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Abstract:

Lentil is a functional food, especially due to its high content in proteins and fibers. Obesity is a risk factor for developing several diseases. The present investigation aimed to study the effect of bread fortified with lentil on obesity and some biochemical parameters of rats. Male albino rats (n=40) were divided into two main groups, Group 1, (8 rats) was kept as a negative control group (-ve) and fed on basal diet, while the second group (32 rats) fed on high fat diet (HFD) for 40 days to induce obesity,then divided into 4 sub-groups, each group contained (8 rats), sub-group 1: Rats fed on HFD control group (+ve), sub-group 2: Rats fed on HFD and wheat bread, sub-groups 3 and 4: Rats fed on HFD and bread fortified with lentil (15 and 30%). Six weeks later, blood samples were collected from rats to estimate lipid profiles, liver enzymes, kidney function, antioxidant enzymes and the level of glucose in serum. Histological of liver tissues are also considered. The results of chemical analysis indicated that orange lentil flour contained a higher level of protein and antioxidant activity compared with wheat flour. The results also showed that bread fortified with lentil 30% had a higher level of protein and antioxidant activity compared with wheat bread. The biochemical parameters of obese rats were improved in HFD fed on bread fortified with lentil 30% and 15% compared to the other groups. The results of liver function were supported by histological studies of hepatocytes. The current study confirms that lentil bread plays a role in reducing obesity and related diseases .

Keywords: Fortified Bread, Lentil, Liver Enzyme, Lipid Profile, Obese Rats.

Introduction:

Obesity is defined as abnormal or too much fat, which can be bad for your health and a risk factor Obesity is a risk factor for developing several diseases **(Cerdó et al., 2019).**

According to **Gregg and Shaw (2017)**, study about 19 million Egyptian adults are overweight. This present about 35% of the total adult population.

Obesity is a long-term disease that increases the chances of getting metabolic syndrome, which includes high blood pressure, hyper triglyceride, lower HDL-cholesterol and high cholesterol, which can lead to type 2 diabetes, heart disease, kidney damage, and fatty liver disease **(Hall et al., 2014).**

Non-oil seeds of legumes such as lentils, beans and dry peas are well-suited to help people lose weight **(Kim et al., 2016).**

Bread is a staple food that most people eat every day. Bread is of particular interest for today's food industry due to its indispensable role as food across the world **(Zhou et al., 2014).** Bread is a popular cereal product and contains many nutrients such as vitamins and minerals, especially phosphorus and copper **(Salehi, 2019).** However, from a nutritional point of view, bread has a low content of essential amino acids, such as. lysine, tryptophan and threonine **(Shen et al., 2019).**

The incorporation of dietary fiber into breads, has received much attention due to their potential use in formulation of functional breads such as gluten-free or fiber-fortified breads **(Struck et al., 2018).** Legume flours are important for making bread and other baked goods healthier because they are high in protein and fiber **(Hefnawy et al., 2012).**

Man and Păucean (2013), prepared different mixtures of bread and lentils (12%, 25% and 40% red lentil flour) to establish the optimal recipes of bakery products with addition of red lentil flour in order to make bread with good nutrients quality characteristics.

Lentils" *Lens culinaris* " are in the Lens genus, which is in the Leguminoseae family. **(Zia-Ul-Haq et al., 2011)**, recommended to eat lentils because they are high in fibre and protein. The lentils flour can be used to many food products such as soups, bread and cakes **(Zia-Ul-Haq et al., 2011)**.

Lentils are a good source of low digestible carbohydrates (prebiotic carbohydrates), which help bacteria to do their jobs in the hind gut. Prebiotic carbohydrates are selectively fermented by beneficial gut microbiome that allow specific biochemical changes in gastrointestinal environment to increase host well-being and health **(Kadyan et al., 2022)**.

prebiotic carbohydrates leads to less obesity and becoming popular health-promoting foods for people to eat **(Migliozzi et al., 2015)**

Compared to foods made from cereal, lentils have a lot more prebiotics Lentil, which called "poor man's meat" and could meet the person daily prebiotic needs **(Thavarajah et al., 2016)**

At the moment, we don't know enough about how probiotics and prebiotics affect obesity in the long run. The aim of this study is to find out how the prebiotic carbohydrates and protein in bread fortified with lentil affects the rats obesity.

Materials and Methods:

Wheat flour and lentil:

Wheat flour (72% extraction) and orange lentil which were used in this investigation was purchased from local market in Alexandria, Egypt. All ingredients used in the bread formulation (sugar, yeast, salt, milk, and corn oil) were commercially available and obtained from the local market in Alexandria, Egypt.

Chemicals and Kits:

- All chemicals which used in analytical to methods were purified grade provided from Merck (Darmstadt, Germany), Sigma-Aldrich Sigma Chemical Co. (St. Louis, MO, USA).
- Commercial kits were obtained from Biosystems S.A. (Spain), Diamond (Germany) and Randox (United Kingdom).

Animals:

Forty male albino rats of Westar strain were used in the present experiment, weighing 120-150gm were obtained from Institute of Graduate Studies and Research, Alexandria University. Approved by the Scientific Research Ethics Committee for experimental animals with a serial code (07).

