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CHEMICAL, NUTRITIONAL AND BIOLOGICAL EVALUATION OF SCHOOL 'S BISCUIT AND FORTIFIED BISSUIT

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ABSTRACT: *This study aimed to determine the effect of school's biscuit and different fortified samples of biscuits on feed intake, body weight, feed efficiency ratio, serum lipids profile, liver functions, kidney functions, and histopathological structure of liver and Pancreas . Forty male albino rats were divided into eight groups, the first group was control, the second group was administered diet with school's biscuits and the other groups were fed on biscuits fortified with 10, 15 and 20mg of zinc, 10, 15 and 20mcg of and selenium for 28 days.*
The results showed that scholar's biscuits was high in fat and iron where as fortified biscuits were highly in protein, ash, zinc and selenium. Liver functions, kidney functions and HDL were higher than the control group but this high in control levels. Other lipid profile as total cholesterol, triglyceride, and LDLcholesterol were lower in fortified biscuits than the control and scholar's bisscuits.There was no histopathological change, from the obtained resultsin fortified biscuits groups. It could be concluded that fortified biscuits with zinc or selenium at the level of 15mg zinc and15mcg selenium improved the organ's functions and histopathological structure of liver and pancrease.

Key words: *Biscuits; zinc ;selenium; lipid profile.*

INTRODUCTION

Micronutrient deficiencies are a major public health problems among school children and preschool children in most low-income countries and developing countries (Rosado *et al.*, 1997 and Tran *et al.,* 2009). Selenium is a trace elemen that is naturally present in many foods, added to others, and available as a dietary supplement. Selenium, which is nutritionally essential for humans, is a constituent of more than two dozen selenoproteins that play a critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection (Sunde, 2012).

Selenium exists in two forms inorganic (selenate and selenite) and organic (selenomethionine and selenocysteine) (Sunde,2006). Both forms can be considered good dietary sources of selenium. Most selenium is in the form of selenomethionine in animal and human tissues, where it can be incorporated nonspecifically with the amino acid methionine in body proteins. Skeletal muscle is the major site of selenium storage, accounting for approximately 28% to 46% of the total selenium pool (Terry and Diamond, 2012). Both selenocysteine and selenite are reduced to generate hydrogen selenide, which in turn is converted to selenophosphate for selenoprotein biosynthesis (Davis, 2012).

Selenium deficiency produces biochemical changes that might predispose people who experience additional stresses to develop certain illnesses (Institute of Medicine, 2000). For example, selenium deficiency in combination with a second stress (possibly a viral infection) leads to Keshan disease, a cardiomyopathy that occurred in parts of China prior to a government-sponsored selenium supplementation program that began in the 1970s (Sunde, 2010). Before the Chinese government supplementation program, adults in the Keshan disease are as had average selenium intakes of no more than 11 mcg/day; intakes of at least 20 mcg/day protect adults from Keshan disease (Institute of Medicine, 2000).

Selenium deficiency is also associated with male infertility and might play a role in

Kashin-Beck disease, a type of osteoarthritis that occurs in certain low-selenium areas of China, Tibet, and Siberia . Selenium deficiency could exacerbate iodine deficiency, potentially increasing the risk of cretinism in infants (Sunde, 2010).

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 (Idpase,2006).

Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes (Institute of Medicine2001) and it plays a role in immune function (Solomons, 1998), protein synthesis , wound healing , DNA synthesis , and cell division (Prasad, 1995). Zinc also supports normal growth and development during pregnancy, childhood, and adolescence and is required for proper sense of taste and smell (Institute of Medicine, 2001).

A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Maret and Sandstead ,2006).

