

## ملخص البحث

### تأثير أوراق المورينجا أوليفيرا على مضادات الأكسدة والدلالات البيوكيميائية للدهون في ذكور جردان الألبينو

تهدف هذه الدراسة إلى دراسة تأثير أوراق المورينجا أوليفيرا على مضادات الأكسدة والدلالات البيوكيميائية للدهون في ذكور جردان الألبينو. وتضمنت هذه الدراسة ٤ مجموعات من الفئران (٧ / مجموعة) بحيث تم تغذية المجموعة الأولى علي الغذاء الأساسي كمجموعة ضابطة سالبة والمجموعة الثانية ( المجموعة الضابطة الموجبة ) تم تغذيتها علي الغذاء الأساسي مضافاً إليه ١٪ الكوليسترول و ٥,٠٪ حمض الكوليك بينما تغذت المجموعتين الثالثة والرابعة علي نفس غذاء المجموعة الثانية مضافاً إليه الخبز البلدي المدعم بـ ٥ و ١٠٪ أوراق المورينجا علي التوالي حسب أفضل نتائج الاختبارات الحسية والتي تمت علي ثلاثة تركيزات من الخبز البلدي المدعم بأوراق المورينجا والذي تم اختباره حسيّاً من قبل بعض طلاب وأعضاء هيئة التدريس بقسم التغذية وعلوم الأطعمة كلية الإقتصاد المنزلي جامعة حلوان. كما تم تحليل نبات المورينجا لمعرفة ما يحتويه من مواد غذائية وفلافونيدات. ثم تم البدء في احتساب استهلاك الغذاء المتناول والزيادة في الوزن ونسبة الكفاءة الغذائية بعد مرور اسبوع علي بداية التجربة (فترة التكيف) وحتى نهاية التجربة (٨ أسابيع). تم تقدير مستويات الدهون المختلفة في الدم، وظائف الكلى، وظائف الكبد، MDA، SOD ومضادات الأكسدة الكلية. ولقد أظهرت النتائج انخفاض معنوي في وزن الجسم، ونسبة كفاءة الغذاء، والكوليسترول الكلي والدهون الثلاثية، ونسبة الدهون الثلاثية إلي البروتينات الدهنية عالية الكثافة والبروتين الدهني منخفض الكثافة، ووظائف الكبد وكذا وظائف الكلى والMDA في المجموعتين ٣ و٤. وأشارت النتائج أيضاً إلي وجود زيادة معنوية في كل من كمية المتناول من الغذاء والبروتين الدهني عالي الكثافة والSOD ومضادات الأكسدة في نفس المجموعات (٣ و٤). وبالإضافة إلى ذلك فقد أظهر الفحص النسيجي للكبد والكلى والقلب تحسناً في فئران المجموعتين والمغذاة علي ٥% مسحوق المورينجا (مجموعة ٣) و ١٠% مسحوق المورينجا (مجموعة ٤). وبناءً علي نتائج هذه الدراسة فإن أوراق المورينجا لها آثار مفيدة علي الدلالات البيوكيميائية للدهون ومضادات الأكسدة ولذا ينبغي التوجه لمزيد من الأبحاث الغذائية العلاجية في المستقبل وتشجيع استخدام أوراق المورينجا وبخاصة لمرضى القلب وتصلب الشرايين.

### الكلمات المفتاحية:

أوراق المورينجا أوليفيرا ، الخبز البلدي ، دهون الدم ، مضادات الأكسدة.

