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# Egyptian Vegetables as Source for Lutein and its Role in Incidence of Cataract in Rats

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## ABSTRACT

The last years the interesting of eye diseases increases and the relationship intervention with nutrients and the common diseases as well cataract. The aim of this study focus on the concern of the effect of lutein on cataract. Therefore, this research was designed to study the use of certain species of vegetables (spinach, leek, parsley and watercress), the most popular and rich in lutein and its relationship to reduce the incidence of cataract. It could be concluded that there is a relationship between lutein consumption and decreasing the risk of cataract. We must eat the fresh vegetables rich in vitamins specially lutein. It could was found that watercress, spinach, parsley and leek provide high content of lutein (12.72, 11.87, 11.03 and 7.30 mg /100g), respectively. Moreover these vegetables could be purchased with low price and available throughout the year. It is noticed that 10 mg lutein daily was effective dose for the beneficial effects of lutein on cataract. Also, it is noticed that feeding on lutein diets increased the serum antioxidant enzymes (SOD, GPx, GR, CAT and GSH) and reduced the proteases activities in rats lenses compared with cataract group and thus its role in reducing the incidence of cataract.

## INTRODUCTION

Lutein is found in natural sources like squash, peaches, mangoes, papayas, yellow and dark green fruits and vegetables including carrots, apricots, cantaloupe, sweet potatoes, spinach, kale, broccoli and mustard greens (BCIEP, 1994).

Kale, turnip greens, spinach, collard greens, watercress, garden peas, broccoli, egg, carrot and avocado are considered good sources of lutein and zeaxanthin *(*SanGiovanni, *et al.*, 2007*).*

Lutein belongs to the xanthophyll family of carotenoids, which are synthesized on dark green leafy plants, such as spinach and kale *(*Subczynski, *et al.*, 2010*).*

USDA, (2015) stated the carotenoids composition of spinach as follows: 5626 µg β-carotene and 12197 µg Lutein+Zeaxanthin (on fresh weight basis).

Perry, *et al.*, (2009) reported that leek contained 36.8 µg lutein /g fresh weight. Lutein and zeaxanthin are the most common xanthophylls in green leafy vegetables (e.g., kale, leek, spinach, broccoli, peas and lettuce) and egg yolks .

USDA, (2015) stated the carotenoids composition of leek as follows: 1000 µg β-carotene and 1900 µg Lutein + Zeaxanthin (on fresh weight basis).

Parsley is a source of flavonoides, and antioxidants , apigenin, (Meyer, *et al.*, 2006) folic acid, vitamin K, vitamin C, and vitamin A. Half a of tablespoon (a gram) of dried parsley contains about 6.0 µg of lycopene and 10.7 µg of alpha carotene well as 82.9 µg of lutein+zeaxanthin and 80.7 µg of beta carotene (USDA, 2013).

USDA, (2015) stated the carotenoids composition of parsley as follows: 5054 µg β-carotene and 5561 µg Lutein+Zeaxanthin (on fresh weight basis).

(Food Standards Agency, 2002) told that watercress contained 18 Kcal/100g, iron, calcium, were: 1.6, 138 respectively and 2016 µg β-carotene, 4614 µg Lutein+Zeaxanthin.

Cataracts is a white cloud affect one or both eyes. Symptoms include blurry vision, difficulty seeing at night, faded colors, *and trouble with the bright lights and halos around light (*NEI, 2015). *Cataracts are the cause of half of blindness and 33% of poor vision worldwide* (WHO, 2015).

Despite the good efficacy of surgical protocols for treating cataracts, there are limitations such as cost, time of diagnosis and inadequate service in some countries which decrease treatment outcome and leads to cataracts-induced inability and blindness (Miller, *et al.* 2005). Surgical intervention for the treatment of cataract is expensive and may be unavailable, so prevention is the use of foods rich in antioxidants can reduce the risk of cataracts. However, as far as lutein is concerned, interventional studies suggest that it might be effective against nuclear cataract but no other kinds of cataract (Ma *et al.*, 2014).

Many studies confirm that the profusion of lutein reduces the incidence of cataracts (SanGiovanni, *et al.*, 2007). Likely in studies that the effective dose in reducing the incidence of cataracts is 6 mg of lutein per day and the dose most commonly used in commercial products is 10 mg / day. Although the optimal dose of lutein supplementation has not been proven yet. (Harikumar, *et al.*, 2008*).*

Lutein is found in the lens of the human eye and they have two main functions there – as an antioxidant to reduce or scavenge free radicals and as a filter against high-energy and harmful blue light *(Landrum and Bone, 2001).* Exposure of the eye to high-energy blue light, results in free radical formation and oxidative stress *(Krinsky, et al., 2003).* Lutein by filtering the hurtful blue light reduces photo-induced oxidation of lens proteins thereby protecting against age-related eye diseases such as cataract.