Methods:

Preparation of lentil flour:

Lentil seeds were washed and soaked for 12 hours then they were dried in an air-oven at 50 °C for ٣٠ min. Lentil seeds were ground in an attrition mill and sieved through a 1.0 mm mesh sieve to make fine flour with particles of the same size. Wheat flour was mixed with 0, 15, and 30% of lentil seeds flour for preparing bread.

Preparation of bread fortified with lentil:

Formulation of fortified bread:

The ingredients used to prepare the two blend formulations of bread fortified with lentil are shown in **table (1)** According to **Juarez-Garcia et al. (2006)**.

Table (1): Ingredients used in preparation of bread fortified with lentil blends

Ingredients	Blend 1	Blend 2	Blend 3
	(gm)		
Wheat flour	100	85	70
Lentil 15%	-	15	-
Lentil 30%	-	-	30
Sugar	2	2	2
Salt	0.5	0.5	0.5
Dry yeast	2.5	2.5	2.5

Bread-Making Procedure:

Wheat and lentil flour or its blends were mixed with salt, yeast and the adequate amount of water. The ingredients were mixed for 7 minutes, then put aside for 10 minutes, split into 100 gm pieces, kneaded, and put aside again (15 min). The dough was rolled out by hand and left to rise until it reached its maximum volume increase at 28°C and 85% relative humidity. After fermentation, dough was baked in an electric oven at 200 °C for 30 min, then cooled at room temperature for 60 min and kept until used **(AACC, 2000).**

Proximate analysis:

Wheat, lentil flours and bread fortified with two ratios of lentil were analyzed for protein, fat, ash, dietary fiber and moisture content were determined using the methods mentioned in Association of Official Analytical Chemists **(AOAC,2000),** while total carbohydrates were calculated by difference according to **(AACC, 2016)**. The caloric value was calculated according to the following equation: Caloric value = 4 (protein%+ carbohydrates %) + 9 (fat %).

Determination of antiradical activity (ARA):

The free radical scavenging capacity of flours and bread extracts were determined using the staple 2,2-diphenyl-1picrylhydrazyl radical (DPPH) according to method by **Wronkowska et al. (2010).** The inhibition percentage of the DPPH free radical is calculated as follows:

$$\textbf{DPPH: scavenging activity (\%)} = [(A0- A1)/A0] \times 100$$

Basal and experimental diets of Rats:

The basal diet was prepared according to **Reeves (1993)**, which consists of sucrose (20%), protein (4.7%), corn oil (10%), choline chloride (2%), vitamin mixture (3.5%) , salt mixture (1%), and fibers (5%). The high-fat diet has been prepared according to **Yao et al. (2011)**, which consists of animal fat (20%) + cholesterol (1%) added to the basal diet.

Design of the experiment:

Rats were divided into 2 groups, Group 1, (8 rats) was kept as a negative control group (-ve) and fed on basal diet, while the second group (32 rats) fed on high-fat diet for 40 days to induce obesity. The second group divided into 4 sub-groups, each group contained (8 rats) as following:

Sub-group 1: Rats fed on high fat diet as a positive control group (+ve)

Sub-group 2: Rats fed on high fat diet and wheat bread 100%

Sub-group 3: Rats fed on high fat diet and bread fortified with (15%) of lentil

Sub-group 4: Rats fed on high fat diet and bread fortified with (30%) of lentil

Blood sampling:

After 6 weeks of feeding, rats were fasted for 12 h and then blood sample were collected after anesthesia by diethyl ether and serum separated. Lipid profiles, liver enzymes, kidney function, antioxidant enzymes and the level of glucose were determined in serum.

Determination of lipid profile:

Triglyceride was determined as described by **Fossati and Prencipe (1982)**. Total cholesterol was determined according to **Richmond (1973)**. High density lipoprotein (HDL-c) and Low density lipoprotein (LDL-c) were determined according to the method of **Jaye et al. (2009)**.

Determination of antioxidant enzymes

Activity of Glutathione peroxidase was determined according to the method described by **Wendel (1980)**. Catalase activity was determined according to the method described by **Hadwan and kadhum Ali (2018)** and Superoxide dismutase activity was determined according to the method described by **Rigo et al. (1975)**.

Determination of liver enzymes:

The liver enzymes include Alanine transaminase (ALT), Aspartate transaminase activity (AST) and Alkaline phosphates (ALP) were measured according to method described by **Williamson (1985), Yagi et al. (1985) and Lavie et al. (2018)** respectively.

Determination of kidney functions:

The kidney functions include Urea and Creatinine were determined according to method of **Andreas and James (2003)**, **Shlipak et al. (2013) .** Albumin was measured according to method described by **Bermeyer and Bernt (1974)**.

Determination of glucose:

Determination of glucose was carried out according to the method of **Lott and Turner (1975)**.

Histology of liver tissues:

Samples of liver tissues were taken from all experimental groups, then fixed in 10% formalin in phosphate buffered for 10 hours and washed in 70% alcohol. After the specimens were fixed, they were dehydrated in graded ethanol, embedded in wax, cut into sections 5 microns thick, stained with Hematoxylin and Eosin, and examined under a microscope according to **Bancroft and Gamble (2008)**.

