



## Evaluation of the effect cholesterolemia on general health condition

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### *ABSTRACT*

This study aimed to evaluate the effect cholesterolemia on general health condition, and investigate the effect of some vegetables such as watermelon, green pepper, rocket and their blend, level to combat with hypercholesterolemia via determination of some biological parameters, biochemical parameters of serum liver and renal function parameters. The study was carried on forty male adult albino rats, weighting(110 ±5) g each, feeding continued for 8 weeks. Watermelon skin, green pepper, rocket and their blend was added to the diet of rats at two levels, 5% and 10% (to basal diet). Body weight and food intake were recorded weekly. At the end of the experiment, all rats were weighed for calculation of body weight gain % and food efficiency ratio. In comparison with control (+ve) group, there was a decrease in cholesterol, TG, LDLC, TL. and R. r in all hypercholesterolemic rats groups treated with vegetables but there was an increase in HDL.c. As for liver function the results indicated that all groups were significantly lower in enzymes actives than positive control. The results proved also the decreasing of renal function parameters (creatinine and urea) in all vegetables treated rats groups. In compared with control (+ve) group, the value of hemoglobin level was increased in all vegetable treated rats groups. On the other hand the histopathological examination or control (+ve) group, of liver tissue showed focal degeneration of hepatic cells and heamorrhage between hepatocytes. The heart tissue showed necrosis of cardiac muscle fibers, odema and inflammatory cells between cardiac muscle fibers. Vegetables, specially watermelon, pepper, rocket and the blend (2.5), (5%) groups showed improvement of pathological changes and the structure was more or less around normal, indicating the value of vegetables and confirming the biological and biochemical analyses. Thus study recommended to Among vegetable sources of value in reducing T.L., T.C., TG, LDL & VLDL in serum, watermelon skin, green pepper and rocket are recommended.



## الملخص العربي

تهدف هذه الدراسة إلى معرفة تأثير الوجبة التي أستبدل فيها زيت الذرة بالدهون الحيوانية و المرتفعة في الكوليستيرول على بعض الوظائف الحيوية والكيميائية في الجسم وتأثير إضافة بعض الخضروات وتناولها على مستوى دهون الدم والصحة العامة لأعضاء الجسم المختلفة وأجريت هذه الدراسة على أربعون من ذكور فئران التجارب التي تتراوح أوزانهم  $110 \pm 5$  جرام واستمرت التجربة لمدة 8 أسابيع. وقد تمت إضافة قشر البطيخ الأبيض والفلل الأخضر والجرجير والخليط بنسبة 2.5% & 5% إلى الغذاء الأساسي وسجل وزن الجسم والغذاء المتناول أسبوعيا وفي نهاية التجربة تم حساب معدل الزيادة في وزن الجسم ومعدل كفاءة الغذاء وأظهرت النتائج المتحصل عليها نقص معنوي في كل من الكوليستيرول والجليسيريدات الثلاثية والليوبروتينات منخفضة الكثافة والدهون الكلية و نسبة الخطر من الإصابة بتصلب الشرايين في جميع المجموعات المعالجة بالخضروات. وعلى الجانب الآخر سجلت النتائج زيادة معنوية في الليوبروتينات مرتفعة الكثافة في جميع الفئران المعالجة وبالنسبة لوظائف الكبد لوحظ وجود انخفاض معنوي في نشاط الأنزيمات لجميع المجموعات التي تغذت على الخضروات مقارنة بالمجموعة الضابطة الموجبة. كما أثبتت نتائج الدراسة أيضا نقص له دلالة إحصائية معنوية في وظائف الكلى المتمثلة في مستوى سيرم الدم من الكرياتين واليوريا في جميع الفئران المتناولة للتركيزات العلاجية للخضروات. بالمقارنة بالمجموعة الضابطة الموجبة أسفرت النتائج على وجود زيادة لها دلالة معنوية في مستوى هيموجلوبين الدم وكذلك نسبته في جميع مجموعات الدراسة. وبالنسبة للنتائج الهستوباثولوجية للمجموعة الضابطة الموجبة أظهرت أنسجة الكبد تغيرات حيوية وتآكل في خلايا الكبد مع وجود نزيف منتشر في جميع خلايا الكبد كما أظهرت في أنسجة القلب موت لبعض الألياف الحيوية في أنسجة العضلات مع ظهور التهاب حاد وأودما دموية بين أنسجة العضلات وذلك بالنسبة لقطاعات من العينة الضابطة الموجبة. أما في مجموعة الدراسة المعالجة بالخضروات فقد أظهرت النتائج الهستوباثولوجية في كل من أنسجة الكبد والقلب تغيرات طفيفة غير ظاهرة بوضوح وكانت القطاعات متشابهة مع أنسجة المجموعة الضابطة السالبة. ولذلك توصي الدراسة بتناول كل من قشر البطيخ الأبيض & الفلفل الأخضر & الجرجير حيث تعتبر من مصادر الخضروات الأكثر شيوعا وذات قيمة حيوية والتي تلعب دورا هاما في الإقلال من الكوليستيرول الكلى & والجليسيريدات الثلاثية & والليوبروتينات المنخفضة الكثافة & والليوبروتينات المنخفضة جدا في الكثافة في سيرم الدم.



## INTROUDYCTION

Cholesterols which when deposit in arteries, on its way from blood stream to cells, causing atherosclerosis leading to heart attack and strokes. Cholesterol, however, serves as raw material for hormones, Vit. D and bile. all the nutrients, fat is implicated most often as contributing factors to disease. **Whitney *et al.*, (1991).**

The Egyptian rates of morbidity and mortality from coronary artery disease seem to be underestimated due to shortage of diagnostic procedures and lack of registration .Most of Egyptian studies about coronary artery disease (CHD) and its risk factors are patient – based, illucidating risk in patient and comparing them with non – patient groups. In spite of these facts, reviewing the Egyptian studies may spotlight roughly on the extent of the CHD problem in Egypt. **WHO, (1979).**

Certain vegetables are rich in antioxidants, valuable to combat with atherosclerosis and coronary heart disease (CHD). Such as rocket, pippier and watermelon **Sanchez *et al.*, (2006).**

Rocket (*Eruca sativa mill.* )is widely distributed all over the world and is usually consumed fresh (leave or sprouts) for its typical spicy taste.And it does contain a number of health promoting agents including carotenoids, vitamin C, fibers, flavonoids and glucosinolates (GLs) wich play role to protect of atheroscaleros. **Barillari *et al.* , (2005) .**

Pippier is a vegetable of importance in human nutrition. Currently, one of the most interesting properties of natural products is their antioxidant content **Matoes *et al.*,( 2003).**

Watermelon is a rich natural source of lycopene, a carotenoid of great interest because of its antioxidant capacity and potential health benefits **Alison *et al.*, (2003).**

Rocket, pippier and watermelon were found to be also beneficial for liver, kidney functions, diabetics and atherosclerotic. Such vegetables a were pronouncedly effective in reducing serum TC,LDL and VLDL and raising the level of HDL.



In this work, the purification and characterisation of peroxisomes from vegetables of a higher plant was carried out, and their antioxidative effect on serum lipid fractions studied.

## MATERIALS AND METHODS

### Materials :

Garden rocket (*Eruce sativa* L), green pepper (*Capsicum annum* L) and watermelon (*Citrulus vulgaris*) skin have been bought from the local market at dokkei, Giza. Fresh and prepared for experiment.