So the main goal of this work was to study Egyptian vegetables that are sources for lutein and its role in cataract in rats.

## MATERIALS AND METHODS

**Materials:**

**Raw vegetables:**

Spinach *(Spinacia oleracea L.),* Leek (*Allium ampeloprasumvar. Porrum),* Parsley (*Petroselinum crispum),* and Watercress (*Nasturtium officinale)* were

obtained from local market in Mansoura city, El-Dakahlia, Egypt.

**Standard lutein:**

Standard lutein was purchased from Sigma Aldrich co., U.S.A.

**Sodium selenite:**

Sodium selenite was purchased from Sigma Aldrich co., U.S.A.

**Methods:**

**Preparation of raw vegetables:**

The vegetables were dried by oven according to *Mary, 1994.*

**Determination of lutein (HPLC):**

Lutein was determined in National Research Center (NRC), Giza, Egypt using HPLC.

**Extraction of lutein:**

By using high-performance liquid chromatography diode array detector lutein was extracted and analyzed. For 15 hours with 10 ml of methanol: tetrahydrofuran (1: 1, v / v), and for another 10 minutes at room temperature two hundred milligrams of the sample was extracted. And then subjecting the extract to room temperature. Then, the liquidation cruised through a filter 0.20 micron membrane and kept in the dark under nitrogen until HPLC analysis of lutein.

**HPLC conditions:**

Hentschel, *et al.,* (2002), HPLC separation was accomplished according to a previously described protocol with modifications. HPLC analysis was performed using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA). An Agilent 1100 series liquid chromatography (Agilent Technologies, Waldbronn, Germany), consisting of a vacuum solvent degassing unit,(a quaternary gradient pump), an automatic sample injector. Column: C18 5µm, 150mm×4.6mm i.d. Chromatogram were monitored at 450nm . In this process it was used at room temperature. lutein was eluted using a mobile phase of solvent A (hexane) and solvent B (1% i-PrOH in EtOAc). The injected volume was 10 µl .

**Biological evaluation:**

**Experimental animals:**

The experimental rats, (suckling white albino rats, Sprague Dawley strain) according to the following design. Albino rats were obtained from the experimental animal house of the Research National Center, Giza, Egypt

**Experimental design:**.

A number of 56 rats with a body weight of 20 to 55 g were divided into 8 groups each comprised 7 rats. Each rat in each group was housed individually into separate stainless steel cage in a room at temperature of 25ºC. Water was admitted freely to rats from glass bottles mounted. Each group of rats was given one of the prepared diets. Group 1(control negative) and 2 (control positive) were given the control diet the others were given other formula . Part of the food provided to groups from G2 to G8 contained vegetable dryer has a recommended daily allowance of lutein (10 mg) and then completed the rest of the quantity by control diet.

Then, rats in group 1 to group 8 were fed as follows:

G1- Rats fed on basal diet (Negative control or normal control).
G2- Rats fed on basal diet (Positive control or cataract group).
G3- Rats fed on spinach .
G4- Rats fed on leek .
G5- Rats fed on parsley .
G6- Rats fed on watercress .
G7- Rats fed on mixed vegetables (25% each) .
G8- Rats fed on mixed vegetables (25% each) .

G2 to G7 were selenite cataract model from the first day while the G8 was injected by selenite after 15 days from the start of the experiment . An amount of food equal to 20 g was weighed and placed in the dish inside the cage. This was allowed for consumption over the day. Animals were weighted twice a week and the weights of the animal were recorded to follow their growth . The animal experiment lasted for 4 weeks.

**Samples Collection:**

At the end of experiment (one month), the rats were fasted overnight, anaesthetized and blood samples were withdrawn from the eye vein. Serum was prepared and kept in deep freezer at –30°C until used assessment of different biochemical parameters .The eyes were enucleated and lenses were excised, washed in saline solution and kept in 10% formalin.

**Cataract model:**

Sodium selenite used in studies and research since 1978, and a quick easy way to cataracts in rats (Ostadalova *et al.,* 1978). suckling rats are injected under the skin at a rate of 19-30 µM/kg of body weight of sodium selenite (Shearer *et al.,* 1997). Repeated injections of smaller doses of selenite (Huang *et al.,* 1992) or oral administration (Shearer *et al.,* 1983) are also cataractogenic.

**Biochemical analysis:**

**Determination of serum triglycerides:**

An enzymatic colorimetric method according to *(Fassati and Prencipe, 1982).*

**Determination of total cholesterol**:

The kits were provided form Biodiagnostic according to (Allain *et. al.,* 1974).

