BUN". According to Pincus and Abraham, (2006); urea nitrogen is what forms when protein breaks down. The BUN test is often done to check kidney function. In consequence of McPherson and Pincus, (2006); Higher-than-normal levels may be due to: Congestive heart failure, Excessive protein levels in the gastrointestinal tract, Gastrointestinal bleeding, Hypovolemia, Heart attack, Kidney disease, including glomerulonephritis, pyelonephritis, and acute tubular necrosis,

Kidney failure, Shock, Urinary tract obstruction. Lower-than-normal levels may be due to: Liver failure, Low protein diet, Malnutrition, Over-hydration.

Owing to a study of Patricia, (2012); createnine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass), serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism, creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion), there is little or no tubular reabsorption of creatinine, if the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR).



(a) (b)

Histograms (7):-Influence of collected and baked bran bread on kidneys functions (a)- Urea & (b)- Createnine.

In depletion period "after 45- days"; no matter whether the collected or the fortified bran breads whether with EDTA or ascorbates were affect urea or createnine of kidneys functions as compared with that of subjected control rats group with no significance differences ($p > 0.05$).

In repletion period "after 90- days"; urea and createnine states of rats were fed whether the collected or the fortified bran breads whether with EDTA or ascorbates as they were in the depletion period with no significance differences ($p > 0.05$) with reference to controller as shown in histograms (7a), (7b) and (7c).

Histopathological investigations for kidney of rat at zero time showing the normal histopathololigical structure of renal parenchyma as illustrated in fig (3a). No histopathological changes to take place for kidneys of rats were fed on collected bran breads through the depletion period as showing in fig (3b). The fortified baked bran breads with whether EDTA salts of iron and zinc in their two levels or that ascorbate salts of iron and zinc not to be affected kidney of rats of the corresponding subjected groups along the experimental periods in view of the fact that no histopathological changes showed in fig (3c).

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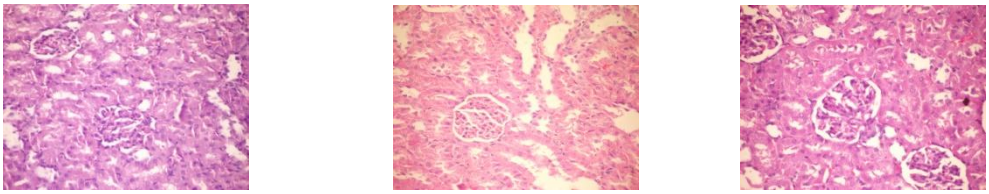
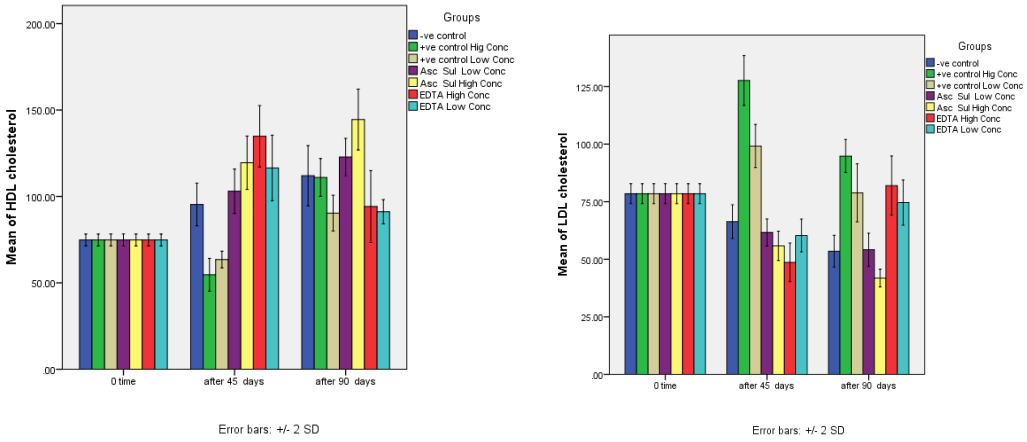


(a) (b) (c)

Fig (3): Histopathological studies for Kidney of the subjected rat groups (H and E × 400).

HDL- & LDL- Cholesterol.

High-density lipoprotein (HDL) is one of the five major groups of lipoproteins, which, in order of sizes, largest to smallest, are chylomicrons, VLDL, IDL, LDL, and HDL, which enable lipids (fats), like cholesterol and triglycerides, to be transported within the water around cells, including the bloodstream. In healthy individuals, about thirty percent of blood cholesterol, along with other fats, is carried by HDL, AHA, (2009). This is often contrasted with the amount of cholesterol estimated to be carried within low-density lipoprotein particles, LDL, and called LDL-C, LDL is often informally called *bad cholesterol,* (as opposed to HDL particles, which are frequently referred to as good cholesterol or healthy cholesterol. HDL particles remove fats, including cholesterol, from cells, including within artery wall atheroma and transport it back to the liver for excretion or re-utilization, the reason why the cholesterol carried within HDL particles (HDL-C) is sometimes called "good cholesterol" despite the fact that it is exactly the same as the cholesterol in LDL particles. Those with higher levels of HDL-C tend to have fewer problems with cardiovascular diseases, while those with low HDL-C cholesterol levels (especially less than 40 mg/dL or about 1 mmol/L) have increased rates for heart disease Toth and Peter (2011). Higher HDL levels are correlated with better cardiovascular health. Further increasing one's HDL has been found to improve cardiovascular health Sirtori and Cesare R, (2006) and NIH, (2011).