### Methods:

#### Preparation of Vegetables:

Green pepper, watermelon skin and rocket leaves, cleaned by washing, dried and milled to fine powder in the Agricultural Research Center at Giza – Egypt.

Cholesterol as a pure with crystalline powder was purchased from Al-Gomhoriya Company for Med Preparations, Chemicals and Medical Equipments, Cairo, Egypt.

#### Experimental animals :

Since cholesterol metabolism in rats closely resembles that of human, albino rats are recommended to be used for atherosclerosis research **Bravo et al., (1994)**. In this concern forty male adult albino rats weighing ( $110 \pm 5$  g) each, rats were housed in individual stainless steel cages under controlled environmental conditions, in the animal house of the Faculty of Home Economics, Minufiya University and fed 7 days on basal diet prior to start feeding on experimental diet for acclimatization.

Animals had access to diets and water ad-libitum. Food and water checked daily, and rats weighed weekly.

#### Experimental design :

After the period of acclimatization, albino rats were assigned to ten groups (4 rats in each group):

The first Group:

(group, 4 rats): Rats fed on the basal diet (control -ve).

Other nine groups (36 rats) were fed first on hypercholesterolemic diet. (basal diet plus 1.5% cholesterol for 3 weeks to obtain hypercholesterolemic rats.

Group 2 (4 rats): hypercholesterolemic rats were fed on the basal diet only (as control +ve).



Group 3 (4 rats): Fed on hypercholesterolemic diet with (2.5%) watermelon skin powder.

Group 4 (4 rats): Fed on hypercholesterolemic diet with (5%) watermelon skin powder.

Group 5 (4 rats): Fed on hypercholesterolemic diet with (2.5%) green pepper powder.

Group 6 (4 rats): Fed on hypercholesterolemic diet with (5%) green pepper powder.

Group 7 (4 rats): Fed on hypercholesterolemic diet with (2.5%) rocket powder.

Group 8 (4 rats): Fed on hypercholesterolemic diet with (5%) rocket powder.

Group 9 (4 rats): Fed on hypercholesterolemic diet with (2.5%) blend of (watermelon skin, green pepper and rocket powders, 1:1:1).

Group 10 (4 rats): Fed on hypercholesterolemic diet with (5%) blend of (watermelon skin, green pepper and rocket powders, 1:1:1).

### **Tissue sample collection:**

During the experiment which lasted for 28 days the total body weight was recorded, also organs weight was recorded (liver, kidney, heart, spleen and lungs). At the end of the feeding period, (4 weeks) animals were fasted overnight, anesthetized (under ether). About 2-ml blood was withdrawn using retro orbital method into clean dry centrifuge plastic tubes, to obtain serum. Heparized micro tubes were also used for the estimation of hemoglobin and hematocrit. Blood samples centrifuged and serum obtained then stored at  $-20^{\circ}\text{C}$  until analysis. Total lipids, total cholesterol, triglycerides, HDL-c, LDL-c, risk rate and liver, function enzymes (ALP, GOT and GPT) were determined in the serum. Moreover, liver, heart, kidney and lung were excised, rinsed, blotted, weighed and kept in formalin solution (10%) till histopathological examinations carried out.

### **Calculation of some parameters:**

Biological evaluation of the different diets was carried by determination of body weight gain% (BWG) and organ/body weight % according to **Chapman *et al.*, (1959)**.

Using the following equations:

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

Organ weight



$$\text{Organ (weight \% of bodyweight)} = \frac{\text{Weight of rat at the end of experiment}}{\text{Weight of rat at the start of experiment}} \times 100$$

FER was calculated by division of daily body weight gain (g)/daily food intake (g) according to **Lee and Nieman, (1996)**.

### **Biological Assay:**

The following determinations were carried out on serum and liver samples.

Serum Triglycerides (TG), total cholesterol (TC), total lipids (TL), (HDLc) were determined by using enzymatic colorimetric methods of **Fassati and Prencipe, (1982); Richmond et al., (1973); and Allain et al., (1974); Zollner, (1962); Burstein et al., (1970) and Lopez et al., (1977)** respectively.

The concentration of VLDL was estimated according to the method of **Lee and Nieman, (1996)** as follows  $\text{VLDL cholesterol (mg/dl)} = \text{triglycerides} / 5$ .

Atherogenic Index were calculated as

$$\text{Atherogenic Index (AI)} = \frac{\text{VLDL} + \text{LDL}}{\text{HDL}}$$

The concentration of (AI) was estimated according to **Kikachi et al., (1998)**.

Serum aspartat and alanine amino transfeeres (AST,ALT and alkaline phosphates ALP) were determind by using enzymatic colorimetric method after **Reitman and Frankel, (1957) and Haussement, (1977)**. Determination of serum creatinine, urea and hemoglobin was carried out by using enzymatic colorimetric methods according to **Schimeister, (1964); Fawcett and Soctt, (1960) and Drabkin, (1932)**.

### **Histopathological Test:**

Specimens from liver, kidney, spleen, heart and lungs were collected and kept in 10% buffered formalin, and dehydrated in ethylalcohol cleared in xylol. Then, embedded in paraffin 4-6 M thick sections were prepared and stained with hematoxylin and eosin accoring to **Bancroft et al., (1996)**.

### **Statistical analysis :**

Statistical analysis were performed by using computer program statistical package for social science **SPSS, (1998)**, and compared with each other using the suitable tests.

## **RESULTS AND DISCUSSION**

This section presents the results of the effect of some antioxidants which found in some vegetables on the hypercholesterolemic rates.

### **Nutrition Results**

Data in table (1) show the mean value of body weight (%), food intake and FER. The hypercholesterolemic rats of different treatments showed





nonsignificant difference in weight gain percent in comparing with control group(+), except rat groups which consumed rocket 5%,green pepper(2.5%) and mixed vegetables (blends 2.5%, 5%) which showed significant decrease in weight gain percent as comparing with control (+ve) group ( $p<0.01$ ).The mean value of daily food intake was ( $14.28\pm 3.48$ g) in control (-ve) group. There were nonsignificant differences between hypercholesterolemic rats groups of different treatments in comparison with control (+ve) group. Meanwhile rats of green pepper 5%, watermelon 5%, rocket 2.5% tended to show higher FER (0.16 , 0.15 & 0.14) respectively than that of control (+ve) rats (0.13); being comparable to control (-ve) group (0.17). There was nonsignificant difference between watermelon (5%) and pepper (5%) treated rats groups which showed significant increase compared with the rest of experimental groups in FER. Anyhow, highest weight gain% & FER was recorded for green pepper( 5%), followed by watermelon (5%) and green rocket (2.5%) groups.