**Determination of high density lipoprotein cholesterol (HDL-c):**

An enzymatic colorimetric method according to *(Lopez, 1977).*

**Determination of LDL-cholesterol and vLDL-c:**

LDL-cholesterol and vLDL-c in serum were performed following the method of (Lopez *et.al.,* 1977).

**Determination of lipid peroxide (MDA):**

Lipid peroxide was determined according to the method of *(Satoh, 1978).*

**Determination of Proteolytic (proteases) activity:**

Proteases activity was determined according to **(**Schoenberger, 1987).

**Determination of catalase activity (CAT):**

Catalase activity was determined according to the method of (Aebi, 1984).

**Determination of reduced glutathione (GSH):**

Reduced glutathione was determined according to the method of (Beutler, *et al.,* 1963).

**Determination of glutathione reductase activity:**

Reductase glutathione was determined according to the method of (Goldberg and Spocner, 1983).

**Determination of glutathione peroxidase (GPx) activity:**

Glutathione peroxidase activity was determined according to the method of (Hafemann, *et al.*, 1974).

**Determination of superoxide dismutase (SOD) activity:**

Superoxide dismutase activity was determined according to the method of (Mc Cord and Fridovich, 1969).

**Statistical analysis:**

The data were subjected to statistical analysis using one way classification least significant differences (L.S.D) according to (Steel and Torrie, 1980).

**Histopathological examinations:**

The eyes were enucleated and lenses were excised, washed in saline solution and kept in 10% formalin, dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin wax. Then, sections (5 µm thick) were stained with haematoxylin and eosin (H&E) *(Bancroft and Gamble, 2002).*

# RESULTS AND DICSSIONS

Fruits and vegetables are the most important source of carotenoids in the diet, and the preventive role effect in the medicine. Several publications reported the qualitative and quantitative content of carotenoids in different fruits and vegetables. There is a large variation in the amount of lutein and zeaxanthin in fruits and vegetables (Mangels, *et al.*, 1993).

So, at first, raw vegetables (spinach, leek parsley and watercress) lutein ratio must be determined as shown in table (1). From these data, it could noticed that watercress had the highest value of lutein and it was 12.72 mg /100g followed by spinach, parsley and leek as follows: 11.87, 11.04 and 7.31 mg /100g , respectively. These results go in parallel with those reported by (USDA, 2015).

These vegetables are available in the Egyptian market and is also cheap and easy in reach of the average consumer, who does not realize its potent effect of lutein and cataract.

**Table 1. lutein ratio in raw vegetables**

| Vegetables | Lutein (mg/100g FW) |
|---|---|
| Spinach | 11.87 |
| Leek | 7.31 |
| Parsley | 11.04 |
| Watercress | 12.72 |

Lutein concentration in serum for all groups of rats to know the lutein absorption as a result of eating vegetables rich in lutein was determined.