Zinc deficiency is characterized by growth retardation, loss of appetite, and impaired immune function. In more severe cases, zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions . Weight loss, delayed healing of wounds, taste abnormalities, and mental lethargy can also occur . Many of these symptoms are non-specific and often associated with other health conditions; therefore, a medical examination is necessary to ascertain whether a zinc deficiency is present (Nishi, 1996). So, Selenium and zinc are essential micronutrients for human health. Deficiencies in these (2) nutrients remain a global problem, especially among children in developing countries (Christa *et al.,* 2005)

School meals improved micronutrient deficiencies and enhanced nutrition and child health, increased learning and decreased morbidity for students school meal programmers provide complete meals while others provide high energy biscuits or snacks, High energy biscuits is the most known meal provide at school. School feeding program used to prevent micronutrients and vitamins deficiency of school age children (WFP.,2009) school feeding program fortified with a premix of vitamins and minerals, to covering the urgent needs in an emergency situation during which population is not able to cook due to a lack of basic facilities (clean water, cooking equipment) School meal fortification is the addition of micronutrients during or after processing a food, raising micronutrient levels above the amounts in the original food product Fortification is sometimes also called enrichment, levels of fortification should be set so that vitamin or mineral added will make significant contribution to nutritional requirements, but not lead to a micronutrient intake above the safe upper limit (WFP.,2010).

Thus the main objectives of this study were to study the effect of school's biscuit and different fortified samples of biscuits on feed intake, body weight, feed efficiency ratio, serum lipids profile, liver functions, kidney functions, and histopathological structure of liver and Pancreas in rats.

MATERIALS AND METHODS :

Materials:

Selenium, zinc and diets components were obtained from Elgornhoriya, Company for Medical Preparations, Chemical and Medical Equipments, Cairo, Egypt. Iron fortified biscuits were obtained from schools at El Qaliobia governorate which offered to school's pupils, every packet weight (50g) consists of (4) pieces, Wheat flour and other ingredients used in sweet biscuits were obtained from the local markets.

Animals:

Forty male albino rats (90 ±5g) of Sprague Dawley strain were obtained from the Laboratory of Animal Colony, Ministry of Health and Population, Helwan, Cairo, Egypt. The rats were kept under controlled conditions in plastic cages.

Diets:

The basal diet consists of casein (12%), corn oil (10%), methionin (0.3%) choline chloride (0.2%), vitamin mixture (I %) according to Campbell (1963), cellulose (5%), salt mixture(4%) according to Hegsted *et al* (1941) and corn starch (up to 100%).

Methods:

Preparation of Biscuits: The sweet biscuits were prepared by using the method of AACC (1984). Preparation of biscuits was carried out using wheat flour samples fortified by adding zinc sulfate and sodium selenite at the level 100, 150 and 200mg /1kg biscuit doug.

Analytical Methods: Proximate composition was estimated according to AOAC (1999) Sensory characteristics of biscuits were determined as described by
Austin and Ram (1971).

Experimental design:

A total 40 male albino rats weighing 90±5 g. were housed individually in stainless steel cages, fitted with wire-mesh bottoms, in a temperature-controlled room (25°C) with 12-h light and dark periods. They were allowed free access to water. In this study, rats fed on normal diet for 2 days as adaptation period, the basal diet according to (AIN .,1993) consisted of casein (12%), corn oil (10%), cellulose (5%) , salt mixture (4%) (Hegsted *et al.*, 1941), vitamin mixture (1%) (Campbell,1963), methionin (0.3%), choline chloride (0.2%) and corn starch (up to 100%).

After the first 2days, rats (the initial weight was 90±5 g.) were divided randomly into eight groups. The rats in group (1) were continued feeding on basal diet as a negative group, The rats in group (2) were feeding on basal diet with 10%scholar*s biscuits as a positive group and the other groups were fed on basal diet with 10%fortified biscuits with zinc and selenium for 4 weeks. The other rats divided into 6 subgroups (5 rats per each) as follow:

Group 3: basal diet+ 10%biscuits fortified with 10mg zinc.
Group 4: basal diet + 10%biscuits fortified with 15mg zinc.
Group 5: basal diet +10%biscuits fortified with 20 mg zinc.
Group 6: basal diet+ 10%biscuits fortified with 10mcg selenium.
Group 7: basal diet + 10%biscuits fortified with 15mcg selenium.
Group 8: basal diet +10%biscuits fortified with 20 mcg selenium.

Biological evaluation:

At the end of the experiment, biological evaluation of the different diets carried out by determination of daily feed intake (consumption), relative organs weights (% of body weight), body weight gain% (BWG %) and feed efficiency ratio (FER) according to Chapman *et al* (1959) using the following formulae:

$$BWG = \frac{\text{Final weight- initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}}$$

Collection of blood: On the last day of the experimental period, blood was collected by retero orbital sinus puncture, under mild ether anesthesia after 8 hr fasting and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20C until biochemical estimations were carried out.