**Table. (1): Mean values of body weight gain, food intake and food efficiency ratio (FER) of control and hypercholesterolemic rat groups treated with vegetables.**

Variables Groups	Weight Gain (%)	Daily Food Intake (g)	Food Efficiency Ratio (FER)
Control (-ve)	$30.93\pm 2.51$	$14.28\pm 3.48$	$0.17\pm 0.02$
Hypercholesterolemic Control (+ve)	$24.79\pm 3.74$	$14.61\pm 3.42$	$0.13\pm 0.09$
Watermelon (2.5%)	$22.34\pm 2.95$	$13.91\pm 3.11$	$0.12\pm 0.01$
Watermelon (5%)	$25.87\pm 3.01$	$13.82\pm 3.07$	$0.15\pm 0.10$
Green Pepper (2.5%)	$22.20\pm 2.88$	$13.52\pm 3.05$	$0.13\pm 0.05$
Green Pepper (5%)	$26.23\pm 2.18$	$13.18\pm 3.02$	$0.16\pm 0.02$
Rocket (2.5%)	$24.09\pm 2.23$	$13.21\pm 4.05$	$0.14\pm 0.06$



<b>Rocket (5%)</b>	21.09*±1.84	13.21±3.01	0.12±0.06
<b>Blend (2.5%)</b>	20.95*±0.98	13.16±3.09	0.12±0.06
<b>Blend (5%)</b>	20.12*±0.82	13.11±3.00	0.12±0.06

Significant with control (+ve) group at levels:

\* p<0.05

\*\*p<0.01

\*\*\*p<0.001

These results (table1) agreed with that of **Kahlon *et al.*, (1997)** and **Salem, (1999)** who claimed that diets containing cholesterol were poorly utilized by the rats or they impaired metabolism, resulting in lower weight gain compared with normal control group. These results are also similar to that obtained by **Owiss, (1999)** who reported that body weight gain of cholesterol supplemented group was lower than that of control (-ve) group but the difference was not significant. **National Research Council, (1995)** was reported that high – energy diet usually leads to decrease food consumption. The fluence of B – carotone on food intake may be attributed to decrease the diet palability as





reported also by Hashim,(1997). This may be the reason for the insignificant decrease of daily food intake by rats on watermelon, green pepper, rocket and the blends, compared to control (-ve) rats.

### **Serum lipid pattern:**

Data presented in table (2) show the serum lipids fraction of rats groups. Therefor serum cholesterol value which greatly increased in hypercholesterolemic rats (control+ve group) in comparison with that of the control (-ve) group showed significant decrease  $p<0.05$  when feeding on watermelon (2.5 & 5%) and pepper (2.5%), ( $162.87\pm 5.6$ ,  $168.63\pm 9.23$  and  $143.79\pm 9.98$ )mg/dl when compared to control (+ve) group ( $197\pm 7.11$ ) mg/dl .Meanwhile lowest TC was recorded for serum of rocket( 5%) rats ( $116.03\pm 6.99$ ) mg/dl followed by rocket (2.5%) group ( $120.55 \pm 6.23$ ) mg / dl, then came green pepper group (5%) ( $129.76 \pm 7.61$ ) mg / dl.

Concerning serum triglyceride, the data showed high significant increase in control (-ve) group ( $p<0.01$ ), as the value being ( $88.61\pm 3.43$ ) mg/dl compared to hypercholesterolemic control (+ve) group ( $115.18 \pm 4.14$ ) mg/dl. The rats group which treated with rocket (2.5&5%), watermelon (5%), green pepper (2.5%) and blend (5%) was showed significant decrease ( $p<0.05$ ) when compared to control (+ve) group, while rats which treated with green pepper (5%), showed high significant decrease ( $P<0.01$ ) when compared to the level of control (+ve) group. Lowest TG level was recorded for green pepper group 5% ( $92.18\pm 7.86$ ) mg/dl.

HDL-c showed significant increase in (control-ve) group. ( $p<0.05$ ) than the control (+ve) group. The rats received watermelon (5%), Pepper (2.5)%, rocket (2.5)% and the blend (2.5)&(5%) showed significant difference increase ( $p<0.05$ ), compared to control (+ve) rats, most increase was found for green pepper (5%) followed by rocket (5% ) diet groups, which showed significant increase ( $p<0.01$ ) when compared to control (+ve) rats.

The mean values of LDL-c in control (-ve) group showed very high significant increase ( $p<0.001$ ) than the hypercholesterolemic (control+ve) group. Rocket (2.5&5%) treated diets showed pronounced decreases ( $p<0.001$ ) than the control (+ve) group, while rats group which consumed watermelon (2.5%) ,blend (2.5&5%) and green pepper (2.5 & 5%) showed less decrease .Least LDL levels were recorded for rocket (5%) followed by rocket (2.5%) diets.

The mean values of VLDL-c in control (-ve) group showed significant increase ( $p<0.05$ ) than hypercholesterolemic control (+ve) group. All groups watermelon (2.5 % & 5 %), and blend (2.5%) treated groups showed nonsignificant difference than for control (+ve) rats, while least levels were found for green pepper (5%) followed by rats group rocket (5%), blend (5%), pepper (2.5%) and rocket (2.5%) diets.



Risk ratio: values were high significantly decreased in control (-ve) rats ( $p < 0.01$ ) when comparing hypercholesterolemic control (+ve) group with, which were (2.25&6.28) respectively.

Least risk ratio was found for rocket (5%), green pepper (5%) & rocket (2.5%), being  $2.40 \pm 0.63$ ,  $2.61 \pm 0.93$  and rocket (5%) being  $2.67 \pm 0.97$  respectively.

Serum Total Lipid (mg/dl) value were low ( $96.44 \pm 9.09$ ) mg/dl in control (-ve) group, but very high significantly increased in control (-ve) rats being ( $96.44 \pm 9.09$ ) mg/dl ( $P < 0.01$ ) in comparison with hypercholesterolemic rats (control +ve group) While rats groups treated with watermelon (2.5%), Rocket (2.5%) and blend (2.5&5%) levels showed nonsignificant difference. Least values are recorded for green pepper (5%) followed by green pepper (2.5%), rocket (5%) and watermelon (5%) diets.

The mean values of (AI) in control (+) hypercholesterolemic rats group were ( $5.32 \pm 6.98$ ). The mean value of (AI) in control (-ve) group was significantly lower being ( $1.25 \pm 1.57$ ).  $p < 0.01$  respectively than control (+ve) group.

While the rats which received watermelon (2.5%) showed nonsignificant (AI) when compared to control (+ve) group. The rats which received green pepper (2.5, 5%) and rocket (2.5%) showed significant decrease in (AI) when compared to control (+ve) group ( $P < 0.01$ ).

Also the rats which received watermelon (5%) and blend (2.5,5%) showed significant decrease in (AI) when compared to control (+ve) group  $P < 0.05$ .

On the other hand the best result showed in rats which received rocket (5%) showed which revealed significant decrease in (AI) when compared to control(+ve) group  $P < 0.001$ .



**Table. (2): Mean values of (TC, TG, HDL,LDL,VLDL, R.R, TL and AI) of control and hypercholesterolemic rats groups treated with vegetables.**

Variables Groups	TC Mg/dl	TG Mg/dl	HDLc Mg/dl	LDLc Mg/dl	VLDLc Mg/dl	Risk Ratio (R.r)	AI	Total Lipids Mg/dl
Control (-ve)	101***±5.59	88.61**±3.43	44.97±4.58	38.31***±3.09	17.722*±4.59	2.25**±1.90	1.25***±1.57	96.44***±9.09
Hypercholesterolemic Control (+ve)	197±7.11	115.18±4.14	31.35±2.38	142.61±7.71	23.036±10.02	6.28±3.52	5.32±6.98	208.85±18.36
Watermelon (2.5%)	162.87*±5.6	107.21±8.64	38.80±4.08	102.63*±6.52	21.442±5.62	4.20*±1.32	3.20±3.30	121.85*±9.21
Watermelon (5%)	168.63*±9.23	101.82*±11.68	42.22*±6.11	106.05*±9.93	20.364±4.49	4.0*±2.02	3.00*±3.01	116.72*±8.11
Green Pepper (2.5%)	143.79*±9.98	95.63*±9.19	47.02*±5.61	77.64**±9.76	19.126*±5.02	3.06**±1.43	2.06**±2.41	112.2**±8.97
Green Pepper (5%)	129.76**±7.61	92.18**±7.86	49.74**±6.00	60.58**±6.63	18.436*±6.30	2.61**±0.93	1.61**±1.87	108.16**±9.92
Rocket (2.5%)	120.55**±6.23	98.61*±7.39	45.15*±6.61	55.68***±4.99	19.722*±5.92	2.67**±0.97	1.67**±1.90	124.37*±9.43
Rocket (5%)	116.03***±6.99	95.11*±9.04	48.42**±5.40	48.59***±5.22	19.022*±3.00	2.40**±0.63	1.40**±1.60	115.11*±8.00
Blend (2.5%)	132.46*±7.83	102.92±8.97	44.82*±5.40	67.06**±7.64	20.584±4.99	2.95**±0.99	1.95**±1.97	135.31*±9.8
Blend (5%)	129.86**±7.71	95.56*±9.11	46.43*±4.85	64.32**±7.82	19.112*±4.05	2.80**±0.45	1.80**±1.40	128.71*±9.22