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## Abstract

### **Influence of *Moringa oleifera* Leaves on Antioxidant and Lipid Biochemical Markers in Male Albino Rats**

The present study aims to investigate the beneficial effect of (*Moringa oleifera*) leaves on antioxidant and lipid biochemical markers in male Albino rats. Rats were divided to 4 groups. Group1 was fed basal diet. Group2 was fed basal diet with 1% cholesterol and 0.5% cholic acid. Groups 3 and 4 were as the same group2 with balady bread fortified with 5 and 10% *Moringa* leaves, respectively. Chemical composition and flavonoids were assayed. Feed intake, body weight gain and feed efficiency ratio were calculated. Lipid profile, kidney functions, liver functions, malondialdehyde, superoxide dismutase and total antioxidant content were determined. The results revealed significant reduction in body weight gain, feed efficiency ratio, total cholesterol, triglycerides, triglycerides/ high density lipoproteins, low density lipoprotein, liver functions, kidney functions and malondialdehyde in groups3 and 4. Feed intake, high density lipoprotein, superoxide dismutase and total antioxidant were significantly increased in the same groups (3 and 4). In addition, the histopathological examination of liver, kidney and heart showed slight improvement in the hepatic tissues, normal histological structure of renal parenchyma and normal cardiac myocytes of rats in groups 3 and 4. In general, the above data indicate that *Moringa* leaves have beneficial effects on lipid biochemical markers and the plant should be considered as therapeutic nutritional researches in future and the usage of *Moringa* leaves must be encouraged by human beings especially atherosclerotic patients.

#### **Key words:**

*Moringa oleifera*, balady bread, lipid profile, antioxidants.

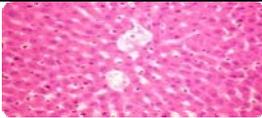
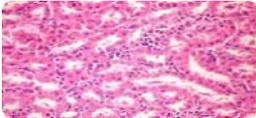
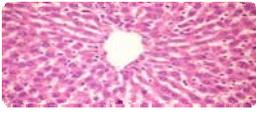
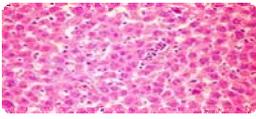
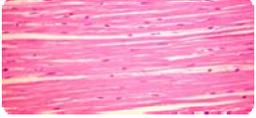
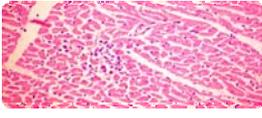
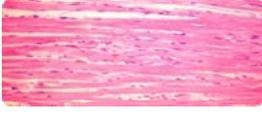
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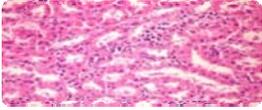
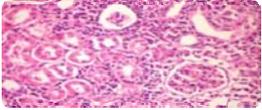
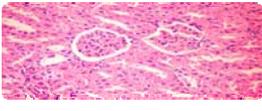
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	Photos No.	Histopathology (H & EX 400)	
<b>Liver</b>	5		Control, untreated rat showing the normal histological structure of hepatic lobule
	6		Control positive rat showing Kupffer cells activation and focal hepatic necrosis associated with inflammatory cells infiltration.
	7		Rat from group 3 showing Kupffer cells activation .
	8		Rat from group 4 showing Kupffer cells activation and sinusoidal leukocytosis .
<b>Heart</b>	9		Control, untreated rat showing normal cardiac myocytes.
	10		Control positive rat showing focal myocarditis with inflammatory cells infiltration between cardiac myocytes.
	11		Rat from group 3 showing slight intermuscularoedema.
	12		Rat from group 4 showing no histopathological changes

**Table (9): Effect of *Moringa oleifera* at different levels on superoxide dismutase (SOD), malondialdehyde (MAD) and total antioxidant of rats**

Parameters Groups	SOD	MAD	Total antioxidant
	n mol/L		
-ve Control	15.12±1.26 <sup>a</sup>	18.93±3.04 <sup>d</sup>	4.23±0.39 <sup>a</sup>
+ve Control	12.88±1.99 <sup>c</sup>	22.02±2.67 <sup>a</sup>	1.99±0.09 <sup>d</sup>
+5% <i>Moringa oleifera</i>	13.97±1.53 <sup>b</sup>	21.05±5.30 <sup>b</sup>	2.76±0.15 <sup>c</sup>
+10% <i>Moringa oleifera</i>	14.04±2.08 <sup>b</sup>	20.27±5.61 <sup>c</sup>	3.53±0.21 <sup>b</sup>

All results are expressed as mean ± SD. Values in each column which have different letters are significantly different (p<0.05).