(a) (b)

Histograms (8):- Influence of collected and baked bran bread on (a) HDL- & (b) LDL-Cholesterol.

The collected bran breads with their concentrations decreased the HDL-C levels and increased the LDL- C levels in the subjected rat groups with highly significance differences (p<

0.01) than that in controllers and zero time intervals as shown in histograms (8a), (8b). In contrast with the fortified bran breads with EDTA in its concentrations were enhance the HDL-C levels and reduced their corresponded LDL- C levels with the highly significance differences ($p< 0.01$) than that controller and zero time corresponded groups. Foods that are high in soluble fiber are excellent sources of beta glucans, a substance that interferes with the absorption and production of cholesterol.

Fortified baked bran breads with ascorbates were help to protect LDL- C from oxidation, ascorbic acid prevent plaque buildup in the coronary arteries. Ascorbic acid may also boost blood levels of HDL- C studies as shown in histograms (8a), (8b) in highly significances ($p< 0.01$) are ongoing to provide definitive evidence of this action. Ascorbic acid also enhances the effect of zinc and another antioxidant commonly taken to fight high cholesterol as well as copper and iron.

Assessment of some minerals in Rats blood serum:

According to Gomella (2007); the amount of circulating iron bound to transferrin is reflected by the serum iron level. The serum iron reference range is 55–160 µg/dL in men and40–155 µg/dL in women. In addition to Ginder (2011) state that; serum iron is a test that measures how much iron is in blood, iron levels are highest in the morning, it's best to do this test in the morning, total iron binding capacity (TIBC) is a blood test to see if may have too much or too little iron in the blood, iron moves through the blood attached to a protein called transferrin, this test helps to know how well that protein can carry iron in the blood, the normal range is iron: 60-170 mcg/dL, TIBC: 240-450 mcg/dL, transferrin saturation: 20-50%.

Due to the studies of BoLönnerdal (2000), zinc is an essential mineral that is naturally present in some foods, added to others and available as a dietary supplement, zinc is also found in many cold lozenges and some over-the-counter drugs sold as cold remedies, zinc is involved in numerous aspects of cellular metabolism, it is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division, zinc also supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste, smell. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system.

Jorge, (2003) stated that; the increasing of copper absorption lead to increase the HDL- C "good cholesterol" and lowered the LDL-C "bad cholesterol" as well as; copper level of rat blood serum is reversibly proportional to zinc absorption in blood, thus due to the aggressive competition between zinc and copper absorption on the same cites of small intestine lumen and high intakes of zinc can induce synthesis of copper-binding metallothionein in the mucosal cell; this protein sequesters copper, making it unavailable for transfer, and thus decreases copper absorption.

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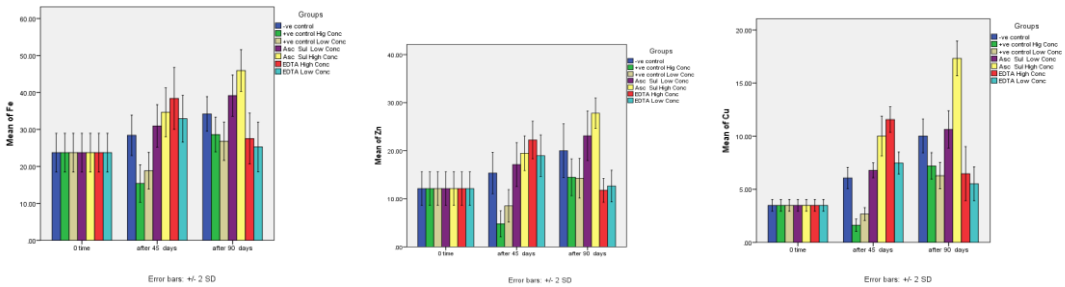

(a) (b) (c)

Histogram (9):- Influence of collected and baked bran bread on absorption of a- iron, b- zinc & c- copper.