Statistical analysis:

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Quantitative data was described using mean and standard deviation. F-test (ANOVA) used for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (LSD) for pairwise comparisons **(Kirkpatrick, 2015)**.

Results and Discussion:

Chemical constituents of raw wheat and lentil flour:

The chemical constituents of wheat and lentil flour are shown in **Table (2)**. The mean values of protein, fat, crude fiber, ash, carbohydrates and energy were 9.44, 1.29, 0.77, 0.78, 87.72% and 400.28 kcal/100gm of wheat flour, respectively. While, the mean values of protein, fat, crude fiber, ash, carbohydrates and energy were 27.03, 1.47, 9.96, 1.74, 58.83% and 356.67 kcal/100gm of lentil flour, respectively.

The data showed that lentil flour has more levels of protein, fat, crude fiber and ash compared with wheat flour. While wheat flour has high levels of carbohydrates and energy compared with lentil flour. Lentil flour has a lot of protein and crude fiber, which makes it a good food. The high values of protein and crude fiber make lentil flour as possible medicines to improve the immune system and control blood lipids **(Bouchenak and Lamri-Senhadji, 2013).**

These results were closed to those reported by **Elhadidy et al. (2020)** in which the proximate compositions of wheat flour (72% extraction) were 11.60, 0.50, 0.45, and 86.85% of protein, ash, fiber and carbohydrates, respectively

Also, the data in agreement with **Elhadidy et al. (2020)** who reported that wheat flour had 0.64, 0.63, 0.70 and 85.78% of crude fiber, ash, fat and total carbohydrates, respectively. Moreover, the current results in the line with that reported by **Abdel-Gawad et al. (2016)** in which the protein, crude fiber, ash content and starch of wheat flours were11.76, 0.59, 0.63 and 82.53%, respectively. However, **Miranda-Ramos and Haros (2020)** reported that wheat flour contained 11.54, 1.0, 0.58 and 83.3% of protein, fat, ash and carbohydrates, respectively.

The current results showed that Lentil had 27.03 and 1.47% of protein and fat. While the values reported by **de Almeida Costa et al. (2006)** were 20.60 and 2.15% of protein and fat, that might due to the variety of lentil.

The percent of protein and carbohydrates of our results were close to that reported by**Rico et al. (2021)**, in which lentil seeds showed a protein content of 25.16% and carbohydrates of 62.96%

The current result showed that lentil flour contained 58.83 and 9.96% of carbohydrate and fiber ,while **Rico et al. (2021)** reported that lentil seeds had a range (62–69%) and (5–20%) at carbohydrate and fiber.

Table (2): Chemical constituents of raw wheat and lentil flour

Treatment groups	Protein	Fat	Crude Fiber	Ash	Carbohydrates	Energy* Kcal
	(%)					
Wheat flour	9.44** ± 1.44	1.29 ± 0.60	0.77 ± 0.11	0.78 ± 0.25	87.72 ± 2.16	400.28±19.78
Lentil flour	27.03 ± 0.63	1.47 ± 0.38	9.96 ± 0.16	1.74 ± 0.69	58.83 ± 0.96	356.67±2.05
t	19.385*	0.423	80.641*	2.289	21.201*	3.798*
P	<0.001*	0.694	<0.001*	0.084	<0.001*	0.019*

****Data was expressed using Mean ± SD (g/100 g dry weight basis)**
t: Student t-test,
*: Statistically significant at $P \leq 0.05$
*kcal.100g-1 in dry weight.

Data in **table (3)** showed the chemical composition of wheat bread (control), bread fortified with 15 and 30% lentil flour. The mean values of protein, fat, fiber, ash, carbohydrates and Energy in control bread were 9.57, 1.29, 0.77, 0.78, 87.32 % and 399.20 kcal/100g, respectively. In the case of bread fortified with 15 % lentil, the mean values of protein, fat, fiber, ash, carbohydrates and Energy were 15.11, 0.99, 4.49, 2.00, 77.40 % and 378.97 kcal/100g, respectively. While the mean values of protein, fat, fiber, ash, carbohydrates and Energy in bread fortified with 30 % lentil were 18.86, 1.19, 7.36, 0.84, 71.75 % and 373.12 kcal/100g, respectively. The data showed significant differences in the percentage of protein and fiber between control bread and bread fortified with 15 % or 30% in which the percentage increased with increasing the percentage of lentil in bread. In contrast the data showed significant decrease in the percentage of carbohydrates and energy between control bread and bread fortified with 15 % or 30% in which the percentage reduced with increasing the percentage of lentil in bread.

The current data are close with that reported by **Bouhlal et al. (2019)** in which the supplementation of bread with lentil flour (30%) led to an increase of proteins (15.80%), fat (1.15%) and total energy content (347.67 kcal/100g) compared to 13.08%, 0.44% and 342.45 kcal/100gm of proteins, fat, and total energy, respectively of wheat flour. While, the carbohydrate content has decreased to (68.66%) and the substitution levels of lentil improving the diabetics. Also, **Portman et al. (2018)** mention that the addition of lentil flour resulted in a significant increase in protein.