	Photos No.	Histopathology (H & EX 400)	
<b>Kidney</b>	1		Control, untreated rat showing the normal histological structure of renal parenchyma.
	2		Control positive rat showing peritubular inflammatory cells infiltration.
	3		Rat from group 3 showing no histopathological changes.
	4		Rat from group 4 showing no histopathological changes.

**Table (7): Effect of *Moringa oleifera* at different levels on uric acid, urea nitrogen and creatinine of rats**

Groups	Parameters	Uric acid	Urea nitrogen	Creatinine
	mg/dl			
-ve Control		3.442±0.121 <sup>d</sup>	15.371±1.162 <sup>d</sup>	1.094±0.004 <sup>b</sup>
+ve Control		4.014±0.108 <sup>a</sup>	22.371±3.342 <sup>a</sup>	1.168±0.172 <sup>a</sup>
+5% <i>Moringa oleifera</i>		3.900±0.180 <sup>b</sup>	17.371±2.742 <sup>b</sup>	0.931±0.152 <sup>c</sup>
+10% <i>Moringa oleifera</i>		3.275±0.151 <sup>c</sup>	16.862±1.912 <sup>c</sup>	0.832±0.057 <sup>d</sup>

All results are expressed as mean ± SD. Values in each column which have different letters are significantly different ( $p < 0.05$ ).

**Table (8): Effect of *Moringa oleifera* at different levels on aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in of rats**

Groups	Parameters	AST	ALT	ALP
	(u/l)			
-ve Control		12.71±2.17 <sup>c</sup>	9.71±1.62 <sup>b</sup>	45.29±3.23 <sup>c</sup>
+ve Control		18.71±4.37 <sup>a</sup>	12.86±4.33 <sup>a</sup>	105.86±2.42 <sup>a</sup>
+5% <i>Moringa oleifera</i>		15.43±4.48 <sup>b</sup>	10.29±1.88 <sup>b</sup>	71.43±2.57 <sup>b</sup>
+10% <i>Moringa oleifera</i>		12.38±3.86 <sup>c</sup>	09.86±1.84 <sup>b</sup>	67.33±2.63 <sup>b</sup>

All results are expressed as mean ± SD. Values in each column which have different letters are significantly different ( $p < 0.05$ ).

**Table (5): Effect of feeding *Moringa oleifera* at different levels on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of rats**

Parameters Groups	FI (g/d)	BWG (g/d)	FER
-ve Control	10.250±1.26 <sup>c</sup>	2.42±3.04 <sup>c</sup>	0.24±0.007 <sup>b</sup>
+ve Control	12.423±1.99 <sup>b</sup>	5.11±2.67 <sup>a</sup>	0.41±0.004 <sup>a</sup>
+5% <i>Moringa oleifera</i>	12.980±1.53 <sup>b</sup>	03.24±5.30 <sup>b</sup>	0.25±0.004 <sup>b</sup>
+10% <i>Moringa oleifera</i>	14.510±2.08 <sup>a</sup>	02.99±5.61 <sup>c</sup>	0.21±0.002 <sup>c</sup>

All results are expressed as mean ± SD. Values in each column which have different letters are significantly different (p<0.05)

**Table (6): Effect of *Moringa oleifera* at different levels on total cholesterol (TC), triglyceride (TG), HDL, TC/HDL ratio, LDL and VLDL of rats**

Parameters Groups	TC	TG	HDL	TG / HDL ratio	LDL	VLDL
	mg/dl					
-ve Control	83.86± 14.25 <sup>c</sup>	105.86± 3.61 <sup>b</sup>	49.81± 3.93 <sup>a</sup>	2.13± 0.09 <sup>b</sup>	12.88± 2.35 <sup>c</sup>	21.17± 4.90 <sup>b</sup>
+ve Control	100.88± 26.24 <sup>a</sup>	122.01± 27.16 <sup>a</sup>	24.65± 2.47 <sup>d</sup>	4.09± 1.05 <sup>a</sup>	51.55± 4.89 <sup>a</sup>	24.68± 7.61 <sup>a</sup>
+5% <i>Moringa oleifera</i>	86.29± 13.45 <sup>b</sup>	107.78± 12.32 <sup>b</sup>	34.71± 3.48 <sup>c</sup>	2.49± 0.08 <sup>b</sup>	30.49± 3.83 <sup>b</sup>	21.09± 3.41 <sup>b</sup>
+10% <i>Moringa oleifera</i>	80.66± 15.83 <sup>c</sup>	103.33± 14.49 <sup>b</sup>	41.12± 2.37 <sup>b</sup>	2.51± 0.11 <sup>b</sup>	18.87± 2.49 <sup>c</sup>	20.67± 3.87 <sup>b</sup>

