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THE ROLE OF CHICKPEA AS A FORTIFIED MATERIAL TO BISCUITS ON CONTROLLING SERUM GLUCOSE OF DIABETIC RATS

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ABSTRACT

The effect of unfortified biscuits, fortified biscuits with chickpea and school children biscuits on some biological and biochemical parameters such as food intake, percent of weight gain, relative organs weight, serum glucose, liver functions, kidney functions and iron status in normal and diabetic rats were studied. Biscuits were fortified with 10, 15 and 20% chickpea. After baking, biscuits samples were evaluated to choose the best samples for the biological part. Sixty albino rats weighing about (110±5.0 g) were divided into two main groups (30 rats/each). The first main group was normal while the second main group was injected with alloxan to induce hyperglycemia. Both groups were divided into six subgroups fed on basal diet containing 2.5 or 5% protein from unfortified biscuits, school children biscuits, and fortified biscuits with 20% chickpea. After 5 weeks rats were sacrificed, some organs were weighed, and blood samples were collected and centrifuged to obtain the serum. Results indicated that the use of 2.5 or 5% protein from fortified biscuits with 20% chickpea showed significant improvement in body weight gain, serum glucose, liver functions, kidney functions and iron status compared with to unfortified biscuits and school children biscuits. From the obtained results, both normal and diabetic children can nutritionally benefit from fortified school biscuits with chickpea because it is suitable for them and improves their nutritional status

especially iron status. It is recommended to use chickpea in diabetic foods and diets through planning and implementing nutrition education programs for diabetic patients in order to explain the important role of chickpea in controlling diabetes.

Key words: Diabetic rats- chickpea- glucose- liver functions- kidney functions- total iron total iron binding capacity- biscuits.

INTRODUCTION

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels (*WHO, 2005*).

Health authorities in many countries recommended regular consumption of legumes as a means of reducing the risk of diet-related disease such as non insulin-dependent diabetes mellitus, coronary heart disease and obesity (*Messina, 1999; Leterme, 2002*). Recently, the potential role of chickpea in diabetes and hyperlipidimia has received much attention. Several compounds with anti-diabetic and hypocholesterolemic activity are found in relatively high concentrations in chickpea.

Compared to cereal grains chickpea is a very good source of dietary fiber (soluble and insoluble) plus resistant starch. In contrast to most other pulses and cereals, chickpeas have a relatively high fat content at 6 g/100 g. This makes them an important energy source for vegans and those without regular access to meat and dairy products. The fat is mostly ω -6 polyunsaturated fatty acids, with some monounsaturated fatty acids and less than 1% saturated fatty acids (*USDA, 2002*)

Chickpeas are a rich source of vitamins, minerals and phytoestrogens. They contain folate, thiamine, riboflavin, niacin, pantothenic acid, vitamins C, A and E. Chickpeas have a higher content of calcium and phosphorus than other pulses and are a good source of iron and zinc. Chickpeas are abundant in the isoflavones formononetin and biochanin A, phytoestrogens common to many pulses (*Wood and Grusak, 2007 ; Rochforts and Panozzo , 2007*).

Biscuits can be used successfully as vehicle for nutrient fortification in school feeding programs. Consumption of the fortified biscuits resulted in a significant improvement in micronutrient status and also appeared to have a favorable effect on the morbidity and cognitive function of the school children in this community. A major advantage of using biscuits as a vehicle for fortification is that it needs no preparation, is easy to distribute, has a long shelf life, and can be easily monitored (*Pineda et al., 1994*).

So, the study was carried out to investigate the effect of fortified biscuits with chickpea compared with school children biscuits on some biological and biochemical parameters in normal and hyperglycemic rats

MATERIALS AND METHODS

Materials

- Chickpea (*ciecer arietinum L.* variety G2) was obtained from the Field Crops research institute, Agricultural Research Center, Giza, Egypt.
- School children biscuits were obtained from 6th of October Company for Food Industries.
- Commercial wheat flour (72% extraction rate), eggs, skimmed milk, salt (sodium chloride), baking powder and corn oil were obtained from the local market.
- Sixty weanling female albino rats(110±5.0g) were obtained from the animal house, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

Methods:

Technological methods:

- **Soaking:** Chickpea seeds were soaked in tap water (1:5, w/v) for 12 h at room temperature. The soaked seeds were dried in a hot air oven maintained at 55°C according to the method of Jood *et al.*, (1988).
- **Production of biscuits:** Biscuits were prepared according to the method of Abd El Salam., (2000) with some modifications. Biscuits composed of 100 gm flour, 3 gm dried skimmed milk powder, 24 gm egg, 4.5 gm baking powder, 17 gm oil, 4 gm salt. The required amount of eggs and oil were beaten for one min.,

and then dried skimmed milk was added and mixed for one min. the flour and baking powder were seared together and added to the above mixture and beaten continuously until the blend got smooth. The dough was cut into circles (1/2 cm thick and 2cm diameter) and transferred to greased plate and baked at 180 °C for 12-15minutes. Biscuits were allowed to cool then evaluated chemically and organoleptically.

- Chickpea flour was used to replace wheat flour at 10, 15 and 20% levels and the fortified biscuits were prepared by the same method.