Moreover, **Miranda-Ramos and Haros (2020)** reported that the wheat supplemented with red lentil bread 30% contained more proteins and double amount of fiber than wheat bread. However, the lentil-wheat bread contained a lower amount of carbohydrates than wheat bread. The lower amount of carbohydrates in wheat-lentil bread might be due to the fact that lentils contain less starch than wheat **(Carcea et al., 2019).** Moreover, **Faris et al. (2013)** reported that legume starch has a higher fraction of amylose than wheat (about 35%). . Really, lentils provide slowly absorption of carbohydrate which gives a flatter blood glucose profile even in non-insulin dependent diabetes mellitus **(Bouchenak & Lamri-Senhadji, 2013).**

Table (3): Chemical constituents of bread fortified with two ratios of lentil

Treatment groups	Protein	Fat	Fiber	Ash	Carbohydrates	Energy * kcal
	(%)					
Control bread	9.57**^{c}±1.46	1.29 ^{a}±0.60	0.77 ^{c}±0.11	0.78^{a}±0.25	87.32^{a}±0.17	399.20^{a}±11.27
Bread fortified with lentil 15 %	15.11^{b}±0.40	0.99^{a}±0.09	4.49^{b}±0.28	2.00 ^{a}±2.49	77.40^{b}±0.52	378.97^{b}±3.98
Bread fortified with 30% lentil	18.86^{a}±0.82	1.19^{a}±0.03	7.36^{a}±0.52	0.84 ^{a}±0.04	71.75^{c}±0.81	373.12^{b}±2.27
F	66.274*	0.564	269.947*	0.696	586.487*	11.393*
P	<0.001*	0.596	<0.001*	0.535	<0.001*	0.009*
LSD 5%	1.986	0.7008	0.6955	2.8891	1.1261	14.03

Data was expressed using Mean ± SD (n=3). (g/100 g dry weight basis)

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**

Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant)

*: Statistically significant at $P \leq 0.05$

*kcal.100g-1 in dry weight.

Antioxidant activity of wheat, lentil flour and bread fortified with two ratios of lentil:

Data in **table (4)** showed the antioxidant activity of wheat, lentil flour and bread fortified with two ratios of lentil (15 and 30%). The results showed significant differences in the antioxidant activity between the wheat flour in one side and the other treatments of lentil flour and bread fortified with two ratios of lentil in another side. The lowest antioxidant activity was observed in wheat flour 23.47% and the highest percentage 76.58% was recorded in lentil flour. The activity in the bread fortified with the with two ratios of lentil 15 and 30% were 34.30 and 44.27 %, respectively. **Bello Pérez (2013)**, reported that legumes considered an important source of polyphenols and have a high antioxidant capacity.

Based on the current results, bread made with wheat-lentil mixtures ratios 15% and 30% have a higher antioxidant activity compared with wheat flour only. Our results confirm those reported by **Turfani et al. (2017)** and are consistent with **Nikolic et al. (2014),** who showed that lentil flour is a good way to improve the anti-oxidative properties of wheat flour. Consequently, the increase of antioxidant activity can help to heal several pathologies which is the main cause of oxidative stress.

Also, our results agreed with those of **Bouhlal et al. (2019)**, who said that antioxidant activity was greatly improved when wheat-lentil fortified flours were used up to 20%.

Table (4): Antioxidant activity of wheat, lentil flour and bread fortified with two ratios of lentil

Treatment groups	Antioxidant activity %
Wheat flour	$23.47^{d}\pm0.25$
Lentil flour	$76.58^{a}\pm0.41$
Bread fortified with lentil 15 %	$34.20^{c}\pm8.52$
Bread fortified with 30% lentil	$44.27^{b}\pm0.4$
F	86.266^{*}
P	$<0.001^{*}$
LSD 5%	8.0524

Data was expressed using Mean ± SD.

Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant)

*: Statistically significant at $P \leq 0.05$

Effect of bread fortified with lentil on body weight gain of obese rats:

Data in Table (5) showed the effect of control and the experimental groups on body weight gain of obese rats. At the end of experiments, the data showed that the percent of body weight gain (BWG) of rat fed on HFD (control +) was 166.08 while, the percent of BWG of rat fed on basal diet (control -) was 107.75. The best treatment for reducing the percent of BWG was 81.93 of bread fortified with lentil (70+30%) followed with 101.51% of bread fortified with lentil (85+15%) compare with 112.10% of wheat bread. The current results revealed that consumption of bread fortified with lentil reduced BWG and the reduction depend on lentil ratio. These results may be due to the higher dietary fiber content present in lentil **(Jarpa-Parra, 2018)**.

In fact, relatively few researches were performed on the effects of lentil diet on body weight of rat **(Siva et al., 2018)**. However, strong evidence exists for a relationship between lentils consumption and weight loss. A systematic review of human trials revealed that lentils have a significant effect on weight loss. **(Kim et al., 2016)**. Moreover, **Hermsdorff et al. (2011)** mentioned that diets of mixed crops (lentils, chickpeas, peas, and beans) significantly reduce the body weight of obese adults**(Siva et al., 2018)**. Further **Mohamed et al. (2014)**, claimed that eating lentil as a dietary mainstay is a successful way to cure obesity.