Enzymes and metabolites assay:

Determination of the activity of liver enzymes:-

Serum Alkaline phosphatase (ALP) was determined according to IFCC methods (1983). Aspartate aminotransferase (AST) was carried out according to the method of Yound (1975). (ALT) Determination was carried out according to the method of Yound (1975).

Determination of the activity of kidney :-

Creatinine was determined according to the method described by Bohmer (1971). Serum uric acid was determined according to the method described by Fassati and Prencipel (1982).

Determination of lipid profile:-

Serum cholesterol levels were determined according to Seary and Bergquist (1960). HDL was determined by Uwajima *et al* (1984). Total triglycerides, and LDL- cholesterol were determined according to Jacobs and Van Denmark (1960).

Statistical Analysis:

Triplicate samples were analyzed for each property. Data were assessed by analysis of variance (ANOVA) as outlined by Sendecor and Cochran (1987).

Histopathological Examination:-

At end of the experiment all rats were sacrificed, tissue samples including liver and Pancreas were fixed in 85% alcohol and they were microscopically examined to evaluate the effects of scholar biscuit's different concentration of fortified biscuits on these organs.

RESULTS:

Proximate composition of substituted biscuits:

Table (1) shows that protein , ash , carbohydrate, selenium and zinc contents increased in tested biscuits samples whereas fat and iron decreased as compared with the scholar's biscuits. On the other side, for fortified biscuits with zinc and selenium contents increased and reached to 19.78±0.14 and 19.50±0.08, respectively.

From Table (2), food intake of rats fed on selenium at the levels10, 15 and 20mcg selenium was nearly significant (P ≤0.05) to the Control group and the best level was 15 mcg selenium but Food intake of rats fed on scholar biscuits was lower significant than (P ≤0.05) the control group, and also groups fed on different levels of fortified biscuits with10,15 and 20 mg zinc was lower significant than (P ≤0.05) the control group.

Table 1: Chemical composition of biscuits fortified with zinc, selenium and scholar's biscuits g/100g dry weight basis

Sample	Protein	Fat	Ash	Carbohydrates	Iron	Zinc	Selenium
Scholar's biscuits	5.88±0.16	24.23±0.21	0.81±0.02	69.08±1.08	8.73±.02	1.75±0.02	0.98±0.01
Biscuits with 10mg zinc	9.48±0.06	17.61±0.17	1.22±0.11	73.69±1.25	3.21±0.31	9.41±0.07	0.90±0.15
Biscuits with 15mg zinc	9.71±.022	17.53±0.15	1.39±0.03	75.37±1.35	3.49±0.36	14.57±0.1	0.92±0.11
Biscuits with20 mg zinc	9.53±.023	17.12±0.25	1.52±0.01	77.83±0.98	3.31±0.26	19.78±0.14	0.93±0.16
Biscuits with 10mcg selenium	9.63±0.08	17.24±0.13	1.13±0.02	75.00±1.11	3.47±.021	1.34±0.09	9.13±0.09
Biscuits with 15mcg selenium	9.23±0.23	17.28±0.19	1.36±0.05	76.13±1.26	3.25±0.28	1.74±0.08	14.51±0.12
Biscuits with 20mcg selenium	9.40±0.13	17.56±0.25	1.48±0.08	78.56±1.23	3.71±0.33	1.21±0.16	19.50±0.08

Table (2). The effect of feeding scholar's biscuits and different levels of zinc, selenium on food intake, body weight gain and feed efficiency ratio .

Groups / Parameters	G 1	G 2	G3	G4	G5	G6	G7	G8
Food intake g/100g	12.9^{a} ±0.38	9.83^{b} ±0.03	8.38^{c} ±0.38	9.78^{b} ± 0.35	8.54^{c} ± 0.35	11.48^{b} ±0.41	12.18^{a} ± 1.14	11.54^{a} ± 2.41
BWG g/100g	42.27^{a} ±2.51	33.58^{b} ±5.71	30.12^{b} ±3.46	33.17^{b} ±4.82	28.28^{c} ±4.69	39.12^{b} ±2.40	41.27^{a} ±4.02	39.99^{a} ±2.09
FER g/100g	0.117^{c} ± 0.011	0.119^{c} ±0.02	0.127^{a} ± 0.007	0.121^{a} ± 0.016	0.118^{c} ±0.031	0.121^{a} ± 0.007	0.121^{a} ± 0.016	0.124^{a} ±0.031