All results are expressed as mean ± SD. Values in each column which have different letters are significantly different (p<0.05).

**Table (3): Minerals and vitamins composition of wheat flour and *Moringa oleifera* as raw materials**

Minerals mg/100g DW	Wheat flour	<i>Moringa oleifera</i>	Vitamins	Wheat flour	<i>Moringa oleifera</i>
Sodium	02.25	01.16	B <sub>1</sub> mg/100gDW	00.09	00.65
Potassium	110.0	1320.00	B <sub>2</sub> mg/100gDW	00.06	04.94
Calcium	20.42	945.25	C mg/100gDW	ND	409.73
Iron	01.81	24.70	E (ppm)	0.60	36.61
Zinc	01.20	01.03	A(μ100g)	ND	221.074
Copper	00.12	00.19	β-carotene mg/100gDW	ND	106.603
Magnesium	35.05	88.32			
Phosphorus	102.00	218.04			

ND = Not detected

**Table (4): Types and concentration of flavonoids (ppm) in *Moringa oleifera* leaves**

Flavonoids (ppm)	<i>Moringa oleifera</i> leaves
Rosmarinic acid	33.507
Rutin	276.78
Quercitrin	4135.00
Querctin	1804.40
Kaempferol	28.514

(Photo 2). However, kidneys of rats were received 5 and 10% *Moringa* leaves powder revealed no histopathological changes (Photo 3 and 4).

Microscopically, liver of control, untreated rat revealed the normal histological structure of hepatic lobule (Photo 5), meanwhile, liver of rat from control positive group showed Kupffer cells activation and focal hepatic necrosis associated with inflammatory cells infiltration (Photo 6). Examined sections from rats were fed 10% *Moringa* leaves powder showed Kupffer cells activation (Photo 7) and sinusoidal leukocytosis (Photo 8).

Microscopically, heart of control, untreated rat revealed normal cardiac myocytes (Photo 9). However, heart of rat from control positive group revealed focal myocarditis with inflammatory cells infiltration between cardiac myocytes (Photos 10 and 11). Some examined sections from rats were fed 10% *Moringa* leaves powder showed no histopathological changes (Photo 12).

**Table (1): Physical characteristics and sensory evaluation of balady bread prepared from soft wheat flour (SWF) and *Moringa oleifera* leaves (M.O.)**

Bread	Characteristics							
	General appearance (15)	Top layer color (10)	Inside layer color (10)	Volume (cm <sup>3</sup> ) (10)	Loaf Texture (15)	Odor (20)	Taste (20)	Total score (100)
Control (SWF)	13.33± 1.83 <sup>a</sup>	09.08± 1.56 <sup>a</sup>	09.17± 1.53 <sup>a</sup>	9.97± 1.47 <sup>a</sup>	13.00± 2.22 <sup>a</sup>	16.83± 3.59 <sup>a</sup>	18.70± 2.12 <sup>a</sup>	90.08
5% M.O.	10.25± 1.71 <sup>b</sup>	8.46± 1.70 <sup>b</sup>	7.96± 1.18 <sup>b</sup>	9.08± 2.78 <sup>a</sup>	11.63± 2.10 <sup>b</sup>	13.92± 3.63 <sup>b</sup>	15.58± 5.38 <sup>b</sup>	76.89
10% M.O.	9.17± 3.54 <sup>b</sup>	7.29± 2.12 <sup>c</sup>	6.50± 2.32 <sup>c</sup>	8.25± 2.80 <sup>a</sup>	10.46± 3.37 <sup>b</sup>	13.50± 5.14 <sup>b</sup>	16.08± 4.89 <sup>b</sup>	71.25
15% M.O.	7.79± 2.95 <sup>c</sup>	6.67± 2.02 <sup>c</sup>	5.83± 1.53 <sup>c</sup>	7.67± 2.90 <sup>b</sup>	8.92± 2.97 <sup>c</sup>	11.08± 4.29 <sup>c</sup>	14.96± 5.71 <sup>c</sup>	62.92