Sensory evaluation:

Thirty three children from AL Cawmia primary school in Hadaec El Koba, Cairo governorate were selected randomly for sensory evaluation of biscuits. Their age ranged between 7 to 12 years. Each child was asked to evaluate biscuits samples fortified with chickpea (10, 15, and 20% levels of fortification) according to the color; odor, taste, texture, and general acceptability by putting a mark beside the statement that best reflect his/her opinion in the evaluation sheets. The statements used were " like it very much, like it sometimes, don't like or hate it, do not like it sometimes, do not like it very much according to Penfield and Campbell, (1990), and results were analyzed statistically in order to know the best percentage for fortification.

Chemical analysis:

Moisture content, crude protein, fat, ash, minerals and fiber were determined in raw materials and all samples of biscuits on dry basis according to the method described in A.O.A.C., (1990). Fiber was determined according to the method described by Pearson, (1970). Total carbohydrate content was determined by difference.

Experimental animal design:

Sixty weanling female albino rats were housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. After this period, rats were divided into two main groups (30 rats each):

The first main group was normal (-) and divided into 6 subgroups fed on:

- Basal diet containing 2.5% protein from unfortified biscuits

- Basal diet containing 5% protein from unfortified biscuits
- Basal diet containing 2.5% protein from fortified biscuits with 20% chickpea.
- Basal diet containing 5% protein from fortified biscuits with 20% chickpea.
- Basal diet containing 2.5% protein from school children biscuits.
- Basal diet containing 5% protein from school children biscuits.

The second main group was hyperglycemic (+):

Rats were injected with 150 mg/kg body weight of recrystallized alloxan to induce hyperglycemia. Rats were kept fasted for 12 hours but allowed free access to water before the injection of alloxan. After alloxan injection, water contains glucose was introduced to rats for drinking to prevent hypoglycemia according to Buko *et al*, (1996). All diabetic rats received basal diet prepared according to Reeves *et al*, (1993) for 48 hours after injection They were divided into six subgroups fed on the same previous diet scheme.

At the end of experimental period (5 weeks), the rats were fasted overnight then sacrificed. Blood samples were collected from the orbital plexus by means of fine capillary glass tubes. Samples were centrifuged for 10 minutes at 3000 rpm to separate serum from the blood. Serum was carefully aspirated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till analysis at -20°C.

Liver, kidney, heart and spleen were carefully removed from each rat, cleaned and weighed for the determination of the relative organs to weight according to the following formula:

$$\text{Relative organ weight} = (\text{Organ weight} / \text{Final weight}) \times 100.$$

Percent Body weight gain (% BWG) and food efficiency ratio (FER) were calculated according to the following formulas:

$$\% \text{ BWG} = [(\text{Final weight} - \text{Initial weight}) / \text{Initial weight}] \times 100.$$

$$\text{FER} = \text{Gain weight (gm)} / \text{Food consumed (gm)}.$$

Serum analysis:

- Blood glucose levels were assessed weekly throughout the study. Enzymatic determination of glucose was carried out colorimetrically according to the method of Trinder, (1969).

- Serum urea nitrogen, uric acid and creatinine were determined colorimetrically according to the method of Tabacco *et al.*, (1979), Fossatti and Prencipe, (1980) and Bartels and Bohmer, (1971) respectively.
- Liver function enzymes were determined using the method of Wilkinson (1976) for aspartate amine transferase (AST) and the method of Bergmeyer and Horder (1986) for alanine amine transferase (ALT). Serum protein was determined colorimetrically according to the method described by Dawson *et al.*, (1986).
- Serum total iron and total iron binding capacity were determined calorimetrically according to the method of Burtis and Edward, (1994).

Statistical analysis:

The statistical analysis was performed according to the methods described by Snedecor and Cochran, (1967) using the computer SPSS software package, version 11, Chicago, USA. Results were expressed as mean \pm standard deviation (SD). Significant differences between groups were tested with one-way analysis of variance (ANOVA) followed by the least significant differences test (LSD) as a post hoc test. Two-tailed student t test was used to examine the differences between blood glucose level at the beginning and at the end of the study among various groups.

RESULTS AND DISCUSSION

Chemical composition of wheat flour, chickpea were presented in table (1). Wheat flour composed of 11.19%, 1.40%, 0.69% 0.64% and 86.08% while chickpea contained 24.31%, 5.98%, 4.04%, 1.97% and 63.7% in protein, fat, fiber, ash and carbohydrate, respectively. From the obtained results it could be observed that wheat flour had lowest percent of all determined parameters compared with chickpea except of carbohydrates. The obtained results were in agreement with the finding reported by Collins and Pangoli (1997); Abo-Zeid (1998) for wheat flour and with Faheid and Hegazi (1991) for chickpea.

Fortification of biscuits with chickpea at levels of 10, 15 and 20% caused general increment in protein, fat and fiber while, it caused a significant reduction in carbohydrates content when compared with unfortified biscuits. These results are in agreement with Faheid and

Hegazi (1991) who found an increase in protein, ash, fiber of supplemented cookies with 5, 15 and 15% chickpea. In addition, Rababah *et al.*, (2006) reported that substituting specified concentrations 3,6,9, and 12 % of chickpea from the total percent of wheat flour resulted in significant increase in protein content of biscuits. School children biscuits had the lowest value of protein and the highest value in carbohydrate when compared with either unfortified biscuits or chickpea fortified biscuits.