Study on human showed that the intake of lentil seed reduces food intake, body weight and lead to maintaining body weight in obese subjects **(Mollard et al., 2012)**. Further, **Chung et al. (2008a)** mentioned that diets of mixed pulse crops (lentils, chickpeas, peas, and beans) significantly reduce the body weight of obese adults and serum lipids.

Table (5): Effect of bread fortified with lentil on body weight gain of obese rats

Treatment	Body weight (gm)		BWG%
	Initial	Final	
Control (-)	$152.65^{a}\pm4.27$	$317.17^{b}\pm28.12$	$107.75^{b}\pm16.75$
High fat diet (HFD) control (+)	$153.97^{a}\pm7.38$	$409.0^{a}\pm7.87$	$166.08^{a}\pm11.98$
(HFD and wheat bread 100%	$153.97^{a}\pm7.02$	$326.33^{b}\pm21.22$	$112.10^{b}\pm13.18$
(HFD and wheat-lentil bread (85+15%)	$153.62^{a}\pm4.73$	$309.50^{b}\pm16.32$	$101.51^{b}\pm9.48$
(HFD and wheat-lentil bread (70+30%)	$153.47^{a}\pm4.79$	$278.67^{c}\pm17.70$	$81.93^{c}\pm15.69$
F	0.052	37.676^{*}	31.639^{*}
P	0.995	$<0.001^{*}$	$<0.001^{*}$
LSD 5%	6.8759	23.073	16.251

Data was expressed using Mean ± SD
F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**
Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant)
*: Statistically significant at $P \leq 0.05$

Effect of bread fortified with lentil on Lipid profile of obese rats:

Lipid profile most frequently induces measurements of total lipids: Total cholesterol (TC), triacylglycerol (TG), high- and low-density lipoprotein-cholesterol (HDL-c and LDL-c). The results listed in **Table (6)** showed that high fat diet significantly (P<0.05) increased the concentrations of all serum lipid profile, while HDL-c decreased. On the other hand, bread fortified with lentil decreased all serum lipid profile, while increased HDL-c compared to the HFD. Moreover, the increase of lentil levels decreased the lipid profile or increased HDL-c. Our results matched with that reported by**Ahmad (2017),** who showed that the administration of lentils significantly increased HDL cholesterol.

Also, **Shams et al. (2008)**, found that addition of cooked lentils to rat diet led to a significant decrease (P<0.05) in TC. **Boualga et al. (2009)**, indicated that lentil proteins prevent access fat accumulation in adipose tissue.

Addition to lentil proteins, lentil prebiotic carbohydrates may have potential to reduce body fat **(Siva et al., 2018)**. Moreover, **Siva (2017)** revealed that lentil reduce risk factors of obesity including body weight, percent body fat, blood plasma TG concentration.

Table (6): Effect of bread fortified with lentil on Lipid profile of obese rats

Treatments groups	Parameters			
	Cholesterol	Triglycerides	HDL-c	LDL-c
	(mg/dl)			
Control (-)	$80.60^{b}\pm1.61$	$66.80^{bc}\pm1.54$	$34.33^{a}\pm1.15$	$32.90^{c}\pm1.01$
HFD control (+)	$165.23^{a}\pm1.15$	$116.0^{a}\pm1.00$	$16.0^{d}\pm1.00$	$124.47^{a}\pm0.76$
High fat diet and wheat bread 100%	$88.0^{b}\pm7.21$	$67.33^{b}\pm10.41$	$21.33^{c}\pm2.08$	$56.13^{b}\pm9.49$
High fat diet and wheat-lentil bread (85+15%)	$81.67^{b}\pm5.51$	$58.0^{cd}\pm2.65$	$26.67^{b}\pm4.04$	$35.33^{c}\pm5.51$
High fat diet and wheat-lentil bread (70+30%)	$77.33^{b}\pm20.60$	$51.33^{d}\pm1.53$	$30.00^{b}\pm1.00$	$31.33^{c}\pm8.50$
F	41.242^{*}	80.798*	32.361^{*}	120.793^{*}
P	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$
LSD 5%	18.384	8.951	3.9858	11.339

LDL-c = [TC − (HDL-c + TG/5)]

Data was expressed using Mean ± SD

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**

Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant)

*: Statistically significant at $P \leq 0.05$

Effect of bread fortified with lentil on liver enzymes activities of obese rats:

Table (7) represented the mean values of the liver enzymes activities of aspartate aminotrasferases(AST), alanine aminotrasferase (ALT), and alkaline phosphatase (ALP) in serum of rats fed on basal diet (control -), (control +) and the experimental groups (control). The data showed that feeding on high fat diet increased the activities of serum ALP, ALT and AST compared to the control.

The other treatment groups showed significantly decreased of ALP (447.70 IU/L), ALT (66.83 40U/ml) and AST (80.40U/ml)) compared with positive control group our results showed that the best values of the previous parameters represented by fortified bread by 30% of lentil.