P ≤0.05

G1: Control group
G2: Rats fed on scholar biscuits
G3: Rats fed on 10mg zinc
G4: Rats fed on 15mg zinc
G5: Rats fed on 20mg zinc
G6: Rats fed on 10mcg se
G7: Rats fed on 15mcg se
G8: Rats fed on 20mcg se

For body weight gain, the rats fed on biscuits with 15, 20 mcg selenium, and 20mg zinc was not significant (P ≤0.05) to the Control group. While, body weight gain of rats feed on scholar's biscuits, and the other fortified levels of zinc biscuits was lower significant (P ≤0.05) to the control group.

In case of food efficiency ratio (FER), scholar's biscuits and fortified biscuits with selenium and zinc at all levels improved the feed efficiency ratio higher significant (P ≤0.05) than the control group.

Form Table (3) the mean values of serum AST, ALP and ALT in all fortified biscuits with zinc and selenuim groups were significantly higher (P ≤0.05) than the mean value of serum AST, ALP and ALT of control group. There is no significant differences between fed on scholar biscuits and the control group .

In Table (4) showed the effect of feeding scholar's and different levels of zinc, and selenium biscuits on kidney function of rats. The mean values of creatinine in all groups was significant (P ≤0.05) higher than group fed on scholar's biscuits. The mean values of uric acid in all groups was significant (P ≤0.05) lower than group fed on scholar's biscuits. There is no significant between the groups fed on biscuits with different levels of selenium and zinc and the group fed on basal diet.

Table (5) showed the mean values of total cholesterol, triglycerides, HDL and LDL which affected by scholar's and different levels of zinc, and selenium biscuits . Adding zinc and selenium at different levels to biscuits led to keep the mean values of lipid profile in the normal range . There is no significant (P ≤0.05) between groups fortified biscuits and the control one while there was significant (P ≤0.05) between the scholar's biscuits group and the others significant to the Control group.

A:- Histopathological results of liver

liver of rat from group (1,3,4) showing the normal histological structure of hepatic lobule while liver of rat from group (2) showing hydropic degeneration of some hepatocytes and liver of rats from groups (5,6,7,8) showing no histopathological changes

Table (3). The effect of feeding scholar's biscuits and different levels of zinc, selenium on liver function of rats (u / L).

Parameters \ Groups	G1	G 2	G3	G4	G5	G6	G7	G8
AST(U/L)	25.1[a] ±0.27	26.2[a] ±1.11	27.1[a] ±2.56	27.5[a] ±0.21	29.7[a] ±0.25	25.1[a] ±0.16	27.5[a] ±0.01	29.7[a] ±0.05
ALT(U/L)	19.8[b] ±0.31	21.9[a] 1.11±	23.7[a] ±0.52	25.4[a] ±2.05	25.7[a] ±1.25	20.7[b] ±0.82	21.4[b] ±2.65	25.7[a] ±0.05
ALP (U/L)	80.1[a] ±0.17	80.7[a] ±2.01	81.7[a] ±1.16	82.7[a] ±6.16	82.7[a] ±1.15	80.7[a] ±0.32	82.7[a] ±0.26	82.7[a] ±0.15

P ≤0.05
G1: Control group
G2: Rats fed on scholar biscuits
G3: Rats fed on 10mg zinc
G4: Rats fed on 15mg zinc
G5: Rats fed on 20mg zinc
G6: Rats fed on 10mcg se
G7: Rats fed on 15mcg se
G8: Rats fed on 20mcg se

Table (4). The effect of feeding scholar's biscuits and different levels of zinc, selenium on kidney function of rats (u / L).