All results are expressed as mean ± SD. Values in each column which have different letters are significantly different (p<0.05).

**Table (2): Chemical composition of wheat flour and *Moringa oleifera* as raw materials (g / 100g dry weight basis)**

Macronutrients (g/100g DW)	Wheat flour	<i>Moringa oleifera</i>
Moisture	11.35	07.52
Protein	10.05	27.81
Ash	00.57	07.83
*Carbohydrate	75.41	37.44
Crude Fiber	01.45	17.10
Fat	01.27	02.30

\*Carbohydrate calculated by difference.

supplementation in rat diets used for natural treating of hyperlipidemia. The changes of these enzymes in all four groups of rats fed different diets are given in Table (8). The mean values of AST, ALT and ALK-P were 12.71, 9.71 and 45.29 u/l in normal rats(group1) fed basal diet, which were significantly increased to the mean values of 18.71, 12.86 and 105.86 u/l for AST, ALT and ALK-P, in the same order, in rats fed high fat-high cholesterol diet(group2). A significant decrease in this tested enzymes in experimental groups 3 and 4 as a result of presence of *Moringa* leaves powder in their diet which profound a desirable effect after supplementation in groups 3 and 4. Increasing the level from 5% to 10% supplementation showed better values, which become near to the values at group1 (control negative group).

Protection from oxidative damage is sufficiently important that biology has evolved independent enzymes for hastening superoxide dismutation. The erythrocyte membrane is prone to lipid peroxidation under oxidative stress that leads to the formation of MDA, a biomarker used for studying the oxidation of lipids under different conditions **López-Revuelta (2005)**. The antioxidant enzymes implicated in the regulation of cell damage induced by reactive oxygen species are superoxide dismutase (SOD), malondialdehyde (MDA) along with total antioxidants are determined and the results obtained can be seen in Table (9). The results revealed that the level of SOD, MDA and total antioxidants in normal rats (group1) were 15.12, 18.93 and 4.23 nmol/l. In group 2, the values are significantly changed to 12.88, 22.02 and 1.99 nmol/l as a result of hyperlipidemia. In groups 3 and 4 which were fed bread supplemented with *Moringa* leaves powder showed a significant increase in the level of total antioxidant compared with group2 which still lower than normal rats which fed basal diet (group1) which in accordance with **(Ghada, 2013)**. These results demonstrate the presence of balance in the oxidant – antioxidant system in the blood of rats fed *Moringa* leaves powder. *Moringa oleifera* is a rich source of antioxidant **(Singh et al.,2009)** such as quercetin and kaempferol (major bioactive compounds of phenolics) and are responsible for antioxidant activity **(Bajpai et al.,2005)**. Flavonoids can exert their antioxidant activity by various mechanisms, e.g., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for free radical generation **(Lukacinova et al.,2008)**. The antioxidant property also can be due to the presence of carotenoids, alkaloids, proanthocyanidins in this plant **(Lukacinova et al.,2008)** or to the high content of flavonoids such as kaempferol, presence of other polyphenols, carotenoids and cinnamic acid derivatives **(Bajpai et al.,2005)**.

Microscopically, kidneys of control, untreated rat revealed the normal histological structure of renal parenchyma (Photo1). Meanwhile, kidneys of rat from control positive group showed peritubular inflammatory cells infiltration

*Moringa* showed insignificant results when compared to the control negative rats.