Significant with control (+ve) group at levels:

\* p<0.05

\*\*p<0.01

\*\*\*p<0.001

These results are in agreement with that of **Cherubini et al., (2005)** who found that intake of vegetables, relation between serum T.C and risk of coronary heart disease, due to consumption of carotenoids. Also **Rimando and Perkins, (2005)** found that watermelon protected body from oxide lipids oxidation, while **Collins et al., (2004)** found that the supplemented of diet with watermelon did



not affect plasma lipid concentrations, and **Hayashi *et al.*, (2001)** observed that watermelon contained a large amount of fibers which play role to reduced the lipid in body. **Osganian *et al.*, (2003)** found a relation between vitamin C intake and coronary heart disease (CHD). Users of vitamin C supplements appear to be at lower risk for CHD. These results are also similar to that obtained by **protogerous *et al.*, (2004)** and **Das *et al.*, (2006)** observed that ascorbic acid reduced hypercholesterolemia. Also **Hachiya *et al.*, (2007)** found that sweet pepper (rich in vit .C) does not effect systolic blood pressure ( BP) or heart rate (HR). **Glass and Witztum, (2001)** recorded that increased plasma cholesterol, particularly LDL-c are one of the most important risk factor of coronary vascular disease. LDL-c particle are taken up by macrophage cells after oxidized or modified and then deposited in the arterial intima leading to formation of atheroma. Low HDL-c levels are considered as a strong risk factor for coronary heart disease as HDL-c act as antioxidant and protect LDL-c from oxidation so that reduce LDL-c from circulation. **Monserenusorn, (1983)** and **Vijayakumar and Nalini, (2006)** found that green pepper significantly decreased total cholesterol, phospholipids ratio, lipids ratio, VLDL, LDL and free fatty acids. But significantly raised the HDL level.

#### **Kidney functions:**

The mean values of creatinine and urea are shown in table (3) in control (-) group were (0.65±0.04 and 39.33±2.27) mg/dl. Control (-) groups showed significant increase than Hypercholesterolemic rats (control +ve) (p<0.05). While rats which received all treatment showed significant decrease (P<0.05) in creatinine.

Although all tested vegetables improved the renal function of hypercholesterolemic rats, watermelon (5%) diet showed the best effect in concern to creatinine, and the blend (5%) diet improved better the urea level.

**Table. (3): Mean values ± SD of serum levels creatinine and urea in control and hypercholesterolemic rats groups treated with vegetables.**

Variables Groups	Creatinine Mg/dl	Urea Mg/dl
Control (-ve)	0.65*±0.04	39.33*±2.27
Control (+ve)	1.62±0.08	68.16±3.71
Watermelon (2.5%)	0.57*±0.08	44.12±2.39
Watermelon (5%)	0.56*±0.06	42.87*±2.74
Green Pepper (2.5%)	0.62*±.01	45.08±2.72
Green Pepper (5%)	0.63*±0.18	42.76*±2.26
Rocket (2.5%)	0.59*±0.06	45.25±2.02
Rocket (5%)	0.57*±0.08	41.06*±3.22



Blend (2.5%)	0.60*±0.01	42.17*±2.03
Blend (5%)	0.59*±0.02	40.38*±2.21

Significant with control (+ve) group at levels:

\* p<0.05

\*\*p<0.01

\*\*\*p<0.001

These results are in agreement with that of Sarwar *et al.*, (2007) found that intake of rocket extract (50 -200) mg/kg body weight for 7days significantly reduced serum creatinine and blood urea nitrogen levels.

**Liver functions:**

The mean value in table (4) of GPT (ALT) in control (-ve) group were (34.2±2.98) U/L while the mean value of (GPT) in control (+ve) group were (55.16±2.28) Supplemented with rocket (5%) and blend (5%) showed significant decrease p<0.01 in comparison with control (+ve) group. Moreover there was nonsignificant differences in GPT and GOT of rats received green pepper (5%), rocket (2.5&5%) and blends (2.5 & 5%) when compared with that of control (-ve) group. Anyhow the mean values of GOT increased significantly in control (-ve) group when compared with hypercholesterolemic rats serum at (p<0.01).

Lowest GPT was found for rocket (5%), followed by blend (5%), blend (2.5%), and rocket (2.5%) group. Lowest GOT was found for treatment rocket (5%). Values of mentioned groups were greatly less than that of control (+ve) rats. The mean value of ALP in control (-ve) group showed significant increase (p<0.05) in comparison with hypercholesterolemic (+ve) group, while there was non significant differences in ALP of pepper (2.5), (5)%, rocket (2.5)%, (5)% and blend (5)% comparing to control (-ve).

Anyhow, lowest ALP was recorded for green pepper (5%), rocket (5%), (2.5%), green pepper (2.5%) and blend (5%) diets as compared with that found for hypercholesterolemic control (+ve) group, indicating improvement of the liver function.

**Table (4): Mean values±SD of serum amino transferase enzymes (GPT, GOT and ALP) in control and hypercholesterolemic rats groups treated with vegetables.**

Variables Groups	GPT U/L	GOT U/L	ALP U/L
Control (-ve)	34.2**±2.98	129**±5.77	122.87*±1.92
Control (+ve)	55.16±2.28	158.04±4.80	134.45±4.96
Watermelon (2.5%)	50.70±4.16	140.15±6.75	130.11±5.60
Watermelon (5%)	47.50*±4.93	137.37*±4.41	128.72±3.44
Green Pepper (2.5%)	48.50±2.72	142.18±9.8	126.96*±2.99



Green Pepper (5%)	45.50*±3.89	135.76*±6.72	124.96*±1.99
Rocket (2.5%)	45.90*±5.97	136.81*±9.44	126.80*±2.78
Rocket (5%)	39.90**±3.49	131.67**±7.92	125.50*±2.32
Blend (2.5%)	42.15*±2.20	137.12*±8.02	129.04±4.74

Variables	H.b g/dl
Groups	
Control (-ve)	13.32*±2.35
Hypercholesterolemic	11.46±2.11

Blend (5%)	40.0**±4.63	134.54*±7.95	127.9*±3.59
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Significant with control (+ve) group at levels

\* p<0.05

\*\*p<0.01

\*\*\*p<0.001

These results in table (4) for GOT were in parallel with that of **Harrison et al., (1966)** who reported that the increase of GOT and GPT are closely correlated in most cases with liver diseases.

All vegetables treatments (Table 5) raised Hb level compared to control (+) group, in particular watermelon (5%) group.