Feng et al. (2021), stated that the activity of ALT and AST are the most sensitive indicators of liver function and when elevated may indicate the presence of inflammation, toxicity, and tissue trauma.

The obtained results in agreement with that reported by **Nwozo et al. (2018)** they found that feeding on HFD for 6 weeks, serum levels of AST, ALT were significantly higher than those in the control group. Also, **Zeng et al. (2015)** reported that levels of ALT and AST in serum were clearly increased in response to feeding on HFD + wheat compared with the control group.

Moreover, **Combe et al. (2004),** reported high levels of liver enzymes (ALT, AST, ALP) in HFD group, while low levels of liver enzymes were observed for control group, and group feed on cooked lentil. They clarify that due to induce a partitioning of the flux for protein synthesis in liver**.**

Further, **Lee et al. (2019).** showed that adzuki bean supplementation significantly inhibited AST and ALT activity and reduced hepatic steatosis caused by HFD, indicating a protective effect of adzuki bean on the liver.

Table (7): Effect of bread fortified with lentil on liver enzymes activities of obese rats

Treatments groups	Parameters		
	ALP (IU/L)	ALT (U/ml)	AST (U/ml)
Control (-)	255.40^{c}±0.53	34.63^{c}±0.57	49.87^{c}±1.00
HFD control (+)	447.70^{a}±1.57	66.83^{a}±1.55	80.40^{a}±1.54
High fat diet and wheat bread 100%	309.67^{b}±17.39	44.33^{b}±2.08	58.67^{b}±3.21
High fat diet and wheat-lentil bread (85+15%)	263.67^{c}±38.08	36.67^{c}±1.53	44.00^{c}±5.29
High fat diet and wheat-lentil bread (70+30%)	163.33^{d}±15.63	31.33^{d}±1.53	37.33^{d}±4.73
F	80.954*	260.639*	65.208*
P	<0.001*	<0.001*	<0.001*
LSD 5%	36.383	2.7873	6.5108

Data was expressed using Mean ± SD

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**

Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant)

*: Statistically significant at $P \leq 0.05$

Effect of bread fortified with lentil on urea, creatinine, albumin and glucose of obese rats:

The mean values of urea, creatinine, albumin and glucose in the serum of rats fed on basal diet (control-) and the experimental groups are shown in **Table (8).** The concentration of urea in HFD rats was high in serum (51.07 mg/dl) compared with control –ve (26.00 mg/dl). The bread fortified with two ratios of lentil treatments showed reduction in urea concentration compare to wheat bread. The concentration of urea was 20.67 and 17.33 mg/dl, in bread fortified with 15% lentil and bread fortified with 30% of lentil while it was 28.67 mg/dl in wheat bread treatment. The data showed no significant variation in the creatinine concentration between all treatments. The HFD rats showed the lowest level of the albumin compared with the other treatment and control.

Whereas the concentration of albumin was 2.07 g/dl in HFD compared with 2.57, 2.73 and 3.04 g/dl in wheat bread, bread fortified with (15%) lentil and bread fortified with (30%) lentil, respectively.

The level of glucose was significantly increased in HFD rats compared with non-obese rat. Whereas level of glucose was 104.73 mg/dl in HFD compared with 69.43 mg/dl in control (-). The treatments of wheat bread, bread fortified with (15%) lentil and bread fortified with (30%) lentil reduced the level of glucose which reached 93.00, 81.33 and 66.67 mg/dl, respectively. The improvement of blood glucose level was more at the high level of lentil.

The enrichment of bread and other cereal with legume flours is recommended particularly when protein utilization is inadequate, and this could be due to the fact that legumes are high in protein and lysine, the limiting amino acid in most cereals **(Jideani & Onwubali, 2009).**

Miranda-Ramos and Haros (2020), found that rats fed on wheat bread and soy (15-20%) a decrease in the creatinine and urea compared with the group of wheat 100% the (control group). Moreover, **Ebuehi and Okafor (2015),** observed that, when rats fed on supplemented soy flour bread at 10- 40% levels, the rats showed decrease in the creatinine and urea compared to control fed on wheat bread and that may enhance the protein quantity and quality of wheat bread especially for those groups who are at risk of protein deficiency **(Hassan, 2017).**

The current results are in line with that reported by **Refaat Hassan (2017)** in which the addition of soy bean at 10, 15 and 20% to wheat resulted in significant increase of serum albumin in comparison with the wheat 100% group.

Moreover, **Tovar et al. (2002),** mentioned that consumption of diet containing soy protein may retard kidney damage by lowering urinary albumin excretion and sustain adequate growth rate in rats.

Chung et al. (2008b) claimed that a diet containing lentils appears to be an effective intervention and management strategy for the prevention of diabetes. Also, **Hodge et al. (2004)** demonstrated that lentils in the diet regulate starch digestibility and the glycemic index, which diminish diabetes complications.

The regular consumption of cooked lentils among diabetic patients leads to significant reductions of fasting blood sugar and glycemic index in diabetic animals **(Al-Tibi et al., 2010)**.