The changes in total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein (VLDL) in all four rat groups are given in Table (6). The mean initial TC was 83.86 mg/dl which was significantly increased to 100.88 mg/dl in high fat high cholesterol diet (group 2). The total cholesterol levels decreased significantly by about 15% and 20% in rats fed bread supplemented with 5% and 10% of *Moringa* leaves powder, respectively. Almost same trend can be seen in the case of LDL-C and VLDL whereas in the case of HDL-C the rats fed basal diet showed values of 49.81mg/dl which was significantly decreased to 24.65mg/dl in rats fed high cholesterol level (group 2), then the values increased significantly as a result of feeding bread supplemented with *Moringa* at the two levels (5 and 10%, of supplementation, respectively).

It was interesting to note that *Moringa* supplementation to diet resulted in a significant modification of lipid profile which becoming almost near the values of rats fed basal diet. The results reported in Table (6) was in agree with the results reported by **Chumark et al.,(2008)** who examined the therapeutic potential of *Moringa* leaves on dyslipidemia induced in rabbits on high cholesterol diet for 12 weeks, and **Ghasi et al.,(2000)** as well as **Jain et al.,(2010)** who examined the anti-dyslipidemic effects of *Moringa* leaves in rats fed a high fat diet for 30 days. In untreated rabbits or rats several fold increases in the levels of total cholesterol, LDL-C and TG. When these animals were concomitantly fed *Moringa* leaves extract, these increases were reduced.

To estimate how well kidneys are working and their function may affected by the chemical components of *Moringa* leaves powder was used for fed supplementation, uric acid, urea nitrogen and creatinine were determined, and the results obtained are presented in Table (7). Data presented in this table showed that the control positive group which fed basal diet has a mean value of 3.442, 15.371 and 1.094 mg/dl for uric acid, urea nitrogen, and creatinine, respectively. Rats with hyperdyslipidemia showed increased values with means of 4.014, 22.371 and 1.168mg/dl in the same order. All treated groups fed on fortified bread at 5% and 10% *Moringa* leaves powder induced significant decrease in all parameters of kidneys functions, compared to the positive control group. The effect of 10% level is better compared with 5% level in terms of kidney functions.

Liver function tests are groups of clinical biochemistry laboratory blood assays designed to give information about the state of liver. Transaminases (ALT, AST) and alkaline phosphatase (ALK-P) are useful biomarker tests in the evaluation of the side effect of feeding *Moringa* leaves powder

outstanding as the best plant source of proteins and total minerals. The moisture content is 11.35g/100g dry weight for wheat flour and 7.52g/100g dry weight for *Moringa* leaves. **Sengev et al., (2012)** reported similar values for wheat flour and *Moringa* leaves powder. The high levels of crude proteins and ash as well as crude fiber and fat reported in Table (2) are in agree with the result reported by **Sengev et al.,(2012)** for wheat flour and *Moringa* leaves powder. *Moringa* leaf powder contain about 12% fiber as reported by (**Joshi and Mehta, 2010**) which are lower than that reported in the results of Table (2). Dietary fiber reduces gastric emptying (**Bortolotti et al., 2008**).

The results in Table (3) of wheat flour and *Moringa* leaf show an increase in all examined vitamins as well as potassium, calcium, iron, magnesium and phosphorus. These results are agreed with the result reported by **Aslam et al., 2005 and Amaglo et al., 2010**, who shown that *Moringa* leaves are particularly rich in potassium, calcium, iron, vitamin A as well as such known antioxidant as  $\beta$ -carotene and vitamin C.

The results reported in Table 4 of *Moringa* leaves contain the highest values of quercitrin and quercetin. On the other hand, rosmarinic acid and kaempferol showed the least values. Biologically, flavonoids are best known for antioxidant properties. The flavonolquercetin concentration was with a mean value of 1804.4 ppm and the flavonolquercitrin was 4135 ppm. Dried *Moringa* leaves had a high concentration of quercetin in the study of **Lako et al., 2007**. Quercetin is a potent antioxidant (**Zhanget al., 2011**). It can reduce hyperlipidemia and atherosclerosis in rabbits (**Kamadaet al., 2005**) and its hypotensive effect has been confirmed in human study (**Edwardset al., 2007**).