**Table.(5): Mean values± SD of hemoglobin (Hb) of control and hypercholesterolemic rats groups treated with some vegetables.**





Control (+ve)	
Watermelon (2.5%)	13.72*±2.66
Watermelon (5%)	13.84*±3.87
Green Pepper (2.5%)	13.43*±2.11
Green Pepper (5%)	13.25*±2.98
Rocket (2.5%)	13.08*±2.29
Rocket (5%)	13.06*±2.21
Blend (2.5%)	13.38*±2.38
Blend (5%)	13.43*±2.90

Significant with control (+ve) group at levels :

\*  $p < 0.05$

\*\*  $p < 0.01$

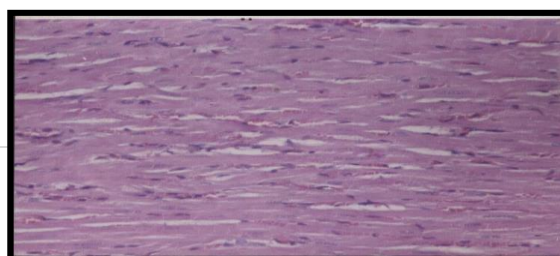
\*\*\*  $p < 0.001$

These results are in agreement with that of **Kahlon et al ., (1997)** as reported for Hb may be resulting from poor unilization of iron and defects in metabolism that occur by feeding the animals diet containing cholesterol which affect the unilization of iron in comparison with basal diet. Also this could be also due to the effect of oxidants mediated cellular damage in hypercholesterolemic rats **Feri et al., (1989)**, and peroxidation damage of red blood cells membrane as reported by **Bieri et al., 1976)**. **Sies and Stahl, (1995) and Gey,(1995)**.

### Histopathological Results

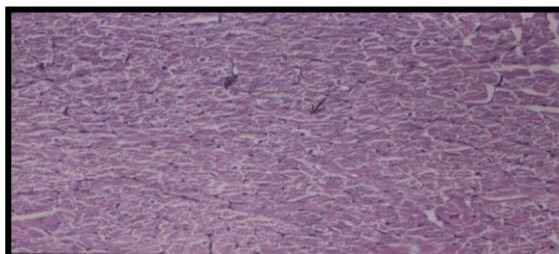
#### Heart:

Microscopically, heart of rat from control (-ve) group, revealed normal myocardial muscle (**Pict.1**). On the other hand, heart of rat from hypercholesterolemic control+ve group showed granularity of the sarcoplasm of myocardial muscle (**Pict.2**), intermuscular edema and focal mononuclear leucocytic cells infiltration (**Pict.3**). Heart of rat from hypercholesterolemic rat treated with watermelon (2.5%) showed zenker's necrosis of sporadic muscle fibers (**Pict.4**) . Meanwhile, heart of rat from hypercholesterolemic rat treated with watermelon (5%) showed no histopathological changes (**Pict.5**). However, the only change observed in heart of rat from hypercholesterolemic group treated with green pepper (2.5%) was slight congestion of myocardial blood vessels (**Pict.6**). Conversely, heart of rats from hypercholesterolemic rat treated with green pepper (5%), green rocket (2.5%), green rocket( 5%), blend (2.5%), blend( 5%), showed no histopathological changes (**Pict. 7, 8, 9, 10,11**) .

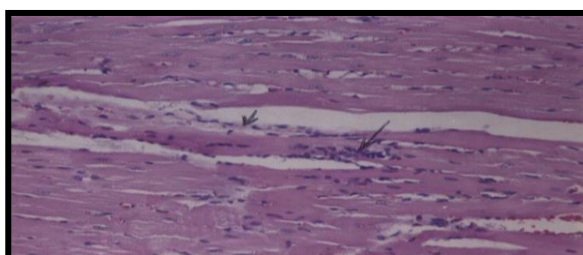




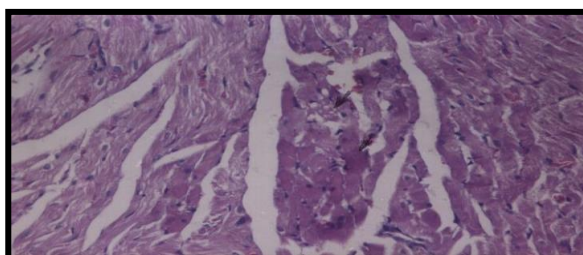
**Pict. (1):** Heart of rat from control (-ve) group, showing normal myocardial muscle fibers (H and E X 200).



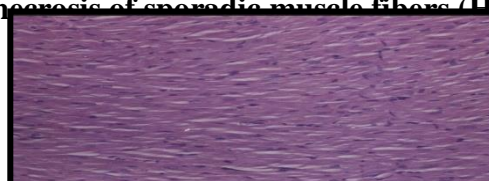
**Pict. (2):** Heart of rat from hypercholesterolemic control (+ve) group showing granularity of the sarcoplasm of myocardial muscle fibers (H and E X200).



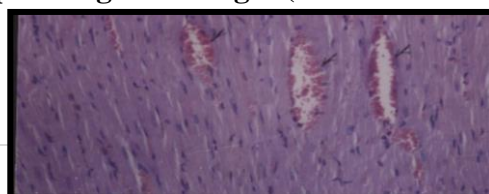
**Pict. (3):** Heart of rat from hypercholesterolemic control (+ve) group showing intermuscular edema and focal mononuclear leucocytic cells infiltration (H and E X 200).



**Pict. (4):** Heart of rat from hypercholesterolemic group treated with watermelon(2.5%) showing zenker, s necrosis of sporadic muscle fibers (H and E X 200).

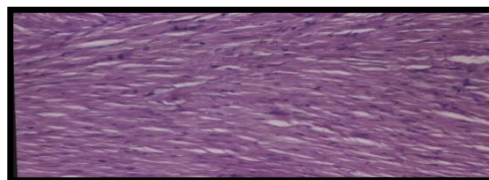


**Pict. (5):** Heart of rat from hypercholesterolemic group treated with watermelon(5%) showing no histopathological changes (H and E X 200).

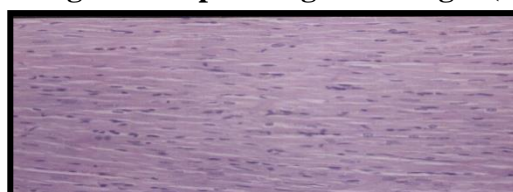




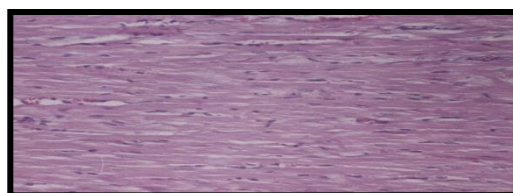
**Pict. (6):** Heart of rat from hypercholesterolemic group treated with green pepper(2.5%) showing slight congestion of myocardial blood vessels (H and E X 200).



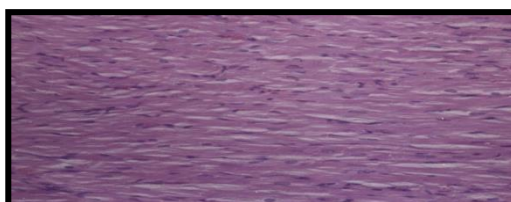
**Pict. (7) :** Heart of rat from hypercholesterolemic group treated with green pepper (5%) showing no histopathological changes (H and E X 200).