Table (8): Effect of bread fortified with lentil on urea, creatinine, albumin and glucose of obese rats

Treatments groups	Parameters			
	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)	Glucose (mg/dl)
Control (-)	26.00b^{c}±1.00	0.72^{a}±0.08	3.47^{a}±0.35	69.43^{bc}±3.84

HFD control (+)	51.07^{a}±0.90	0.84^{a}±0.13	2.07^{c}±0.15	104.73^{a}±1.42
High fat diet and wheat bread 100%	28.67^{b}±1.53	0.74^{a}±0.18	2.57^{b}±0.42	93.0^{ab}±20.42
High fat diet and wheat-lentil bread (85+15%)	20.67^{cd}±2.52	0.69^{a}±0.29	2.73^{b}±0.15	81.33^{abc}±15.14
High fat diet and wheat-lentil bread (70+30%)	17.33^{d}±8.39	0.65^{a}±0.41	3.04^{ab}±0.14	66.67^{c}±18.82
F	32.542*	0.242*	11.282*	3.781*
P	<0.001*	0.907	0.001*	0.040*
LSD 5%	7.314	0.4499	0.491	25.951

Data was expressed using Mean ± SD

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**

Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant)

*: Statistically significant at $P \leq 0.05$

Effect of bread fortified with lentil on activities of antioxidant enzymes of obese rats:

The data in **Table (9)** showed that HFD rats had the lowest level of SOD activity 6.73 U/g compared with control 9.23 U/g and the other treatments. The same trend of CAT and GPx activities were observed between HFD rats which had the lowest levels of activity compared with control and the other treatments. The highest levels of antioxidant enzymes were observed in bread fortified with the two ratios of lentil treatments. The current data in agreement with **Shawky (2015),** who mentioned that HFD exhibited a significant decrease in SOD activity. These findings are also, in accordance with those of **Galaly et al. (2014),** who stated that male rats fed with a high fat diet had significantly lower levels of SOD compared with the. **Okafor and Ebuehi (2016),** reported that the administration of wheat bread and wheat-soy bread for 4 weeks showed a significant difference in the levels of GPX, SOD and CAT of experimental rats liver in wheat-soy bread group compared to control. They claimed that the levels might be protective against the oxidative damage **(Okafor and Ebuehi, 2016)**.

The current study, showed increasing of antioxidant capacities for bread fortified with to ratio of lentil, and may protect against obesity.

Table (9): Effect of bread fortified with lentil on activities of antioxidant enzymes of obese rats

Treatments groups	Parameters		
	SOD	CAT	GPX
	(U/g)		
Control (-)	9.23b^{c}±1.34	6.97^{b}±0.21	9.60^{ab}±0.36
HFD control (+)	6.73^{c}±0.40	3.20^{c}±0.89	3.97^{c}±0.42
High fat diet and wheat bread 100%	8.40b^{c}±0.62	6.53^{bc}±0.70	5.07^{bc}±2.57
High fat diet and wheat-lentil bread (85+15%)	11.13^{ab}±1.04	11.73^{a}±2.00	8.23^{abc}±1.99
High fat diet and wheat-lentil bread	14.0^{a}±4.00	14.0^{a}±3.61	12.33^{a}±4.93

(70+30%)			
F	5.990*	15.338*	4.916*
p	0.010*	<0.001*	0.019*
LSD 5%	3.5872	3.4843	4.8266

Data was expressed using Mean ± SD

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (LSD)**

Means in the **same column** with **common letters** are not significant (i.e. Means with **Different letters** are significant)

*: Statistically significant at $P \leq 0.05$

Histology of liver tissues:

This part of the study explains the effect of the previous treatments on the tissues of the liver of treated rats.

Liver is important organs of storage, detoxification and metabolism of many metabolites, so liver is particularly vulnerable to oxidative damage **(Babu et al., 2000)**.

The control group of rat (fed on basal diet) showed a normal histological structure of hepatic lobule with normal portal vein, bile ductile around which the liver cells are organized in cell spans arranged in radial way, while, HFD fat group showed fatty changes in sporadic hepatocytes. Liver of HFD with wheat bread showed mild dilation in the central vein around and cells are organized in cell spans arranged in radial. Photomicrograph of liver tissues of HFD fed on bread fortified with lentil (30%) showed normal architecture of liver tissue and normal hepatic.

The mechanism of liver destruction due to of the oxidative stress that involves secretion of cytokines, mainly the tumor necrosis factor **(Michaelis IV et al., 1984).** The current results showed supplement of bread with lentil reduced the hepatic lipids dependently on the level of lentil. The results in line with that reported by **Hoek and Pastorino (2002)**, they observed higher hepatic fat portions in rats fed on high amylose corn diet than lentil and control. Moreover, **Roberfroid (2000)**, indicated that the addition of prebiotics in lentil may play vital roles in modifying fat metabolism.