In depletion period "after 45- days"; the market places collected bran breads depleted whatsoever iron, zinc and copper contents of rats blood where their levels to be diminished with highly significance ($p< 0.01$) diminution than that other groups and zero time interval as shown in histograms (9a), (9b) and(9c). These rat groups undergo vigorous depreciation in RBC'S and HGB as previously discussed in histograms (4b) and (6b) respectively; due to their sever iron deficiency as illustrated in histogram (9a). The rats were fed on the collected bran breads diet group [unutilized zinc intake owing to phytate rich diet] as illustrated in histogram (9a) were less active, had lost hair, had acrodermatitis, had diarrhea, and had typical skin lesions on the tail and paws and are in agreement with (ElzbietaI *et al.,* 2001). No such lesions occurred in the other rats groups were fed *adlibitum* on the zinc fortified baked bran breads and control diets this result is in agreement with (Pang *et al.,* 1992 and NRC, 1995). Also; the alkaline phosphatase level which is one of the liver indicators of functions and the strongest indicator for zinc level in the blood was highly increased ($p< 0.01$) by increasing in zinc absorption into rats blood stream as shown in histogram (6c) an(9c) respectively, these results are in agreement with Nicola *et al.,* (2004). Rats were fed on on the collected bran breads diets group were the lowest of the experimental groups in alkaline phosphatase level than controller, on the other side; alkaline phosphatase levels in rat's blood of groups fed on fortified baked bran breads were gradually increased in accordance to zinc absorption in agreement with Sherif *et al.,* (2013).

The presence of collected bran breads in the *adlibitum* diet of rat groups were decreased the bio availability and the utilization of copper which resulted in lowered the copper level in rats blood, as shown in histogram (9c) and decreased the HDL- C and increased the corresponding LDL- C as early discussed in histograms (8a) and (8b) respectively; these are in agreement with (Jorge, 2003). Fe, Zn and Cu of EDTA complexes were more absorbed in rat blood stream than that ascorbates complexes with significance differences ($p < 0.05$) as shown in histograms (9a), (9b) and (9c).

In repletion period "after 90- days"; fortified baked bran breads with NaFeEDTA improved the absorption of iron in rats blood with high significance differences than that depleted as obviously shown in histogram (9a), these results were in agreement with those concluded by (Christine *et al.,* 2003). Even, the stability of the EDTA complexes whether zinc or copper was

at the same gastric optimal p^H [p^H = 4] increased the size of the competition between them. But the highly significant increasing in copper level of blood of rats group fed $ZnNa_2EDTA$ fortified baked bran breads *addlibtum* diet than the depleted groups with ($p<0.01$) that is due to $ZnNa_2EDTA$ is a strong complex and is partially dissociated and resulted in free zinc binding metallothionein and highly absorbed ($p< 0.01$) in blood serum as in histogram (9b), in agreement with (Jorge, 2003), while the dissociated Na_2EDTA made re-chelation with copper and formed strong complex of $CuNa_2EDTA$ which also dissociated into free copper bound with metallothionine in the mucosal cell, this protein making it available for copper transfer and increase its level ($p< 0.01$) in the blood as in histogram (9c) in the same manner of increasing the zinc level in the blood these results are in agreement with those conclusion by Christine *et al.,* (2003). The documented explanations for the undoubted effects of bran phytates on the absorption of the last nutrient metals by: phytic acid molecule has a high phosphorous content "28.2 %" and chelating potential to form a wide variety of insoluble salts with di- and trivalent cations at neutral p^H. Zn, Cu and Fe can be complexed to form insoluble phytates at the p^H of the small intestine but Zn and Cu have the strongest binding affinity, this binding potentially renders these minerals unavailable for intestinal absorption due to formation of un utilized strong insoluble minerals phytate complex excreted in rat feces and make sharp decreasing in mineral blood level.

Ultimately, ascorbic acid is the most potent enhancer of Fe, Zn and Cu absorptions. By adding substantial quantities of ascorbic acid to a baked bran breads, Fe, Zn and Cu absorptions may be increased directly proportional to the time of 45- days and 90- days with ($p< 0.05$) and ($p< 0.01$) respectively. This influence is most pronounced in bran breads that contain high levels of phytates. The solubilizing effect of ascorbic acid counterbalances the negative consequences of dietary fiber of phytic acid.

CONCLUSION

Despite the collected bran breads from different markets and bakeries of different suburbs for Egyptian fourteen governorates; have high iron, zinc and copper contents; nevertheless their utilizations are very low due to their highly phytate contents. The strong belief that bran bread is useful to diminish the body weight is blunder belief in addition to their vigorously declines in each of hemoglobin, RBC's, WBC's PLT's, HDL- C, as well as, Fe-, Zn-, Cu-deficiencies which play important role in whatsoever, hair loss, immunologic abnormalities and loss of appetite an acne-like rash, mental confusion, impaired wound-healing, slow or impaired growth, decrease resistance to infections, reduce senses of taste and smell, skin disorder, mental lethargy and reduced fertility in addition to; diarrhea, adverse effects on fetal brain function and Low birth-weight infants by pregnant women, induced ulcerations, intestine inflammation, liver and heart disorders and the decreasing in water and sodium transport. We recommend that to fortify the bran breads with irrespective of whether EDTA or ascorbates salts of iron and zinc to annihilation the signs mentioned above.

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