Table (1): Chemical composition (g/100g) of wheat flour, chickpea, unfortified biscuits, fortified biscuits with chickpea and school children biscuits.

Components samples	protein	fat	fiber	Ash	Total Carbohydrate
Wheat flour	11.19±0.14 ^A	1.40±0.04 ^A	0.69±0.07 ^A	0.64±0.07 ^A	86.08 ^A
Chickpea	24.31±0.46 ^B	5.98±0.12 ^B	4.04±0.09 ^B	1.97±0.06 ^B	63.7 ^B
Unfortified biscuits	13.80±0.29 ^b	12.8±1.77 ^a	0.52±0.06 ^a	1.90±0.06 ^b	70.98 ^c
Fortified biscuits with chickpea					
10%	14.2±0.36 ^{bc}	13.80±0.28 ^b	1.45±0.09 ^b	1.99±0.12 ^b	68.56 ^b
15%	15.4±1.14 ^c	14.33±0.25 ^c	1.68±0.30 ^c	2.11±0.06 ^{bc}	66.48 ^a
20%	16.03±0.32 ^d	15.20±0.15 ^d	1.90±0.05 ^c	2.30±0.03 ^c	64.57 ^a
School children biscuits	10.6±0.36 ^a	13.28±0.16 ^e	0.40±0.03 ^a	0.97±0.15 ^a	74.75 ^d

Results are expressed as mean±SD

Values sharing the same superscript capital letters in column indicate non significant differences between wheat flour and chickpea.

Values sharing the same superscript small letters in column indicate non significant differences between various kinds of biscuits

Table (2) showed minerals content of wheat flour, chickpea and unfortified biscuits, fortified biscuits with chickpea and school children biscuits. Minerals content of wheat flour were significantly lower than that of chickpea.

In this concept, Abo-Zeid, (1998) studied the minerals content (mg/100g) of wheat flour 72% and reported 26.7 for Ca, 122.3 for K, 83.1 for P, 3.4 for Na, 127.8 for Mg, 1.5 for Mn and 0.3 for Zn. Ibanez *et al.*, (1998) studied the minerals content of chickpea from different varieties. They found that the Fe content ranged from 3.20 to 5.88, Zn 2.96 to 4.38, Mn 1.41 to 2.33 and Na 14.1 to 39.2 mg/100g. The slight difference in mineral content between the present study and these

studies may be due to the differences in varieties and methods of analysis used.

Table (2): Minerals content (mg/100 g) of wheat flour, unfortified biscuits, fortified biscuits with chickpea and school children biscuits.

Components samples	Mg	Na	Zn	Mn	Fe	Ca	K
Wheat flour	126.5±130 ^A	3.6±0.15 ^A	0.6±0.06 ^A	1.40±0.14 ^A	0.90±0.03 ^A	29.8±0.08 ^A	123.70±1.66 ^A
Chickpea	255.16±3.60 ^B	26.41±1.03 ^B	3.05±0.41 ^B	1.90±0.24 ^B	4.95±0.28 ^B	167.00±7.13 ^B	362.00±14.11 ^B
Unfortified Biscuits	32.38±0.74 ^b	89.15±1.02 ^b	4.37±0.94 ^{ab}	0.92±0.07 ^b	1.21±0.10 ^a	31.49±0.60 ^b	77.26±1.44 ^a
Fortified biscuits with chickpea							
10%	40.17±0.82 ^c	93.24±0.53 ^c	4.95±0.11 ^{bc}	0.98±0.08 ^{bd}	1.47±0.05 ^b	38.25±0.33 ^c	89.18±0.21 ^a
15%	53.87±0.03 ^d	119.16±0.17 ^d	5.02±0.11 ^c	1.02±0.08 ^d	1.59±0.03 ^c	47.10±0.14 ^d	93.06±0.48 ^a
20%	66.24±0.32 ^e	128.44±0.57 ^e	5.17±0.07 ^c	1.35±0.05 ^e	1.70±0.02 ^d	58.00±0.10 ^e	106.21±0.8 ^a
School children biscuits	31.23±0.04 ^a	70.26±0.77 ^a	3.82±0.03 ^a	0.47±0.02 ^a	4.01±0.04 ^e	25.06±0.06 ^a	69.12±0.09 ^a

Results are expressed as mean±SD

Values sharing the same superscript capital letters in column indicate non significant differences between wheat flour, and chickpea.

Values sharing the same superscript small letters in column indicate non significant differences between various kinds of biscuits.

When chickpea was used in biscuits fortification at levels of 10, 15 and 20%, gradual increase in most minerals was observed, compared to unfortified biscuits and school children biscuits. Among all studied minerals, only iron content of school children biscuits was significantly higher than that of fortified biscuits with chickpea.

Sensory evaluation of unfortified biscuits (control) and fortified biscuits with chickpea (table 3) revealed that no statistically significant differences were observed between the unfortified biscuits and the other samples fortified with chickpea (10, 15 and 20%) for all the studied sensory characteristics. Fortified biscuits with 10 and 20% showed significant differences in texture and general acceptability.