Siva (2017), reported that lentil diet significantly reduced percent of body fat and plasma TG concentration than corn starch diet**.**

The currant results matched with that reported by **Zhao et al. (2021)** in which the liver tissue weight and fat-cell size in epididymal fat of the HFD group mice increased significantly compared with control group and these effects were ameliorated by adzuki bean supplementation. Also, obese rats fed on soy protein lowered the liver fat **(Bhathena et al., 2003).**

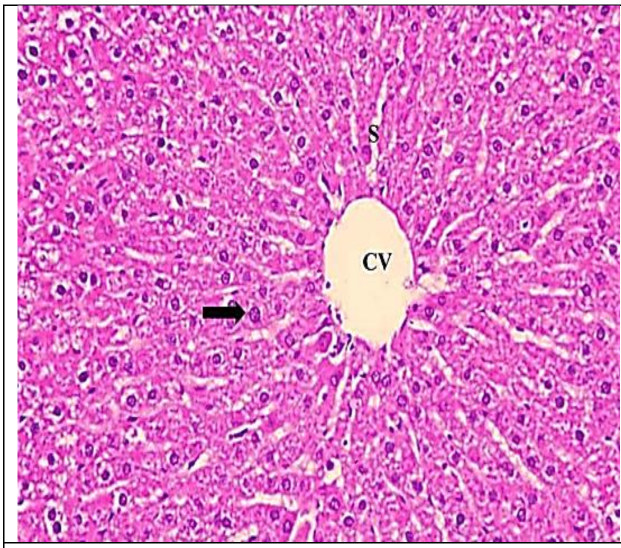

Photo (1): Photomicrograph of liver rats fed on basal diet (HandE stain X 400).

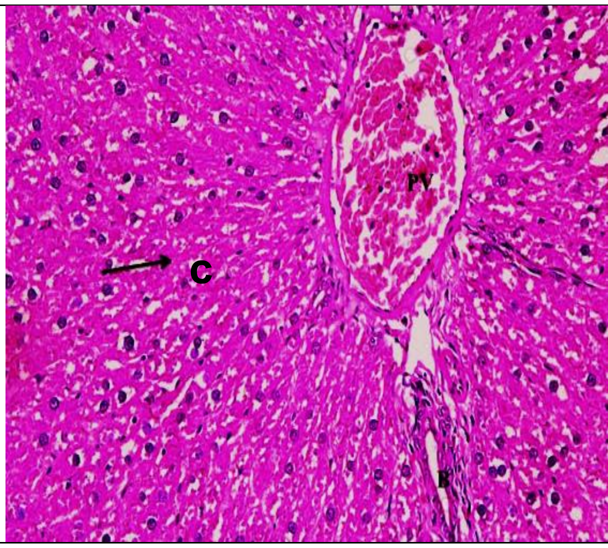

Photo (2): Photomicrograph of liver sections of high fat diet group (HandE stain X 400).

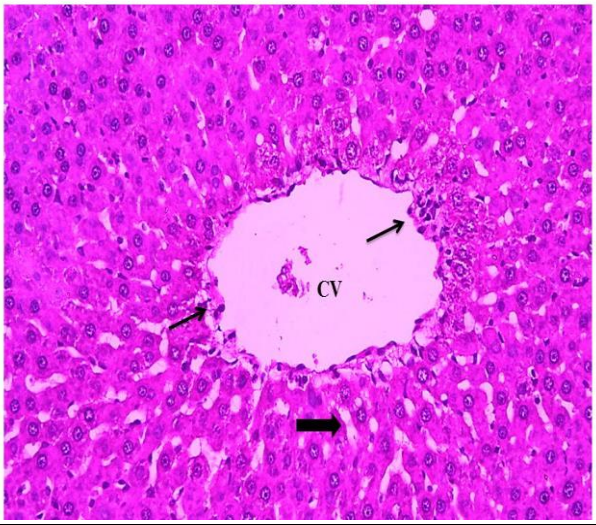

photo (3): Photomicrograph of liver of group HFD fed on wheat bread

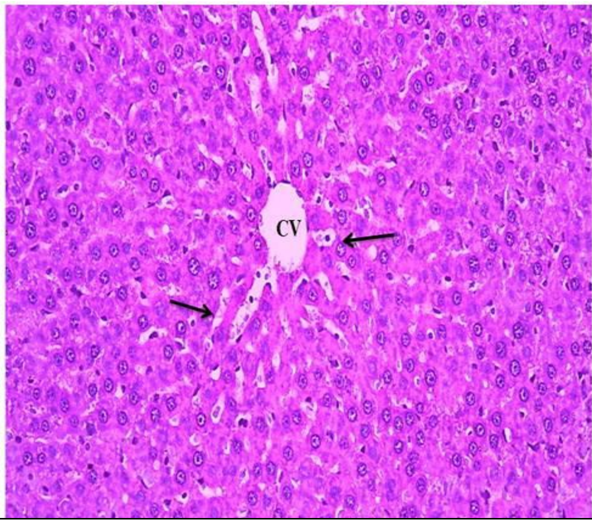

photo (4): Photomicrograph of liver of group HFD fed on bread fortified with lentil (30%)

Conclusion:

The current results revealed that consumption of bread fortified with lentil reduced BWG and improved blood sugar, antioxidant enzymes, liver and kidney functions. Moreover, showed that bread fortified with lentil is beneficial for preventing fatty liver and diabetes and the effect depend on lentil ratio. These results may be due to the protein and higher dietary fiber content present in lentil .The present study suggests that lentil might serve as a good product for reducing obesity and could be a candidate for use in future for dietary applications.

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