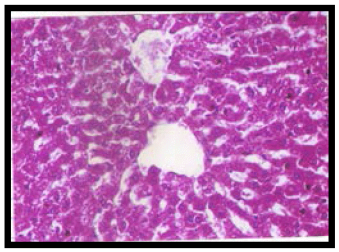
Parameters \ Groups	G1	G 2	G3	G4	G5	G6	G7	G8
Creatinine mg/100ml	0.69[b] ±0.31	0.66[b] ±0.21	0.69[b] ±0.14	0.72[b] ±0.07	0.77[b] ±0.21	0.69[b] ±0.14	0.72[b] ±0.07	0.77[b] ±0.21
Uric Acid mg/100ml	2.35[a] ±0.15	2.35[a] ±0.22	2.56[a] ±1.2	2.72[a] ±1.00	2.75[a] ±2.5	2.56[a] ±1.2	2.72[a] ±1.00	2.75[a] ±2.5

P ≤0.05
G1: Control group
G2: Rats fed on scholar biscuits
G3: Rats fed on 10mg zinc
G4: Rats fed on 15mg zinc
G5: Rats fed on 20mg zinc
G6: Rats fed on 10mcg se
G7: Rats fed on 15mcg se
G8: Rats fed on 20mcg se

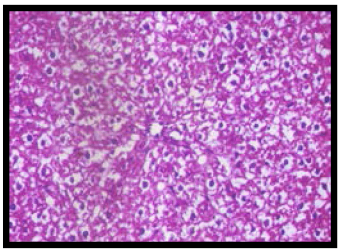
Table (5): The effect of feeding scholar's biscuits and different levels of zinc, selenium on blood lipid profile of rats (mg / dL).

Parameters \ Groups	G 1	G 2	G3	G4	G5	G6	G7	G8
Total cholesterol	86.26[b] ±1.19	81.2[b] ±2.15	81.7[b] ±0.12	86.1[b] ±0.13	87.7[b] ±3.21	81.7[b] ±0.12	86.1[b] ±0.13	87.7[b] ±3.21
Triglycerides	6.48[a] ±0.13	6.8[a] 0.03±	6.68[a] ±0.63	6.4[a] ±2.01	6.96[a] ±1.56	6.68[a] ±0.63	6.4[a] ±2.01	6.96[a] ±1.56
HDL-cholesterol	53.94[b] ±0.12	53.87[a] ±1.15	55.92[a] ±0.03	56.89[a] ±0.04	57.94[a] ±0.05	55.92[a] ±0.03	56.89[a] ±0.04	57.94[a] ±0.05
LDL-cholesterol	20.2[a] ±1.17	20.9[a] ±4.34	20.1[a] ±0.91	19.5[a] ±0.74	19.1[a] ±0.91	20.1[a] ±0.91	19.5[a] ±0.74	19.1[a] ±0.91

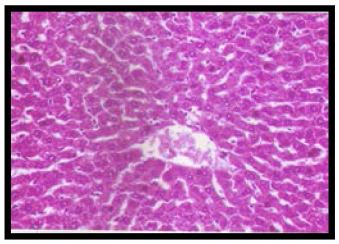
P ≤0.05
G1: Control group
G2: Rats fed on scholar biscuits
G3: Rats fed on 10mg zinc
G4: Rats fed on 15mg zinc
G5: Rats fed on 20mg zinc
G6: Rats fed on 10mcg se
G7: Rats fed on 15mcg se
G8: Rats fed on 20mcg se



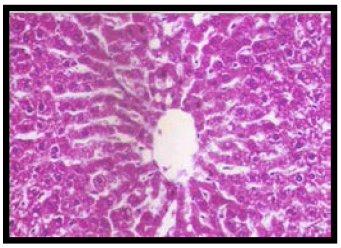
G(1)
Normal histological structure of hepatic lobule



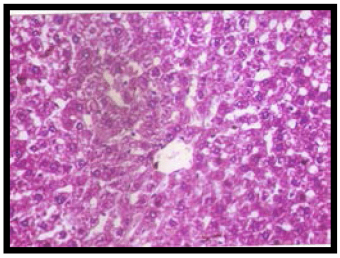
G (2)
Hydropic degeneration of some hepatocytes



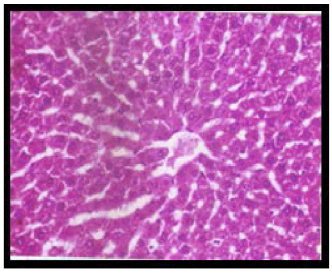
G(3)
Normal histological structure of hepatic lobule