No significant differences in feed intake(FI) of healthy groups fed on basal diet containing 2.5 or 5% protein from unfortified biscuits, fortified biscuits with chickpea and school children biscuits were noticed (table 4). On the other hand, diabetic groups fed on diet containing 2.5 or 5% protein from fortified biscuits with chickpea

showed significant elevation in FI compared to diabetic groups fortified with 2.5 or 5% protein from control biscuits.

Table (3): Sensory evaluation of fortified biscuits with chickpea.

Sample Characteristics	Control	Chickpea		
		10%	15%	20%
Color	4.56±0.92 ^a	4.48±1.09 ^a	4.60±0.76 ^a	4.48±0.92 ^a
Odor	4.44±0.77 ^a	4.40±0.95 ^a	4.48±0.87 ^a	4.32±1.07 ^a
Taste	4.40±0.58 ^a	4.48±0.77 ^a	4.64±0.76 ^a	4.36±0.70 ^a
Texture	4.64±0.91 ^{ab}	4.76±0.59 ^b	4.68±0.90 ^{ab}	4.24±0.78 ^a
General acceptability	7.64±1.38 ^{ab}	8.04±1.71 ^b	7.88±1.54 ^{ab}	7.00±2.16 ^a

Results are expressed as mean±SD

Values sharing the same superscript letters in the same raw are not statistically significant.

No significant differences were observed in feed intake levels among all normal groups fed on basal diets containing unfortified biscuits, fortified biscuits with chickpea and school children biscuits and diabetic groups fed on basal diet containing 5% protein from fortified biscuits with chickpea.

Allam, (2001) revealed that the values of food intake for rats fed on flat bread prepared from raw chickpea or soaked chickpea blended with wheat flour extraction 72% were insignificantly altered than those of normal control. Furthermore, Johnson *et al.* ,(2005) stated that adding chickpea flour or extruded chickpea flour to white bread had no effects on satiety or food intake.

Results showed that, BWG% of diabetic groups fed on basal diet containing 2.5 or 5% protein from unfortified biscuits and school children biscuits were significantly decreased in BWG% as compared to healthy groups fed on the same diets. Diabetic group fed on basal diet containing 5% protein from fortified biscuits with 20% chickpea showed non significant difference compared to the healthy group fed on the same diet.

Moreover, BWG% of all healthy groups of rats fed on basal diet containing 2.5 or 5% protein from unfortified biscuits and school children biscuits did not significantly differ, except for rats group fed on basal diet containing 2.5 or 5% protein from biscuits fortified with 20% chickpea, which showed significant increase. These results are in the same vain with those reported by Louz, (1997) who found a

significant decrease in daily gain in body weight in case of alloxan-induced diabetic rats fed on basal diet. Furthermore, Allam, (2001) revealed that feeding diabetic rats on flat bread supplemented with chickpea meal resulted in increase in rats weight. In addition, feeding rats on soaked chickpea or a blend of soaked chickpea with gunate and wheat flour (20:2:78%) showed almost the same weight (99.29%) or increased to about (114.15%) compared with control.

Feed efficiency ratio of diabetic groups fed on basal diet containing 2.5 or 5% protein from fortified biscuits with 20% chickpea was significantly high compared to diabetic groups fed on basal diet containing 2.5 or 5% protein from unfortified biscuits and school children biscuits. Similar trend was noticed concerning non diabetic groups.

In agreement with the present study, Bell and Hye, (1992) found a decrease in body weight, food intake, food efficiency ratio and digestive efficiency in the injected rats with alloxan compared to non diabetic rats. In addition, Morita *et al.*, (1997) reported that, there was an increase in growth, food intake and body weight gain of rats fed on chickpea than in rats fed on casein diet.

Diabetic groups fed on basal diets containing either 2.5 or 5% protein from unfortified biscuits, revealed a significant increase in the mean values of most organs relative weight compared with non diabetic groups fed on the same diets (table 4). Consistence with the present results Abdel-Megeid *et al.*, (2001) found that, kidney, heart, and spleen relative weights of diabetic group were greater than those of non diabetic groups. Also Allam, (2001) observed that liver, kidney weight/body weight% of control diabetic rats were greater than those of non diabetic groups. Teixeira *et al.*, (2003) reported that the kidneys of patient with diabetes were larger than those of control subjects.

Feeding diabetic rats with 2.5 or 5% protein from fortified biscuits with 20% chickpea resulted in significant decrease in liver and a kidney relative weight compared to other diabetic groups and resultd in non significant differences with non diabetic groups. In the light of this, Allam, (2001) revealed that feeding diabetic rats on supplemented bread with chickpea reduced adverse of hyperglycemia on liver relative weight. On the other hand, feeding diabetic rats with 2.5 or 5% protein from school biscuits did not improve organs relative

weight in relation to diabetic groups fed on 2.5 or 5% protein from unfortified biscuits.

Table (4): Effect of unfortified biscuits, fortified biscuits with chickpea (CP) and school children biscuits (SCB) on some biological parameters of normal and diabetic rats.