Most flavonoids function in the human body as antioxidants. In this capacity, they help neutralize excess reactive oxygen containing molecules and prevent these excess reactive molecules from damaging parts of the cells. While most flavonoids may exert their cell structure protection through a variety of mechanisms, one of their potent effects may be through their ability to increase levels of glutathione, a powerful antioxidant, as suggested by **Myhrsted et al., 2002**.

Mean values of feed intake, body weight gain and feed efficiency ratio of rats as affected by *Moringa* fortified bread are shown in Table (5). There was no significant differences in feed intake among rats fed bread with 5% *Moringa* leaves and control positive group but when the *Moringa* leaves was increased to 10% supplementation feed intake showed a significant increase. Body weight gain and feed efficiency ratio in control negative and control positive groups showed a significant mean values (Table 5). When rats were fed *Morina* at 10% replacement BWG reached to the normal control negative rats which could not be observed at the 5% replacement, but on other hand 5%

Feed intake was recorded daily for 8 weeks of the experimental period. Body weight gain and feed efficiency ratio were calculated according to **Hsu et al., (1978)**. At the end of the experiment period, the rats were fasted over night before sacrifice. Heparinized blood and whole blood samples were immediately collected from the abdominal aorta and then centrifuged for 10 min. at 3000 rpm to separate the plasma and serum from the cells. The plasma and serum were carefully separated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till analysis at -20C°.

#### **Methods of analysis:**

Urea, creatinine and uric acid were measured according to **Kaplan , 1984 ; Murray,1984 and Fossati et al.,1980**, respectively. Total cholesterol and triglycerides were measured at 500-550nm according to the method of **Wahlefeld and Bergmeyer, 1974**. High-density, very low-density, and low-density lipoprotein cholesterol were calorimetrically determined according to the method of **Friedewald et al.,1972**. Serum aspartate aminotransferase, alkaline aminotransferase and alkaline phosphatase were measured according to the method of **Murray,1984**, at 505 nm. Malondialdehyde was measured according to **Ohkawa et al. 1979**. Superoxide dismutase was measured according to **Nishkimi et al., 1972**

#### **Histopathological Examination:**

Liver, kidney and heart of the sacrificed rats were taken and immersed in 10% formalin solution. The specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Then cleared in xylol, embedded in paraffin, sectioned (4-6 microns) and stained with Heamtoxylin and Eosin for histopathological examination according to the method of **Carlton, (1979)**.

#### **Statistical analysis of data:-**

Data were statistically analyzed using statistical analysis system (**SAS, 2006**). One way analysis of variance (ANOVA) was used to test the variations among groups.

### **Results and Discussion**

The physical characteristics and sensory evaluation of balady bread produced from soft wheat flour and *Moringa* leaves are presented in Table (1). It can be seen that the total score of 5% and 10% replacement of *Moringa* leaves in the manufacture of balady bread almost the same which differ significantly of 15% replacement. So, this experiment was designed using only 5% and 10% concentrations.

Raw materials (g/100g dry weight basis) were analyzed for gross chemical composition, and the result obtained presented in Table (2). *Moringa* leaves powder are rich in protein, ash, fat, and crude fibers whereas wheat flours are rich in protein, carbohydrate and moisture. The leaves are

## Materials and Methods

### Materials:

Twenty eight adult male Albino rats (130-140 g) were obtained from Animal House Colony of Vacsera, Helwan, Egypt. They were housed in stainless steel cages under a 12 h light- dark cycle at  $20\pm 5^{\circ}\text{C}$ . Animals were maintained at free access to tap water and were fed a standard pelleted feed for at least 7 days before starting the experiment.

Casein, cholesterol, cellulose, all vitamins and minerals were obtained from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch and soy oil were obtained from the local market.

### Preparation of basal diet:

The basal diet was prepared according to **Reeves et al., 1993**.