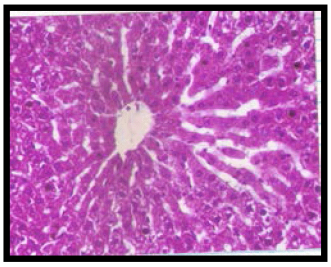
G(4)
Normal histological structure of hepatic lobule



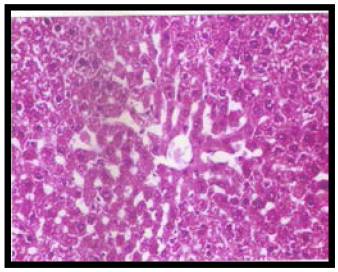
G(5)
No histopathological changes



G6
No histopathological changes



G(7)
No histopathological changes

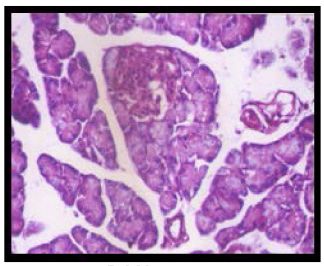


G(8)
No histopathological change

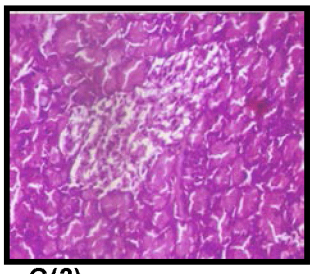
photos (A) :- Liver of rats fed on basal diet (group 1), scholar's biscuits (group 2) and fortified biscuits with different levels of zinc and selenium (groups 3,4,5,6,7,8, and 9).

b) Histopathological structure of Pancreas:-

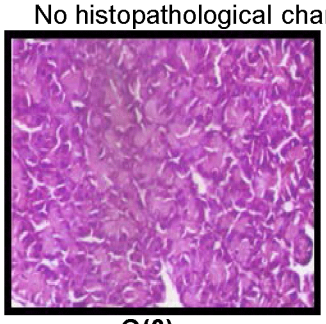
Pancreas of rat from group (1,3,4,5,6,7) showing no histopathological changes while Pancreas of rat from group (2,8) showing vacuolations of B cells of islets of langerhan's.



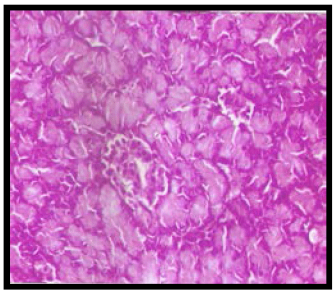
G(1)
No histopathological changes



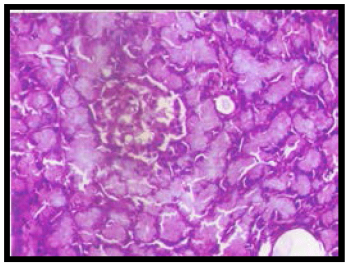
G(2)
Vacuolations of B cells of islets of of langerhan's



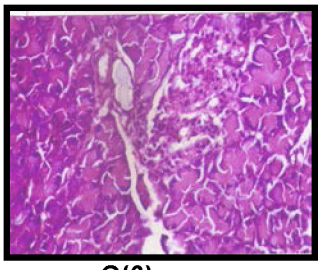
G(3)
No histopathological changes



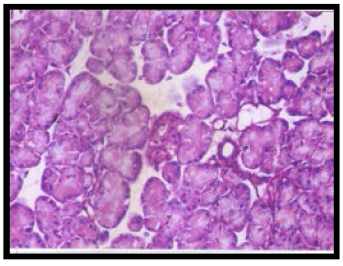
G(4)
No histopathological changes



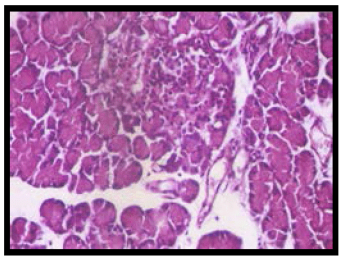
G(5)
No histopathological changes



G(6)
No histopathological changes



G(7)
No histopathological changes



G(8)
Vacuolations of B cells of islets of langerhan's

photos (B):- Pancreas of rats fed on basal diet (group 1), scholar's biscuits (group 2) and fortified biscuits with different levels of zinc and selenium (groups 3,4,5,6,7,8, and 9).