Parameters Groups	Feed intake (gm/day)	BWG (%)	FEI
2.5% protein from unfortified biscuit (-)	13.87±3.25 ^c	32.87±13.18 ^b	3.61±0.
2.5% protein from unfortified biscuit (+)	12.16±2.24 ^a	2.17±1.64 ^a	0.27±0
5% protein from unfortified biscuits (-)	12.82±1.30 ^c	27.69±7.46 ^b	3.40±0
5% protein from unfortified biscuits (+)	12.04±1.19 ^a	1.35±0.64 ^a	0.18±0
2.5% protein from fortified biscuits with 20% CP (-)	13.84±3.20 ^c	51.71±13.76 ^c	6.29±0
2.5% protein from fortified biscuits with 20% CP (+)	12.27±2.60 ^b	36.39±11.01 ^b	4.03±0.
5% protein from fortified biscuits with 20% CP (-)	14.01±2.04 ^c	51.76±10.22 ^c	6.13±0
5% protein from fortified biscuits with 20% CP (+)	13.15±2.70 ^c	39.09±8.62 ^{ac}	4.42±0
2.5% protein from SCB (-)	12.84±3.10 ^c	32.63±3.04 ^b	4.02±0.
2.5% protein from SCB (+)	12.92±1.90 ^{ab}	2.48±1.45 ^a	0.30±0
5% protein from SCB (-)	13.56±2.60 ^c	30.11±7.46 ^b	3.43±0
5% protein from SCB (+)	13.58±2.10 ^c	1.24±2.46 ^a	0.15±0

Results are expressed as mean±SD BWG: Body Weight Gain FER: Feed Efficiency
Values sharing the same superscript letters in the same column are not statistically significant

From table (5) it could be recognized that final serum glucose levels of diabetic rats fed on basal diet containing 2.5 or 5% from fortified biscuits with 20% chickpea were significantly lower than their initial levels. It is important to notice that serum glucose level in diabetic group fed on basal diet containing 5% protein from fortified biscuits with 20% chickpea was comparable to initial and final serum glucose levels of negative control group. Unfortunately, no significant differences were found between the initial and final serum glucose levels of normal or diabetic rats fed on basal diet containing 2.5 or 5% protein from school children biscuits.

Allam, (2001) found that rats fed on diet containing chickpea meal or supplemented bread with chickpea resulted in decreasing serum glucose for diabetic rats compared with control. Jenkins *et al.*, (2002) stated that significantly and substantially lower plasma glucose

and insulin concentrations after the single chickpea meals may reflect the higher amylase content and the botanical structure of chickpeas, it is likely that the starch was digested and absorbed more slowly in the small intestine from chickpeas than from wheat. Johnson *et al.*, (2005) concluded that adding chickpea flour to white bread demonstrated some hypoglycemic effect. In addition, Yang *et al.*, (2007) showed that chickpeas significantly improved insulin resistance, and prevented postprandial hyperglycemia and hyperinsulinaemia induced by the chronic high fat diet.

Table (5): Effect of unfortified biscuits, fortified biscuits with chickpea (CP) and school children biscuits (SCB) on relative organs weight of normal and diabetic rats.

Parameters Groups	Liver	Kidney	Spleen	Heart
2.5% protein from unfortified biscuits (-)	3.96±0.20 ^a	0.62±0.03 ^a	0.24±0.03 ^{ab}	0.28±0.04 ^a
2.5% protein from unfortified biscuits (+)	5.02±0.35 ^b	0.99±0.02 ^b	0.38±0.04 ^a	0.35±0.02 ^c
5% protein from unfortified biscuits (-)	3.77±0.10 ^a	0.72±0.12 ^a	0.21±0.02 ^a	0.27±0.01 ^a
5% protein from unfortified biscuits (+)	5.14±0.09 ^b	0.98±0.04 ^b	0.37±0.03 ^a	0.36±0.02 ^c
2.5% protein from fortified biscuits with 20% CP (-)	3.71±0.17 ^a	0.64±0.04 ^a	0.25±0.02 ^{bc}	0.31±0.01 ^a
2.5% protein from fortified biscuits with 20% CP (+)	3.62±0.18 ^a	0.70±0.06 ^a	0.25±0.03 ^{bc}	0.32±0.02 ^b
5% protein from fortified biscuits with 20% CP (-)	3.80±0.32 ^a	0.63±0.03 ^a	0.31±0.02 ^d	0.28±0.05 ^a
5% protein from fortified biscuits with 20% CP (+)	4.02±0.09 ^a	0.62±0.02 ^a	0.28±0.02 ^{cd}	0.31±0.03 ^a
2.5% protein from SCB(-)	3.80±0.18 ^a	0.69±0.17 ^a	0.26±0.02 ^{bc}	0.30±0.03 ^a
2.5% protein from SCB(+)	4.02±0.26 ^b	1.09±0.11 ^{bc}	0.36±0.05 ^a	0.36±0.02 ^c
5% protein from SCB (-)	3.75±0.12 ^a	0.65±0.04 ^a	0.24±0.01 ^{bc}	0.30±0.02 ^a
5% protein from SCB (+)	5.02±0.28 ^b	1.17±0.11 ^c	0.38±0.06 ^a	0.37±0.07 ^c

Results are expressed as mean±SD

Values sharing the same superscript letters in the same column are not statistically significant.