**Figures of lutein HPLC chromatogram of raw vegetables.**

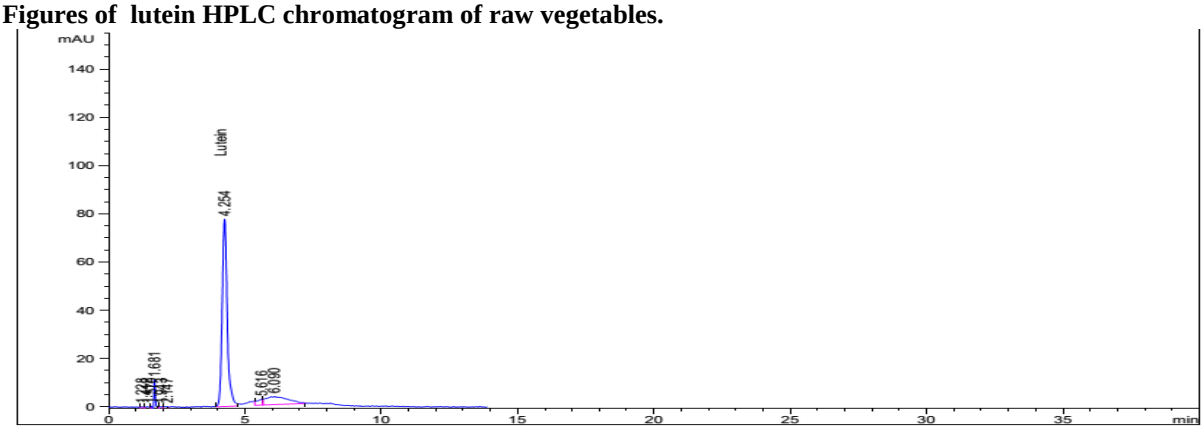


**Fig. 1. HPLC chromatogram of standard lutein**

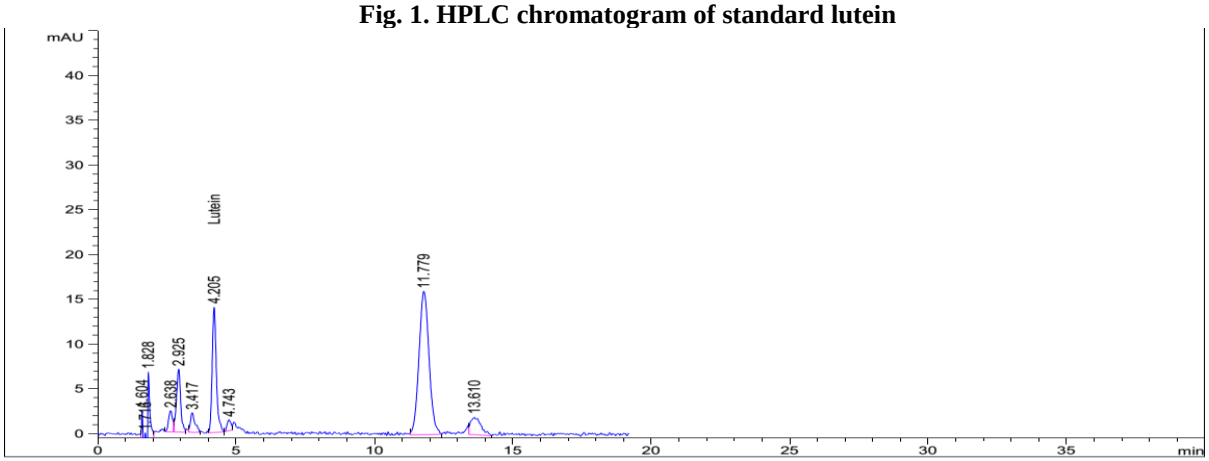


**Fig. 2. HPLC chromatogram of lutein in spinach**

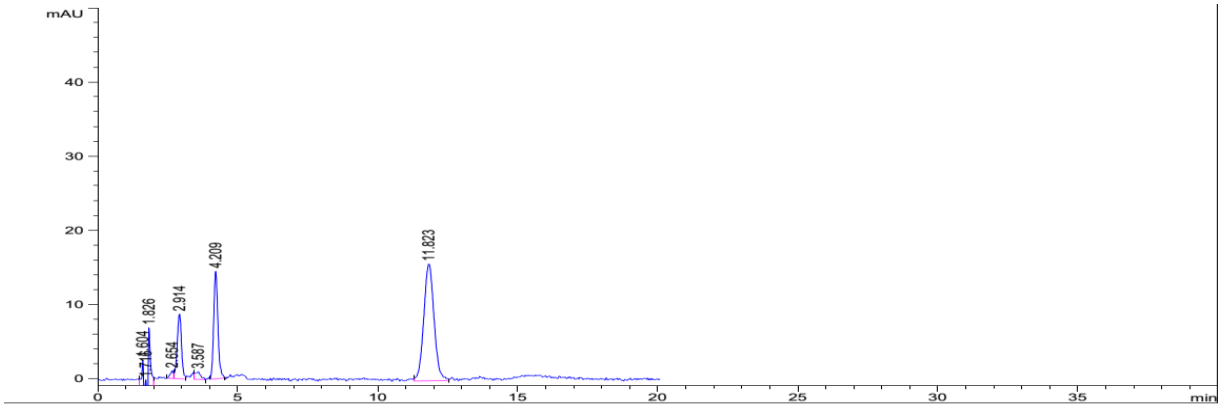


**Fig. 3. HPLC chromatogram of lutein in leek.**

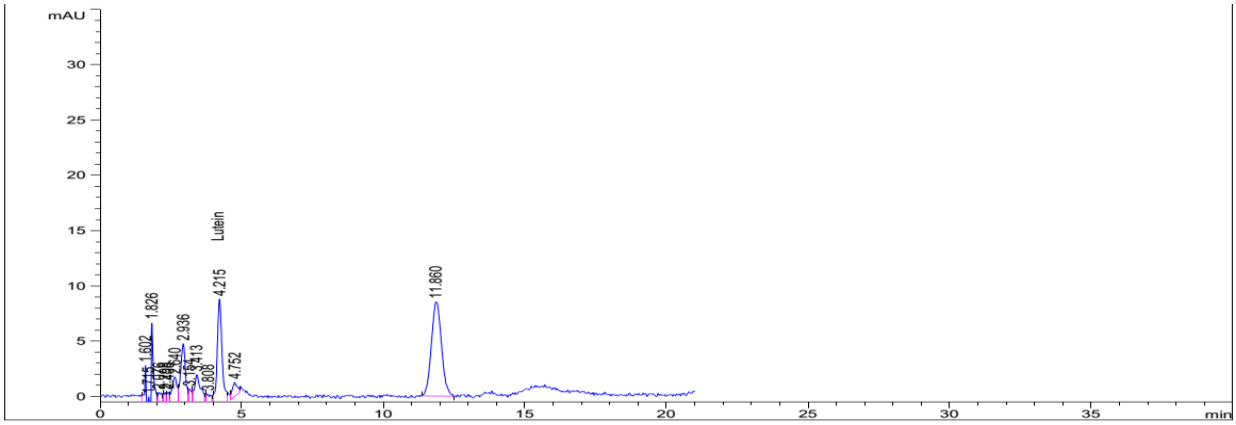


**Fig. 4. HPLC chromatogram of lutein in parsley.**

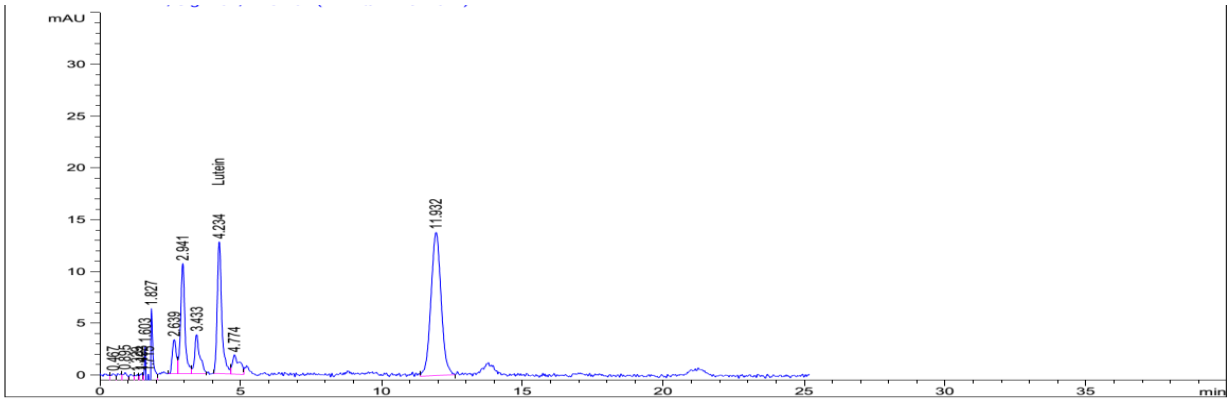


**Fig.5. HPLC chromatogram of lutein in watercress.**

Results in table (2) showed that lutein determined in blood serum in all groups of animals from group 1 to group 8 approximately were : (0.128), (0.114), (0.222), (0.218), (0.214), (0.211), (0.218) and (0.218) ug/ml serum , respectively. It could be noticed that G3 (spinach group) contained the highest level of lutein (0.222 ug/ml serum) while G2 (control positive) had the lowest level of lutein (0.114 ug/ml serum) , This result shows that the oxidative stress reduces the bioavailability of lutein as a result of the presence of sodium selenite in group G2 (control positive) which had 0.114 ug/ml serum, while increasing the bioavailability of lutein as a result of its rich vegetable intake in groups from G3 to G8, the highest value in G3 (spinach group) which had 0.222 ug/ml serum, while the lowest value in G6 (watercress group) which had 0.211ug/ml serum. These results go in agreement with those found by (Leela *et al.*, 2014 and Mamatha and Baskaran 2011).

**Table 2.Lutein concentration in different groups of rats serum**

| Group No. | Lutein concentration( ug/ml Serum) |
|---|---|
| G1 (Negative control) | 0.128385096 |
| G2 (Positive control) | 0.114007448 |
| G3 (spinach) | 0.221551695 |
| G4 (leek) | 0.218241766 |
| G5 (parsley) | 0.214397977 |
| G6 (watercress) | 0.210927891 |
| G7 (mixed vegetables) | 0.218348538 |
| G8 (mixed vegetables) | 0.217707906 |

Long-term lutein supplementation could increase serum lutein concentration, macular pigment optical density (MPOD), and visual sensitivity in early age-related macular degeneration (AMD) patients. The advisable long-term lutein dosage for early AMD treatment is 10 mg daily *(Huang et al., 2015).*

In a research by *(Berendschot et al., 2000)* the influence of daily consumption with 10 mg lutein derived from marigold during 12 weeks on macular pigment density was investigated. This study also showed that plasma lutein concentrations reached a plateau after 4 weeks. Mean plasma lutein concentration increased from 0.18 to 0.90 µmol/L within these 4 weeks and stayed at this level during the supplementation period.