DISCUSSION:

Concerning chemical composition of biscuits fortified with zinc, selenium and scholar's biscuits g/100g dry weight basis. it could be stated that these products had a higher protein , ash , carbohydrate, selenium and zinc while it had low content of fat and iron. These results are in agreement with those reported by Sunde (2010). Form the data concerning feed intake, feed efficiency ratio and body weight gain of rats, it could be noticed that there was not significant (P ≤0.05) between the obtained results and the control group. The best level was 15 mcg of selenium and 20 mg of zinc which had the best effect on feed intake, there was significant (P ≤0.05) between the rats fed on scholar's biscuits, the other fortified level biscuits and the rats fed on basal diet. The fortified biscuits with selenium at the level 15, 20mcg and zinc at all levels improved the feed efficiency ratio higher than the scholar's samples. These values are similar to those measured by Christa *et al* (2005) and Davis (2012). Tested serum liver and kidney functions showed that, addition of different levels of selenium and zinc mainly kept these functions in normal range. There are no significant differences between groups fed fortified biscuits and the control group. The best level of adding was 15 mcg selenium or 15mg zinc. On the other side, it was found that there was a significantly increased in lipid profile of rats fed on scholar's biscuits. This may be due to higher contents of saturate fat and low quality ingredients .For the histological structure of liver 's rats fed control and fortified biscuits showed normal histological structure but there was hydropic degeneration of some hepatocytes of scholar's biscuit group . It could be concluded that biscuit fortified with15mg zinc or15mcg selenium in bakery products as biscuits led to improve the health status of rats.

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التقييم الكيميائى والغذائى والجيولوجى لبسكويت المدارس والبسكويت المدعم

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الملخص العربى:

أجرى البحث لتقدير كفاءة البسكويت المدرسى والعينات المختلفة من البسكويت المدعم على المأخوذ الغذائى ، وزن الجسم ، ومدى كفاءة الوجبة وكذلك على دهون السيرم، وعلى وظائف الكبد والكلى مع التشريح الهستوباثولوجى للكبد والبنكرياس فى فئران التجارب.

استخدم فى التجربة عدد (٤٠) فأر ألبينوتتراوح أوزانهم (٩٠±٥جرام) تم تقسيمها لثمانى مجموعات ،المجموعة الاولى هى المجموعة الضابطه ،المجموعة الثانية دعمت الوجبة فيها بالبسكويت المدرسى ،أما باقى المجموعات فقد دعمت فيها الوجبات بالبسكويت المدعم بالزنك بنسبة ١٠,١٥,٢٠مللى جرام وبالسيلنيوم بنسبة ١٠,١٥,٢٠ ميكروجرام لمدة ثمانية وعشرين يوما.

وقد أظهرت النتائج الأتى، أن البسكويت المدرسى أعلى فى نسبة الدهون والحديد، على حين أن البسكويت المدعم بالزنك والسيلنيوم كان أعلى فى نسبة البروتين و الرماد ،الزنك والسيلنيوم.

زيادة كفاءة وظائف الكبد والكلى وHDL فى جميع المجموعات عن المجموعة الضابطة ، وعلى العكس من ذلك قلت نسبة سائر الدهون فى السيرم كالكليسترول والجليسريدات الثلاثية وLDL فى المجموعات التى تغذت على البسكويت المدعم بالزنك والسيلنيوم عن المجموعتين الضابطة والمجموعة التى تغذت على البسكويت المدرسى، لم يحدث أى تغيير فى التشريح الهستو باثولوجى للمجموعات التى تغذت على البسكويت المدعم بالزنك والسيلنيوم عن المجموعة الضابطة.

وتوصى النتائج المتحصل عليها من المجموعات التى تغذت على البسكويت المدعم بالزنك والسيلنيوم بأن المعدل الأفضل لدعم البسكويت بالزنك هو١٥مللى جرام/١٠٠جرام وكذلك المعدل الأفضل لدعم البسكويت بالسيلنيوم هو ١٥ ميكروجرام/١٠٠جرام ، حيث تحسنت وظائف الأعضاء عند تلك المستويات وهو ما أكده التشريح الهستوباثولوجى.