Table (6) showed that diabetic group fed on basal diet containing 2.5% and 5% protein from unfortified biscuits, revealed a significant increase in the levels of AST and ALT enzymes compared with those of the non diabetic group fed on the same diets (table 7). In the light of this, Anthony *et al.*, (2004) found that markers of liver injury, including AST and ALT were significantly associated with risk of incidence of type 2 diabetes. Vidro *et al.*, (1999) revealed that liver dysfunction associated with diabetes can be attributed to elevated rate

of lipid peroxidase and decreased levels or activates of endogenous antioxidant enzymes in liver. Similar results were reported by Allam, (2001) who found that AST and ALT activity was significantly increased in hyperglycemic control than negative control group.

Table (6): Effect of unfortified biscuits, fortified biscuits with chickpea (CP) and school children biscuits (SCB) on serum glucose levels of normal and diabetic rats.

Groups	parameters	Initial level (mg/dl)	Final level (mg/dl)
2.5% protein from unfortified biscuits(-)		88.88±3.57 ^a	90.57±2.74 ^a
2.5% protein from unfortified biscuits(+)		230.42±39.11 ^b	250.64±2.94 ^b
5% protein from unfortified biscuits (-)		90.68±3.36 ^a	91.53±2.66 ^a
5% protein from unfortified biscuits (+)		222.21±21.85 ^b	246.41±5.37 ^b
2.5% protein from fortified biscuits with 20% CP (-)		89.18±2.39 ^a	89.58±3.26 ^a
2.5% protein from fortified biscuits with 20% CP (+)		230.33±12.54 ^b	106.97±13.22 ^d
5% protein from fortified biscuits with 20% CP (-)		88.63±1.09 ^a	88.98±2.01 ^a
5% protein from fortified biscuits with 20% CP (+)		244.19±13.72 ^b	87.36±5.83 ^a
2.5% protein from SCB (-)		89.94±1.57 ^a	91.78±4.15 ^a
2.5% protein from SCB (+)		221.12±13.25 ^b	231.53±16.20 ^b
5% protein from SCB (-)		88.29±4.96 ^a	92.95±1.66 ^a
5% protein from SCB (+)		230.02±16.01 ^b	230.37±22.08 ^b

Results are expressed as mean±SD

Values sharing the same superscript letters in the same column are not statistically significant

There were non significant differences in serum AST, ALT and total protein between all healthy groups fed on basal diets containing unfortified biscuits, fortified biscuits with 20% chickpea and school children biscuits. Diabetic groups fed on basal diet containing 2.5 or 5% protein from fortified biscuits with 20% chickpea had significantly lower means of AST, ALT and total protein compared to diabetic group that fed unfortified biscuits. On the other hand, feeding diabetic rats either 2.5 or 5% protein from school children biscuits did not improve AST, ALT and total protein compared to positive control group.

Table (7): Effect of unfortified biscuits, fortified biscuits with chickpea (CP) and school children biscuits (SCB) on liver functions of normal and diabetic rats.

Groups	Parameters	AST(U/L)	ALT (U/L)	Total protein (Mg/dl)
	2.5% protein from unfortified biscuits (-).		30.55±5.96 ^a	19.74±2.95 ^a
2.5% protein from unfortified biscuits (+).		47.1±10.73 ^b	36.01±0.08 ^c	5.22±0.08 ^{ab}
5% protein from unfortified biscuits (-).		29.37±7.97 ^a	23.44±2.26 ^{ab}	6.17±0.73 ^c
5% protein from unfortified biscuits (+).		45.04±3.84 ^b	37.27±2.56 ^c	5.23±0.10 ^a
2.5% protein from fortified biscuits with 20% CP (-).		29.59±4.58 ^a	23.31±1.08 ^{ab}	5.79±0.26 ^{bc}
2.5% protein from fortified biscuits with 20% CP (+)		32.40±6.42 ^a	25.93±6.94 ^b	6.15±0.17 ^c
5% protein from fortified biscuits with 20% CP (-).		27.97±4.47 ^a	22.40±1.27 ^{ab}	5.89±0.02 ^c
5% protein from fortified biscuits with 20% CP (+).		27.35±4.19 ^a	26.31±2.17 ^b	6.00±0.02 ^c
2.5% protein from SCB (-).		27.01±5.24 ^a	21.27±2.11 ^a	5.92±0.06 ^c
2.5% protein from SCB (+).		47.19±7.37 ^b	35.37±1.32 ^c	5.20±0.01 ^a
5% protein from SCB (-).		29.38±3.54 ^a	21.25±3.21 ^a	5.89±0.04 ^c
5% protein from SCB (+).		43.58±4.88 ^b	35.81±0.49 ^c	5.30±0.02 ^{ab}

Results are expressed as mean±SD

AST: aspartate amino transferase.

ALT: alanine amino transferase

Values sharing the same superscript letters in the same column are not statistically significant.