Influence of feeding rats lutein diets on antioxidants enzymes is an important parameter when studying the effect of lutein on cataract.

There exists a group of oxygen eliminators in the lens, including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), glutathione reductase (GR), and glutathione S-transferase (GST), protecting crystallins from oxidative damage *(Manikandan, et al., 2010).* Selenite-induced cataract is a cataract model that is causally related to oxidative stress, where oxidation of the critical sulfhydryl groups is essential for the initiation of cataractogenesis. Primary defenses, including non enzymatic antioxidants and enzymatic antioxidants such as glutathione, SOD, CAT, GPx, GR, and GST, neutralize free radicals and repair, recover, or degrade molecules that are damaged *(Shearer, et al., 1997).*It has been demonstrated that antioxidant enzymes levels are altered in cataracts. Some reports showed that the activity of SOD, GSH, GPx, and CAT decreased in cataract *(Ozmen, et al., 2002).*

Effect of lutein on the antioxidant enzymes and glutathione in the serum of rats after administration for a period of 30 days is shown in Table (3).

The activity of superoxide dismutase (SOD), glutathione perodxidase (GPx), glutathione reductase (GR), catalase (CAT), and glutathione (GSH) were significantly increased in all groups of animals which treated with 10 mg lutein daily when compared with treated control (+) group G2 (selenite-induced cataract group).

**Table 3. Effect of feeding lutein diets on antioxidants enzymes**

| GROUP | SOD(U/ml) | GPx(U/l) | GR(U/l) | Cat(U/l) | GSH(mg/dl) |
|---|---|---|---|---|---|
| G1 | 32.57±4.96$^{a}$ | 179.29±20.70$^{a}$ | 210.00±15.81$^{a}$ | 43.40±7.32 | 29.43±8.06$^{a}$ |
| G2 | 18.86±2.04$^{d}$ | 142.86±17.53$^{c}$ | 175.71±25.40$^{b}$ | 30.57±5.35 | 20.29±2.69$^{d}$ |
| G3 | 21.43±5.38$^{cd}$ | 148.57±17.96$^{c}$ | 192.86±23.95$^{ab}$ | 38.57±9.11 | 22.17±3.18$^{dc}$ |
| G4 | 19.14±3.13$^{d}$ | 166.43±8.52$^{ab}$ | 190.00±13.23$^{ab}$ | 41.71±8.36 | 25.29±4.35a$^{bcd}$ |
| G5 | 21.00±3.42$^{d}$ | 156.00±14.55$^{bc}$ | 181.00±18.12$^{b}$ | 36.86±8.59 | 27.71±4.54$^{ab}$ |
| G6 | 19.71±3.35$^{d}$ | 169.29±13.97$^{ab}$ | 190.71±12.72$^{ab}$ | 32.57±6.65 | 23.86±3.58$^{bcd}$ |
| G7 | 26.14±5.08$^{bc}$ | 172.14±7.56$^{ab}$ | 188.57±18.19$^{b}$ | 38.33±9.07 | 26.14±4.26$^{abc}$ |
| G8 | 27.00±6.30$^{b}$ | 169.17±20.09$^{ab}$ | 187.86±19.33$^{b}$ | 37.14±7.69 | 25.40±6.67$^{abcd}$ |
| P value | 0.0001 | 0.0008 | 0.0790 | 0.0668 | 0.0322 |
| LSD | 4.7444 | 16.986 | 20.246 | 8.4494 | 5.3371 |

**a-f = Means with the same letter in each column are not significantly different P≤0.05.**
**LSD = Least Significant Difference SOD: Superoxide dismutase GPx: Glutathione peroxidase GR: Glutathione reductase Cat: Catalase GSH: reduced glutathion**

The data in table (3) showed that G2 (selenite-induced cataract group) has the lowest values in SOD, GPx, GR, CAT and GSH (18.86±2.04$^{d}$ U/ml, 142.86±17.53$^{c}$ U/l, 175.71±25.40$^{b}$ U/l, 30.57±5.35 U/l and 20.29±2.69$^{d}$ mg/dl), respectively while G1 has the highest values (32.57±4.96$^{a}$ U/ml, 179.29±20.70$^{a}$ U/l, 210.00±15.81$^{a}$ U/l, 43.40±7.32 U/l and 29.43±8.06$^{a}$ mg/dl), respectively.