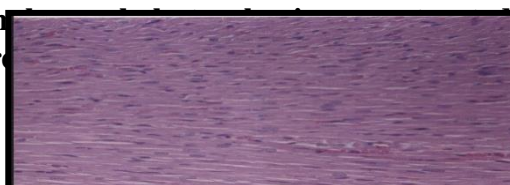
**Pict. (8) :** Heart of rat from hypercholesterolemic group treated with green rocket (2.5%) showing no histopathological changes (H and E X 200).



**Pict. (9):** Heart of rat from hypercholesterolemic group treated with rocket (5%) showing no histopathological changes (H and E X 200).



**Pict. (10):** Heart of rat from hypercholesterolemic group treated with blend (2.5%) showing apparent normal cardiac muscle fibers (H and E X 200).

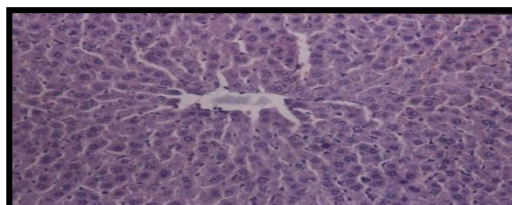


**Pict. (11):** Heart of rat from hypercholesterolemic group treated with blend (5%) showing apparent normal cardiac muscle fibers (H and E X 200).

**Liver:**

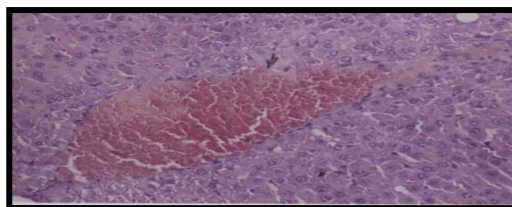


Histopathological examination of liver of rat from control-ve group revealed the normal histology of hepatic lobule (**Pict.12**). However, Liver of rat from hypercholesterolemic control+ve group showed marked dilatation and congestion of central vein (**Pict.13**). As well as vacuolar degeneration of some hepatocytes (**Pict.14**). Meanwhile, the only change observed in liver of rat from hypercholesterolemic rat treated with watermelon (2.5%) was hydropic degeneration of centrolobular hepatocytes (**Pict.15**). No histopathological changes were noticed in liver of rat from hypercholesterolemic rat treated with watermelon (5%), green pepper (5%). (**Pict.16,18**). Mean while, liver of rat from hypercholesterolemic rat treated with green pepper (2.5%) showed appearance of newly formed bile ductules (**Pict.17**). Examined liver of rat from hypercholesterolemic rat treated with green rocket (2.5%) revealed focal area of hepatic necrosis (**Pict.19**). Moreover, liver of rat from hypercholesterolemic rat treated with green rocket (5%) showed minute vacuoles in the cytoplasm of hepatocytes (**Pict.20**). However, liver of rat from hypercholesterolemic rat treated with blend (2.5%) showed focal areas of hepatic necrosis associated with mononuclear cells infiltration (**Pict.21**). Some examined sections of the same group showed apparent normal hepatocytes (**Pict.22**). Some examined sections of rat from hypercholesterolemic rat treated with mixed (5%) showed proliferation of epithelial lining bile duct associated with appearance of newly formed bile ductules (**Pict.23**). While, other examined sections revealed no histopathological changes (**Pict.24**).



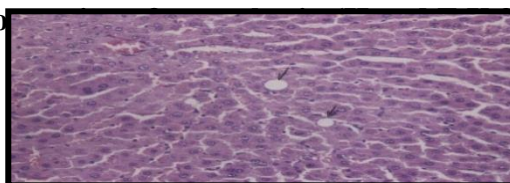
**Pict. (12):** Liver of rat from control (-ve) group, showing normal histology of hepatic lobule.

(H and E X 200).



**Pict.(13) :**Liver of rat from hypercholesterolemic control (+ve) group showing marked

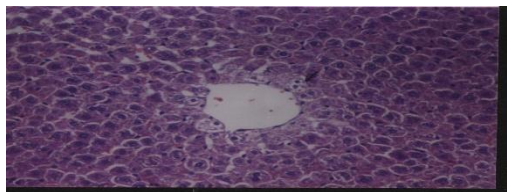
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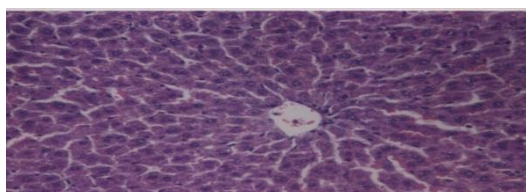




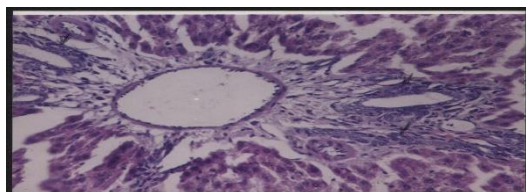
**Pict.(14):** Liver of rat from hypercholesterolemic control (+ve) group showing vacuolar degeneration of some hepatocytes (H and E X 200).



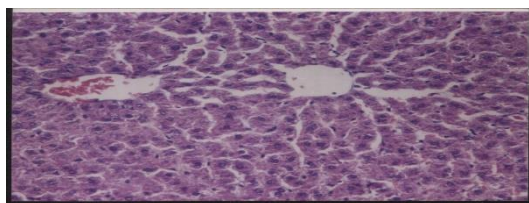
**Pict .(15) :** Liver of rat from hypercholesterolemic group treated with watermelon(2.5%) showing hydropic degeneration of centrolobular hepatocytes (H and E X200).



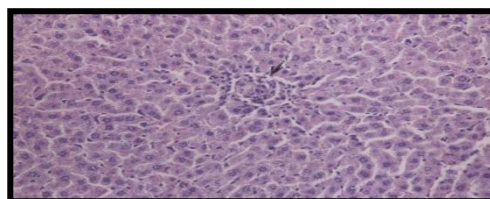
**Pict. (16):** Liver of rat from hypercholesterolemic group treated with watermelon(5%) showing no histopathological changes (H and E X 200).



**Pict. (17):** Liver of rat from hypercholesterolemic group treated with green pepper(2.5%) showing newly formed bile ductules (H and E X 200).

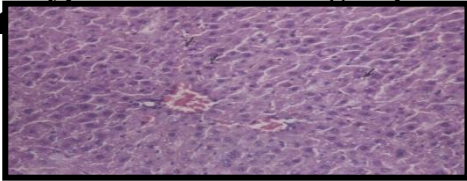


**Pict. (18):** Liver of rat from hypercholesterolemic group treated with green pepper(5%) showing apparent normal hepatocytes (H and E X 200).

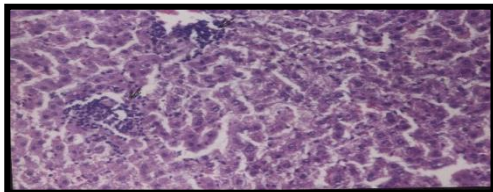




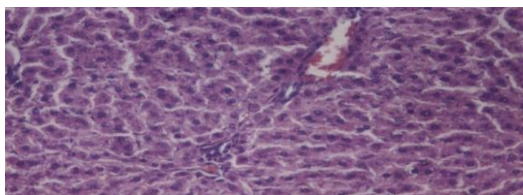
**Pict. (19):** Liver of rat from hypercholesterolemic group treated with green rocket (2.5%) showing  (H and E X 200)



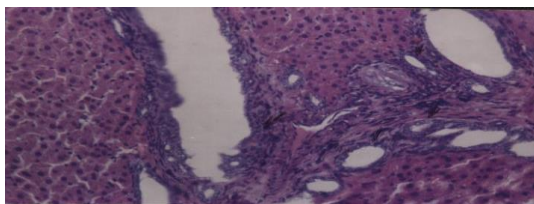
**Pict . (20):** Liver of rat from hypercholesterolemic group treated with rocket (5% ) showing minute vacuoles in the cytoplasm of hepatocytes (H and E X 200).