Results in table (8) represented the effect of unfortified biscuits, fortified biscuits with chickpea and school children biscuits on kidney function of normal and diabetic rats. Diabetic group which was fed on basal diets containing 2.5 or 5% protein from unfortified biscuits, revealed a significant increase in the mean value of uric acid, creatinin and urea nitrogen when compared with non diabetic group fed on the same diets. All normal groups fed on basal diets containing unfortified biscuits, school children biscuits showed non-significant differences in uric acid and creatinin.

It could be noticed that there were a significant increase in the mean value of uric acid, creatinin and urea nitrogen in diabetic groups fed on basal diets containing 2.5 or 5% protein from school children biscuits, compared with diabetic group fed on control biscuits.

Devlin, (1986) reported that the increase in blood glucose level in diabetic patient stimulates the activity of aldolase reductase in

kidneys and then sorbitol was formed from glucose. It was known that in diabetes the sorbitol content of glomerulus was elevated, which result in damage for these tissues. Moreover, the degradation of muscular proteins was increased in diabetes mellitus and therefore the plasma creatinine . Administration of insulin or tolbutamide does not return the renal lesion in diabetic patients to the normal state. In addition, Gabr,(1998) and Abdel-Megeid *et al*, .(2001)stated that, alloxan injection caused a highly significant increase in serum uric acid, blood urea and creatinine relative to the normal control. Moreover, Rockel, (2001) reported that inadequate adjustment of blood sugar and blood pressure levels in diabetic patients tended to cause abnormal protein metabolism, an impairment of kidney functions and eventually diabetic glomerulosclerosis. This is caused by hyperfusion and hyperfiltration of glomruli which could consequently lead to microalbuminuria and acute renal failure.

Table (8): Effect of unfortified biscuits, fortified biscuits with chickpea (CP) and school children biscuits (SCB) on kidney functions of normal and diabetic rats

Parameters Groups	Uric acid (mg/dl)	Createnin (mg/dl)	Urea nitrogen (mg/dl)
	Mean±SD	Mean±SD	Mean±SD
2.5% protein from unfortified biscuits (-).	1.68±0.10 ^a	0.51±0.06 ^a	56.70±1.73 ^a
2.5% protein from unfortified biscuits (+).	2.46±0.23 ^b	1.64±0.05 ^c	67.54±2.28 ^b
5% protein from unfortified biscuits (-).	1.61±0.02 ^a	0.54±0.03 ^a	57.56±1.06 ^a
5% protein from unfortified biscuits (+).	2.42±0.11 ^b	1.65±0.04 ^c	65.61±4.04 ^b
2.5% protein from fortified biscuits with 20% CP (-).	1.63±0.04 ^a	0.59±0.02 ^a	44.52±1.92 ^d
B.D. + 2.5% protein from fortified biscuits with 20% CP (+).	1.63±0.15 ^a	0.91±0.05 ^b	56.24±3.38 ^a
5% protein from fortified biscuits with 20% CP (-).	1.55±0.04 ^a	0.57±0.06 ^a	41.25±0.61 ^d
5% protein from fortified biscuits with 20% CP (+).	1.59±0.20 ^a	0.83±0.02 ^b	55.84±4.84 ^a
2.5% protein from SCB (-).	1.63±0.03 ^a	0.53±0.02 ^a	58.39±2.34 ^a
2.5% protein from SCB (+).	2.52±0.26 ^b	1.68±0.04 ^c	68.84±3.40 ^b
5% protein from SCB (-).	1.63±0.05 ^a	0.53±0.02 ^a	58.68±1.46 ^a
5% protein from SCB (+).	2.57±0.24 ^b	1.71±0.02 ^c	66.47±3.13 ^b

Results are expressed as mean±SD

Values sharing the same superscript letters in the same column are not statistically significant

Treating diabetic groups with basal diet containing 2.5% or 5% protein from fortified biscuits with 20% chickpea resulted in significant decrease in uric acid, creatinin and urea nitrogen values compared to diabetic groups treated with B.D. containing the same amount of protein from unfortified biscuits or school children biscuits. These results are supported by Allam, (2001) who found that the higher levels of urea, uric acid and creatinine in hyperglycemic rats was decreased by feeding on flat bread supplemented with chickpea in comparison to diabetic control.

Table (9): Effect of unfortified biscuits, fortified biscuits with chickpea (CP) and school children biscuits (SCB) on iron status of normal and diabetic rats.

Groups	Parameters	TI ($\mu\text{g/dl}$)	TIBC ($\mu\text{g/dl}$)
2.5% protein from unfortified biscuits (-).		114.60 \pm 6.693 ^c	375.3 \pm 8.715 ^e
2.5% protein from unfortified biscuits (+).		96.00 \pm 3.807 ^b	308.2 \pm 5.805 ^b
5% protein from unfortified biscuits (-).		111.60 \pm 4.774 ^c	345.6 \pm 3.493 ^c
5% protein from unfortified biscuits (+).		81.420 \pm 2.175 ^a	295.4 \pm 5.549 ^a
2.5% protein from fortified biscuits with 20% CP (-).		149.40 \pm 8.142 ^f	373.34 \pm 6.586 ^e
2.5% protein from fortified biscuits with 20% CP (+).		132.80 \pm 3.033 ^d	356.47 \pm 6.59 ^d
5% protein from fortified biscuits with 20% CP (-).		146.80 \pm 3.834 ^f	375.6 \pm 7.369 ^e
5% protein from fortified biscuits with 20% CP (+).		140.20 \pm 2.863 ^e	384.5 \pm 4.387 ^f
2.5% protein from SCB (-).		160.60 \pm 6.426 ^g	403.8 \pm 11.05 ^g
2.5% protein from SCB (+).		148.20 \pm 2.774 ^f	386.4 \pm 7.232 ^f
5% protein from SCB (-).		171.00 \pm 6.855 ^h	419.2 \pm 4.549 ^h
5% protein from SCB (+).		146.40 \pm 5.177 ^f	396.0 \pm 8.944 ^g