### Methods:

*Moringa* leaves were obtained from Agriculture Research Center, Giza, Egypt then washed, air dried, crushed using pestle and mortar followed by high speed laboratory blender and then sieved to obtain fine powder. *Moringa* leaves powder was stored under refrigeration until processing and analyses.

### Dough Preparation and properties:

Balady bread (control) was prepared according to the common method described by **Khorshid et al., (1989)**. Control balady bread was made from 100% wheat flour. Different formulas were made from a mixture of wheat and *Moringa* leaves powder at various ratios 5%, 10% and 15%, respectively. Then according to the panel test, Table (1), we used the best two concentrations on the experimental animals (5 and 10%). The scoring scheme was established according to **A.A.C.C. (2002)** by 15 trained panelists (staff members and students) from Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University.

### Chemical Constituents of wheat flour and *Moringa* leaves:

Moisture, ash, protein, fat, dietary fiber, were determined according to the method outlined in **A.O.A.C. (2005)**. Total carbohydrates were calculated by difference. Minerals contents including Ca, Mg, Na, K, Cu, Fe, P and Zn were assayed as recommended by **A.O.A.C. (2005)** using atomic absorption Spectrophotometer. Flavonoid content was assayed in *M. oleifera* leaves according to **J.og.Agric.and Food Chem., (2000)**.

### Experimental design:

After one week of adaptation, rats were divided into 4 main groups (7rats of each). Group (1) is a normal control group (-ve), fed on the basal diet. Group (2) is the positive control group (+ve) fed on diet with 1% cholesterol and 0.5% cholic acid (**Takako et al.,2006**). Groups 3 and 4 were as the same of group 2, fortified with *Moringa* balady bread 5%(group3) and 10%(group4).

## **Influence of *Moringa oleifera* Leaves on Antioxidant and Lipid Biochemical Markers in Male Albino Rats**

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### **Introduction**

Plants as medicinal agents were mentioned in historic documents dating back many thousands of years (**Rasonavivo et al., 1992**). Currently, medicinal herbs as a whole were reported to be used against a wide range of health problems such as cough, cold, stomach, cataract, constipation and many other ailments (**Jimenez et al., 2003**). The plant *M. oleifera* as one of these herbs was reported to prevent effectively morphological changes and oxidative damage in lens of rats by enhancing the activities of anti-oxidant enzymes, reducing the intensity of lipid peroxidation and inhibiting generation of free radicals (**Sreelatha and Padma, 2009**). In addition, blood parameters namely: packed cell volume(PCV)and white blood cell (WBC)counts, differentiation of WBC, hemoglobin (Hb) and platelets (PLT) were also found to be positively affected by using this plant (**Chinwe and Isitua, 2010**). *M. oleifera* was also found to be of a nutritional value as it contains a number of important vitamins, including: vitamins A, B complex (B1, B3, B6 and B7), C, D, E and K (**Dorga et al., 1975; Booth and Wickens, 1988**). For treatment, it was used against high blood pressure, diarrhea, inflammation of colon, intestinal worms, skin antiseptic, as a diuretic agent (**Lowell, 2002**) and to maintain the level of blood glucose in diabetic patients (**Jaiswal et al., 2009; Chinwe and Isitua, 2010**). Moreover, *M. oleifera* was used as antimicrobial agent (**Caceres et al., 1990**), to treat ulcers (**Pal and Sahib, 1995**) and to promote the immune system against various infections (**Jaiswal et al., 2009**). So far, most of the work about the effect of *M. oleifera* was carried out on the seeds of this plant.

In a research done by **Kasolo, 2010** *M. oleifera* was found to contain phytochemicals which are non-nutritive chemicals that plants produce as a self-defense mechanism. Phytochemicals present in *M. oleifera* include catechol tannins, gallic tannins, steroids, triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars. These phytochemicals are known to have medicinal values for humans such as detoxification and purification of water, antibiotics, skin treatment, anti-inflammatory, ulcers, blood pressure, diabetes, anemia and many other uses. The presence of this chemical indicates the possible healing properties of this species leaves and other parts of its tree.

The aim of this manuscript is to find out the effect of *M. oleifera* leaves on various parameters in male Albino rats.