It noticed that the values of SOD, GPx, GR, CAT and GSH were normal in G1(control negative), and then decreased in G2 (control positive) this is may be due to oxidative stress as a result of doses of sodium selenite then these values increased again in the remaining groups from G3 to G8 as a result of eating vegetables rich in lutein content.

These results are in parallel with those obtained by Seham, *et al.,* (2013), Shearer, *et al.,* (1997), Ozmen, *et al.,* (2002), and Chang, *et al.,* (2013).

Proteolytic (proteases) enzymes activity also important criteria when identifying the nature of the relationship between lutein and cataract.

Cataract is accompanied by low lens protein (Kuck, J. and Kuck, K. 1983). This low protein content could partly be due to loss by proteolysis, and the evidence presented may suggest proteolysis due to increased enzyme activities in cataract lenses. This has also been shown in previous studies for human cataract lenses (Swanson, *et al.,* 1981).

(Gao, *et al.,* 2011), have shown that lutein or zeaxanthin supplementation protects lens protein, lipid and DNA from oxidative damage and improves intracellular redox status upon oxidative stress**.**

The data tabulated in table (4) showed that the G2 control positive (selenite-induced cataract group) showed the highest value it was (3.200±0.798$^{a}$ U/l), while G1 (control negative) was the lowest value of proteases activity (1.414±0.414$^{b}$ U/l). The mean activities of proteases was significantly increased in G2 cataract group (sodium selenite induced group) of animals. While, the enzyme activities were restored decreased on lutein treatment (G3 to G8). From these data ; it could be noticed that feeding on lutein diets reduced the proteases activities in rat lenses.

**Table 4. Proteases activity in rats lenses**

| Group No. | **Proteases(U/l)** |
|---|---|
| G1 (Negative control) | 1.414±0.414[b] |
| G2 (Positive control) | 3.200±0.798[a] |
| G3 (spinach) | 2.871±0.692[a] |
| G4 (leek) | 2.029±0.860[b] |
| G5 (parsley) | 1.443±0.591[b] |
| G6 (watercress) | 2.014±0.626[b] |
| G7 (mixed vegetables) | 1.729±1.106[b] |
| G8 (mixed vegetables) | 2.943±0.577[a] |
| P value | 0.0001 |
| LSD | 0.79 |

**a-f = Means with the same letter in each column are not significantly different P≤0.05.**
**LSD = Least Significant Difference**

These results are in parallel with those obtained by Gao, *et al.*, (2011), Shearer, *et al.*, (1997) and Swanson, *et al.*, (1981).

Histopathological and microscopic examinations of lens show very clearly the absence of any change in the tissues of eyes and lenses in the first group G1(control negative ; untreated) normal (Fig. 1), while many changes (Fig, 2,3 and 4) have emerged in the second group G2 control positive (selenite induced cataract group). On other hand the rest of the groups (from G3 to G8), some of them had been influenced by factors oxidation and others had a protection from oxidation and various pathological changes are heading for a lutein.