Results are expressed as mean \pm SD TI: total iron TIBC: total iron binding capacity
Values sharing the same superscript letters in the same column are not statistically significant

Control negative groups which were fed on basal diets containing 2.5 or 5% protein from unfortified biscuits, revealed a significant increase in the mean values of total iron compared with those of the positive control groups fed on the same diets.

Significant increase in the mean value of total iron in normal groups fed on basal diets containing 2.5 or 5% protein from chickpea

when compared with normal group fed on the same percentage from unfortified biscuits. The same trend was observed regarding diabetic group fed on unfortified biscuits and chickpea biscuits (Table 9). Although school children biscuit was supplemented with iron, no significant differences were observed between diabetic groups fed on basal diet containing 2.5 or 5% protein from chickpea and school children biscuits.

Results of total iron binding capacity (TIBC) indicated that feeding normal group fed on basal diet containing 2.5 or 5% protein from unfortified biscuits revealed a significant increase when compared with diabetic group fed on the same diet. There is a significant increase in the mean value of TIBC in healthy groups fed on school children biscuits provided the diet with 2.5 or 5% protein when compared with the diabetic groups fed on the same content of diet. Feeding diabetic rats on basal diet containing 2.5 and 5% protein from fortified biscuits with 20% chickpea resulted in significant increase in TIBC when compared with those of the diabetic group fed on unfortified biscuits.

From the obtained results, both normal and diabetic children can nutritionally benefit from fortified school biscuits with chickpea because it is suitable for them and improves their nutritional status especially iron status. It is recommended to use chickpea in diabetic foods and diets through planning and implementing nutrition education programs for diabetic patients in order to explain the important role of chickpea in controlling diabetes especially type II. Combination of wheat flour with another protein sources such as chickpea can offers economical improvement in protein quality of wheat flour biscuits and increase its protein content. Finally, further studies are needed to investigate the effect of chickpea fortification on other biological and biochemical parameters.

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دور الحمص كمادة مدعمة للبسكوت في التحكم في مستوى جلوكوز الدم للفنران المصابة بمرض البول السكرى

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تهدف هذه الدراسة إلى اختبار البسكوت المدعم بالحمص، أو بسكوت المدارس على بعض المعاملات البيولوجية و الكيميائية مثل الطعام المتناول، نسبة الزيادة في وزن الجسم ، جلوكوز الدم، وكذلك وظائف الكلى و الكبد و حالة الحديد في فنران التجارب الطبيعية و المصابة بارتفاع في مستوى السكر في الدم. و قد تم تدعيم البسكوت بنسبة 10%، 15% و 20% حمص وبعد إجراء عملية الخبز تم تقييم هذه الأنواع من المخبوزات تقييماً حسيًا لإختيار أفضل التركيزات ليتم استخدامها في التجربة البيولوجية. اخذ 60 فأر البينو متوسط اوزانها 110 ± 5.0 جم قسمت الى مجموعتين رئيسيتين (كلا 30 فأر) المجموعة الأولى تحتوي على ستة مجموعات فرعية وتم تغذيتها على غذاء أساسي مضاف إليه (2.5% أ و 5% بروتين) إما من البسكوت غير المدعم ، البسكوت المدعم بالحمص 20% ، أو من بسكوت المدارس . المجموعة الرئيسية الثانية فقد تم حقنها بمادة الألوكسان لإحداث ارتفاع في مستوى السكر بالدم وتم تغذيتها على نفس مجاميع الغذاء السنه السابقة. استمرت التجربة لمدة خمسة أسابيع بعدها تم الحصول على عينات الدم لتحليلها و تم وزن بعض اعضاء جسم الفنران . و قد أظهرت النتائج أن المجاميع التي تغذت على غذاء أساسي يحتوي على البسكوت المدعم بالحمص (2.5% و 5% بروتين) أدت إلى زيادة في الوزن الجسم كذلك تحسن في مستوى السكر ووظائف الكبد و الكلى و حالة الحديد بالدم. من النتائج توصى الدراسة بإمكانية استفادة كلا من الاطفال الاصحاء او المرضى بمرض السكر من تدعيم بسكوت المدارس بالحمص غذائياً و صحياً. كما يوصى البحث بضرورة ادخال الحمص في اطعمة مرضى السكر و اظهار اهمية تناول الحمص للتحكم في مرض السكر من خلال عمل برامج التوعية الغذائية المختلفة.