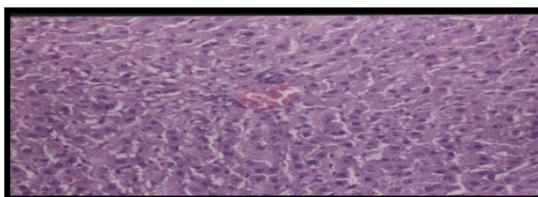
**Pict. (21):** Liver of rat from hypercholesterolemic group treated with blend (2.5%) of hepatic necrosis associated with mononuclear cells infiltration. (H and E X 200).



**Pict. (22):** Liver of rat from hypercholesterolemic group treated with blend (2.5% )showing apparent normal hepatocytes (H and E X 200).



**Pict. (23):** Liver of rat from hypercholesterolemic group treated with blend( 5%) showing proliferation of epithelial lining bile duct associated with appearance of newly formed bile ductules (H and E X 200).







**Pict. (24) : Liver of rat from hypercholesterolemic group treated with blend (5%) showing no histopathological changes (H and E X 200).**



These results are in agreement with those reported by **Brigelius *et al.*, (2005)** found that antioxidants, preferentially those of dietary origin, have for along time been considered to help against diseases that are presumably aggravated by oxidative stress, such as cardiovascular diseases, cancer and neurodegenerative disorder. **Leborgne *et al.*, (2005)** demonstrated that Antioxidant supplementation resulted in a 50% decrease in plaque area of both control and irradiated animals. Antioxidants reduced both the cholesterol-induced and radiation-enhanced circulating and tissue oxidized LDL levels, resulting in reduced plaque. **Sanchez *et al.*, (2006)** reported that consumption of pepper and vegetables is associated with a reduced risk of death from all causes including heart disease and stroke. **Liu *et al.*, (2001)** established research data suggest that higher intake of vegetables may be protective against CVD and support current dietary guidelines to increase vegetable intake. **Sarwar *et al.*, (2007)** found that rocket extract contained large amounts of antioxidant such as carotene, polyphenols and flavonoids. And rocket extract diet significantly reduced the lipids peroxidation. Previously, **EL-Missiry and EL-Gindy, (2000)** and **Martizen *et al.*, (2007)** found that the rocket decreased low density lipoprotein cholesterol in serum.

**Leborgne *et al.*, (2005)** found that rabbits fed with 1 % cholesterol diet for 7 days and a mixture of antioxidants 4 weeks later had plasma significantly higher plasma antioxidants levels. and accordingly lowered the oxidized LDL levels. Moreover antioxidant supplementation resulted in a 50% decrease in artery plaque area. Antioxidants reduced both the cholesterol and oxidized LDL levels.

## REFERENCES

- Alison, J.E.; Bryan, T.V.; Eugene, R.; Wiley, E.D.; Brown, J.K.; Collins, P.; Robert, A.B. and Beverly, A.C. (2003):** Consumption of watermelon Juice increases plasma concentrations of lycopene and B – carotene in humans . Journal Nutrition, 133 : 1043 –1050.
- Allain, C.Z. ;Poon,L.S.and Chan,C.S.(1974)** :Enzymatic determination of total serum cholesterol . Clin.Chem.,20 , 470-475.
- Bancroft, D. ;Steven, A .and Turner , R .(1996)** : Theory and practice of Histological Techniques 4<sup>th</sup> ED.Churchill Living stone, Edinburgh, London, Melbourne.
- Barillari, J.; Canistro, D.; Paolini, M.; Ferroni, F.; Pedulli, G.F.; Iori, R. and Valgimigli, L. (2005):** Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (*Eruca stiva L.*) seeds and sprouts .J. Agric Food Chem. ,53 (7) : 2475-82.



- Bieri, J.G.; Evarts, R.P. and Gart, J. J. (1976):** Relation activity of  $\alpha$ -tocopherol and  $\gamma$ - tocopherol in preventing oxidative red cell hemolysis. J. Nutr., 106: 124-127.
- Bravo,E. ;Cantafora, A.;Calcabvini,A.and Ortu,G.(1994) :** Why prefer the godlen syrian hamster (*Mesocricetus auratus*) to the wistar rat in experimenal studies on plasma lipoprotein metabolism?. Comp Biochem.Physiol., 107(B):347-255.
- Brigelius,F.R.;Kluth,D.and Banning,A.(2005):** Is there a future for antioxidants in atherogenesis? Mol. Nutr. Food Res., 49(11): 1083-9.
- Burstein, M. (1970):** HDL cholesterol and risk ratio determination after separation high density lipoprotein. Lipid. Res., 11: 583. *Capsicum* in rats .Res. Commun. Chem Pathol. Pharmaced., 41(1):95-110.
- Chapman ,D.G. ;Castilla,R.and Compell,J.A.(1959):** Evaluation of protein in food .I.A method for the determination of protein efficiency ratio. Can. J.Biochem.Physiol., 37:679-686.
- Cherubini, A.; Viqna, G.B.; Zuliani, G.; Ruqqiero, C.; Senin, U. and Fellin, R. (2005):** Role of antioxidants in atherosclerosis: Epidemiological and clinical update. Pharm.Des., 11(16):201732.
- Collins, J.K.; Arjmandi, B. H.; Claypool, P.L.; Perkins, V. P.; Baker , R.A . and Celvidence ,B.A . (2004 ):** Lycopene from two food sources does not affect antioxidant or cholesterol status of middle – aged adults . Nutr .J., 15; 3:15 .
- Das,S. ; Snehlata,D.N. and Srivastava,L.M.(2006):** Role of ascorbic acid on in vitro oxidation of low density lipoprotein derived from hypercholesterolemic patients.Clinica Chimica .Acta.,10(3):16.
- Drabkin, D.L. (1932):** Haemoglobin determination after enzymatic hydrolysis. J . Biol . Chem . ,98:719.
- EL-Missiry, M.A. and EL-Gindy, A.M.(2000):** Amelioration of alloxan induced diabetes mellitus and oxidative stress in rats by oil of *Eruca sativa* seeds. Ann.Nutr.Metab.,44(3):97-100.
- Fassati, P. and Prencipe, L. (1982):** Triglycerides determination after enzymatic hydrolysis . Clin .Chem., 28 :2077.
- Fawcett, J.K. and Soctt, J.E. (1960):** Urea determination after enzymatic hydrolysis. J. Clin. Path., 13:156 – 159.
- Feri, B.; England, L. and Ames, B.N. (1989):** Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Nat. Acad. Sci., 86: 6377-6381.
- Gey, K.F. (1995):** Ten years retrospective on the antioxidant hypothesis of Arteriosclerosis: Threshold plasma level of antioxidant micronutrients related to minimum cardiovascular risk. J. Nutr. Biochem., 6: 206-236.
- Glass, C.K. and Witztum, J.L. (2001):** Atherosclarosis: The road ahead cell, 104(4): 503-516.