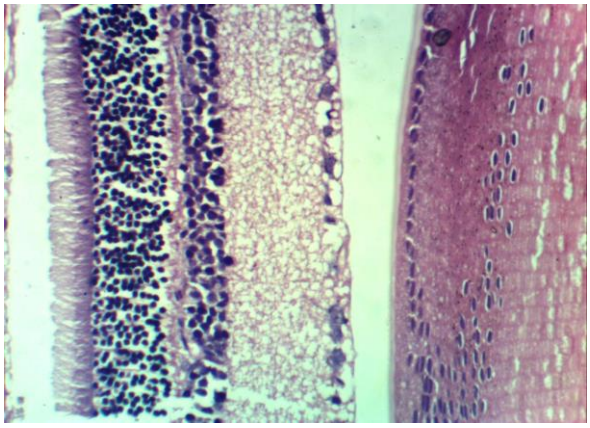

**Fig. (1)**

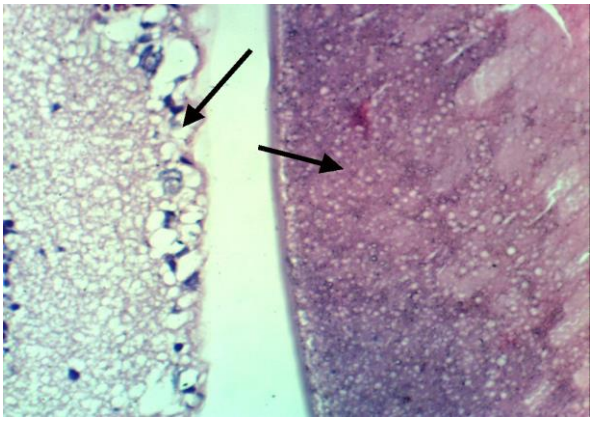

**Fig. (2)**

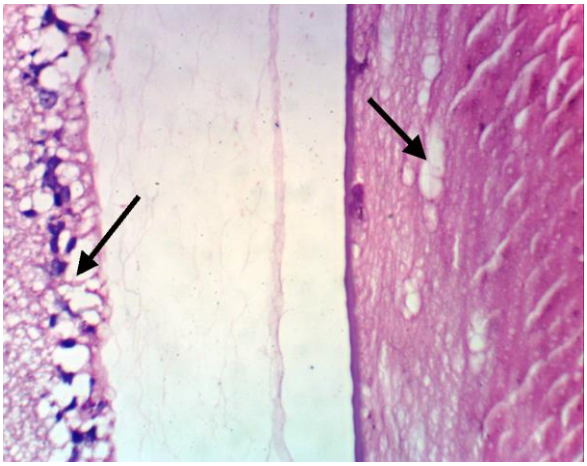

**Fig. (3)**

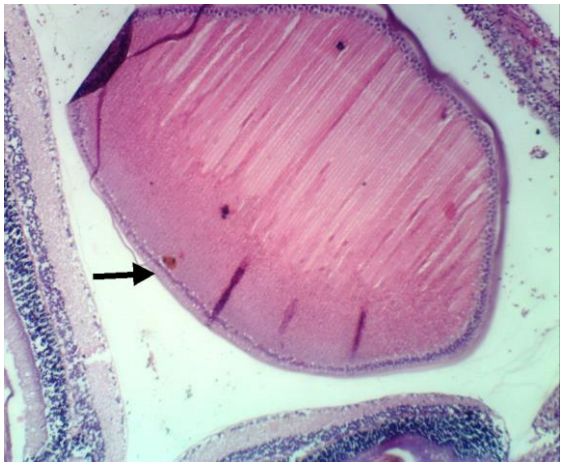

**Fig. (4)**

Finally, it could be concluded that there is a relationship between lutein consumption and decreasing the risk of cataract. We must eat the fresh vegetables rich in vitamins specially lutein. It could be also concluded that watercress, spinach, parsley and leek provide high content of lutein which were (12.72, 11.87, 11.03 and 7.30 mg /100g), respectively. Moreover these vegetables could be purchased with low price and available throughout the year

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## الخضروات المصرية كمصدر لليوتين و دوره فى الإصابة بالمياه البيضاء فى الفئران

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تعتبر الخضروات الطازجة غذاءاً شعبياً تستهلكه يوميا الأعمار المختلفة من الأغنياء والفقراء لإنخفاض سعرها و طيب نكهتها. وبالرغم من السعر المنخفض لهذه الخضروات إلا أنها ذات قيمة غذائية عالية لاحتوائها على تركيزات عالية من الفيتامينات خاصة الليوتين و الألياف و الكالسيوم و البوتاسيوم و الماغنسيوم و الحديد إلى جانب أن هذه الخضروات تتوافر طول العام. في السنوات الأخيرة كانت زيادة أمراض العيون وتداخل العلاقة مع المواد الغذائية مثيرة للاهتمام والأمراض الشائعة خاصة المياه البيضاء. ولذلك تم تصميم هذا البحث لدراسة استخدام بعض أصناف الخضر (السبانخ و الكرات المصرى و البقدونس و الجرجير) الأكثر شعبية و الغنية في الليوتين و علاقتها فى الحد من الإصابة بالمياة البيضاء و لذلك تم تقدير نسبة الليوتين فى هذه الخضروات ثم تصميم التجربة البيولوجية لمدة شهر واحد و فى نهاية التجربة تم أخذ عينات الدم لتقدير القياسات المختلفة كما تم نزع العيون و إزالة العدسات لعمل الإختبارات الهستولوحية و الميكروسكوبية. أوضحت الدراسة أن الجرجير، السبانخ، البقدونس والكراث تحتوى على نسبة عالية من اللوتين (12.72، 11.87، 11.03 و 7.30 ملليحم / 100جم)، على التوالي. كما أظهرت الدراسة العلاقة بين الليوتين و كلا من إنزيمات الأكسدة و نشاط الإنزيمات المحللة للبروتين و بالتالى الحد من الإصابة بالمياه البيضاء فى الفئران لذا تؤكد هذه الدراسة على العلاقة بين إستهلاك الليوتين (10 ملليجم يوميا) من هذه الخضروات الطازجة و الإصابة بالمياه البيضاء وتوصى بالإكثار من تناول هذه الخضروات الغنية بالفيتامينات خاصة الليوتين ونظرا لإنخفاض سعرها و توفرها على مدار العام.