- Hachiya, S.; Kawabata, F.; Ohnuki, K.; Inoue, N.; Yoneda, H.; Yazawa, S. and Fuskiki, T. (2007):** Effects of CH-19.Sweet, a non – Pungent cultivar of red pepper on sympathetic nervous activity , body temperature, heart rate, and blood pressure in humans. *Biosci Biotechnol Biochem.*, 71(3):671 – 6.
- Harrison, T.R.; Adams, R.D.; Bennett, I.L.; Resink, W.H.; Thorn, G.W. and Wintrobe, N.M. (1966):** Principles of Internal Medicine. Fourth edition, P. 164-165. Mc Grawth Hill Book Company. Inc. New York.
- Hashim, A.M. (1997):** Safety Aspects of Some Synthetic Antioxidants Used in the Oils in Egypt.MS.C Thesis, Faculty of Home Economics, Helwan Univeristy .
- Haussement , T. U.(1977) :** Determination of alkaline phosphatase. *Clin.Chem.Acta* , 35: 271 -273.
- Hayashi, K.; Hara, H.; Asvarujanon, P.; Aoyama, Y. and Luanapituksa, P. (2001):** Ingestion of soluble dietary fibere increased zinc and iron absorption and restored growth rate and zinc absorption suppressed by dietary phytate in rats .*J.Nut .*, 86(4):443-57.
- Kahlon, T. S.; Chow, F. I.; Trving, D.W. and Sayre, R.N. (1997):** Cholesterol response and fatty streak formation in hamsters fed two levels of saturated fat and various levels of cholesterol . *Nutr. Res .*, (11/12) : 1693 – 1707.
- Kikachi, K.H.; Onder, N.; Natubara, S.; Yasuda, E.; Chonan, O.; Takahas, R. and Ishikawa, F.(1998):** Effect of Soymilk and bifidobacterium in aged ovarictomized rats. *Bioscience Biotech-nology and Biochemistry.*, 62(9):1688-1692.
- Leborgne, L.; Pakala, R.; Dilcher, C.H.; Hellinga, D.; Seabron, R.; Tio, F.O. and Waksman, R. (2005) :**Effect of antioxidants on atherosclerotic plaque formation in ballon– denuded and irradiated hypercholestrolemic rabbits. *journal of Cardiovascular pharm-acology.*, 46(4):540 –547.
- Lee, R. and Nieman, D. (1996):** Nutritional Assessment .2nd Ed., Mosby, Missouri, USA.
- Liu, S.; Lee, I.M.; Ajani, U.; Cole, S.R.; Buring, J.E. and Manson, J.E. (2001):** Intake of vegetables rich in carotenoids and risk of coronary heart disease in men. *Int. J. Epidemiol* , 30(1) : 130-5.
- Lopez, M.F. (1977):**HDL and Risk Ratio determination after enzymatic Hydrolysis. *J . Clin .Chem.*, 23,882.
- Martinez, S. ; Sanchez, A.; Llorach, R.; Gil, M.I. and Ferreres, F. (2007):** Identification of new flavonoid glycosides and flavonoid profiles to characterize rocket leafy salads (*Eruca vesicaria* and *Diplotaxis tenuifolia*) .*J.Agric.Food Chem.*, 21,55(4):1356-63.
- Mateos, R .M.; Leon, A . M.; Sandalio, L. M.; Gomez, M.; Del, R.L. and Palma, J.M. (2003):** Peroxisomes from pepper fruits (*Capsicum annuum L.*):



Purification characterisation and antioxidant activity. *Journal of plant physiology*, 160 (21): 1507 – 16.

**Monserenusorn, Y. (1983)** : Subchronic toxicity studies of *Capsaicin* and *Capsicum* in rats. *Res. Commun. Chem. Pathol. Pharmacol.*, 41(1):95-110.

**National Research Council, NRC. (1995)**: Nutrient Requirements of Laboratory Animals, Fourth Revised Edition, p.4. Subcommittee on Laboratory Animal Nutrition, National Academy Press, Washington, D.C.

**Osganian, S. K.; Stampfer, M. J.; Rimm, E.; Spiegelman, D.; Hu, F. B.; Manson, J. E. and Willett, W. C. (2003)** : Vitamin C and risk of coronary heart disease in women. *J. Am. Coll. Cardiol.*, 42 (2) : 253 – 5 .

**Owiss, N. M. (1999)**: The Effect of Dietary Barley on Lowering Plasma Cholesterol Level in Rats. M.S.C. Thesis, Faculty of Home Economics, Helwan University.

**Protogerous, A.D.; Lekakis, J.P.; Kontoyanni, D.D.; Stamatelopoulos, K. S.; Tsotsoros, N.D.; Papaioannou, T. G.; Tryfonopoulos, D. J., Papamichal, C.M. and Stamatelopoulos, S.F. (2004)**: Effect of ascorbic acid on forearm reactive hyperaemia in patients with hypercholesterolaemia. *Journal of Cardiovascular Prevention*, 11(2) : 149 – 154.

**Reitman, A. and Frankel, S. (1957)** : GPT and GOT determination after enzymatic hydrolysis. *Amer. J. Clin. Pathol.*, 28:56.

**Richmond, W. and Allain, C.C. (1973)**: Total cholesterol determination after enzymatic hydrolysis. *J. Clin. Chem.*, 19: 1350.

**Rimando, A.M. and Perkins, P.M. (2005)**: Determination of *Citrulline* in watermelon rind. *J. Chromatogr. A.*, 17:1078(1-2) :196-200.

**Salem, I. S. (1999)** : Comparative Evaluation of Two Different Forms of Dietary Fiber on Atherosclerosis in Rats. MS.C. Thesis, Faculty of Home Economics, Helwan University.

**Sanchez, C.; Cano, M. P.; Ancos, B.; Plaza, L.; Olmedilla, B.; Granado, F. and Martin, A. (2006)** : Mediterranean vegetable soup consumption increases plasma vitamin C and decreases F<sub>2</sub>-isoprostanes, prostaglandin E<sub>2</sub> and monocyte chemoattractant protein-1 in healthy human. *J. Nutr. Biochem.*, 17 (3) : 183 – 9 .

**Sarwar, M.; Kaur, G.; Jabbar, Z.; Javed, K. and Athar, M. (2007)**: *Eruca sativa L* seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. *J. Food Chem. Toxicol.*, 45 (6) : 910-20.

**Schimeister, J. (1964)**: Creatinine determination after enzymatic hydrolysis. *Dtsch. Med. Wschr.*, 89:1940.

**Sies, H. and Stahl, W. (1995)**: Vitamins E and C; B-Carotene and other carotenoids as antioxidants. *Am. J. Clin. Nutr.*, 62-87.

**SPSS. (1998)**: Statistical package for Social Science, Computer Software, Ver.10, SPSS Company. London, UK.



**Vijayakumar, R.S. and Nalini, N. (2006b):** Efficacy of piperine; an alkaloidal constituent from *Piper nigrum* on erythrocyte antioxidant status in high fat diet and antithyroid drug induced hyperlipidemic rats .Cell Biochem. Funct.,24(6):491-8.

**W.H.O. (1979) :** Deaths according to cause of death. Annual World Health statistics. Quoted from Mahmoud , S. (1989) : Epidemiological study of the problem of IHD in Mansoura ,. M . Sc . Thesis in Public Health . Fac . of Med., Mansoura Univ., Egypt

**Whitney, E.N.; Catoldo, B. and Relfes, S. (1991) :** Understanding Normal and Clinical Nutrition. 3<sup>rd</sup> .Ed., West Publishing. Company, ST. Paul. New York , Los Angeles , San Francisco.

**Zollner, N.K. (1962):** Total Lipids determination after enzymatic hydrolysis . Z .Ges. Exp. Med., 135 :545.