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# BIOCHEMICAL AND HISTOPATHOLOGICAL STUDIES ON HYPERCHOLESTEROLEMIC RATS FED ON DIET SUPPLEMENTED WITH FRESH BROCCOLI

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**Abstract:**

**The present study was designed to investigate the effect of feeding different levels (10 & 20 & 30%) of fresh broccoli on liver and kidney functions, serum lipid profiles, blood sugar, serum total protein and albumin. In addition to determined of bioactive compounds (flavonoid and saponine). Thirty adult Sprague-Dawley strain male healthy albino rats weighting (80±5g) were used as experimental animals and divided into 5 groups (6 rats each). The experimental animals (30 rats) were fed on basal diet for one week to adapt with the basal diet. After that, rats (30) were divided into two main groups. The first group (6 rats) was fed on basal diet for 7 weeks to be as a negative control group. The second group (24 rats) was divided into 4 sub-groups (6 rats each). The first sub-group (B1) was fed on hypercholesterolemic diet (diet containing 10% animal fat+1% cholesterol) for 7 weeks and was left as a positive control group (untreated group). The other sub-groups were fed on hypercholestrolemic diet for 3 weeks to be as hypercholesterolemic rats. After that, animals were fed on diet supplement with different levels (10&20 &30 %) of fresh broccoli for 4 weeks. At the end of the experimental period animals were killed, blood samples were collected to determine the following parameters, blood sugar, lipid**

**profiles, liver functions, serum total protein, albumin, kidney functions (urea, uric acid and createnine). Liver, kidney were taken for histopathological examination. The obtained results concluded that, 30% levels of fresh broccoli improve of all biochemical parameters.**

**Key words: Broccoli, Hypercholesterolemic rats, liver functions, kidney functions, lipid profiles, blood sugar.**

## INTRODUCTION

Broccoli is widely cultivated vegetable crop in North America and many European countries. Broccoli is grown in very limited scattered areas and the total cultivated area is not exactly known **(Tolba, 2005).** Brassica vegetables are highly regarded for their nutritional value. They provide high amounts of vitamin C and soluble fiber and small amount of selenium **(Finley *et al.*, 2005, George, 2009 and Banerjee *et al.*, 2012)**. Broccoli has the highest levels of carotenoids. It is particularly rich in lutein and also provides a modest amount of beta-carotene **(Science Daily, 2009)**. The chemical composition of broccoli was varied between countries.

**Karmas and Harris (1988)** stated that chemical composition of broccoli were 89.1, 5.9, 3.6 and 0.3% for moisture, carbohydrates, protein and fat, respectively. **Burghagen *et al.* (1999)** showed that, chemical composition of broccoli were 4.4; 0.4; 1.5 and 1.1% for carbohydrates, lipids, dietary fiber and ash, respectively. **Kaviarasan *et al.* (2005)** investigated lipid profile, oxidant-antioxidant status, and glycoprotein component in hyperlipidemic patients with/without diabetes. The results illustrated that a significant increase was observed in the levels of total cholesterol (TC), very low density lipoprotein (VLDL-C), triglycerides (TG), lipid peroxidation, glycoprotein components and glucose in hyperlipidemic patients with/without diabetic. **Diagbare (2005)** illustrated that broccoli is one of the richest food sources of the trace metal chromium, a life extender and protector against the ravages out - of control insulin and blood sugar.

Broccoli also contains vitamin C, an antioxidant. According to World's Healthiest Foods. Vitamin C reduces LDL cholesterol by preventing its formation. Vitamin C also prevents free radical formation,

which can help increase HDL cholesterol. Beta carotene, another type of antioxidant in broccoli, plays a similar role as vitamin C in terms of prevention of LDL cholesterol. In addition, World's Healthiest Foods explains that beta carotene and vitamin C both help blood vessels to dilate. This prevents arterial blockage. **Kristeen (2013)** and **Glore *et al.* (1994)** showed that, oat bran, rice bran, legumes, broccoli, cabbage, carrots cauliflower and corn are good sources of soluble fiber which bind to excess cholesterol and carries it out of the body. Therefor soluble fiber has ability to reduce LDL cholesterol, total cholesterol without reducing the level of HDL cholesterol. The authors concluded that, people who consume more fruits and vegetables often have a lower prevalence of important risk factors for cardiovascular diseases, including hypertension, obesity, and type II diabetes. Antioxidants such as ascorbic acid, vitamin E and β-carotene prevented LDL oxidation in vitro. However, in vivo studies have yielded contradictory results were reported. Beta-carotene supplementation inhibited LDL oxidation. Where as others did not find an inhibition of LDL oxidation **(Gaziano *et al.*, 1995)**.

## MATERIALS AND METHODS

### Broccoli:

Broccoli: (*Brassic oleracea var Italica*) broccoli was obtained from the local market, Cairo, Egypt. and was added to the basal diet as fresh.

### Cholesterol:

Cholesterol was obtained as pure with crystalline powder from Elgomhoria Company for medical Preparations chemicals and Medical Equipments, Cairo, Egypt.

### Animals:

Thirty adult Sprague-Dawley strain male healthy albino rats weighting (80±5g) were purchased from the National Center for Research Cairo, Egypt. Rats were housed as groups (6 rats of each) in wire cages. Under hygienic conditions, at an air conditioned in animal house of Faculty of Science, Zagazig University.

### Chemical analysis of broccoli:

Broccoli samples were subjected to chemical analysis in order to determine, moisture, ash, protein, fat and fiber, according to **A.O.A.C.**

**(1995)**

Carbohydrates were calculated by deferent as follows:

**Carbohydrates %** = 100 – (Moisture**%** +protein**%**+ fat**%**+ ash**%** +fiber**%**) according to the equation of **Chatffeld and Admas (1940).**

**Determination of flavonoids and saponine compounds:**

Flavonoid compounds were determined by HPLC according to the method of **Mattila *et al.* (2000**) and saponine were determined according to the method of **Edeoga *et al.* (2005).**

**Diets:**

Diets were given in an non scattering feed cups to minimize food loss, water was provided to the rats by mains of glass tube projecting through the wire cage from an in vetted bottle supported to one side of the cage food and water provided *ad-labtam.* Standard basal diet was preparing from fine ingredients 100g according to **AIN (1993)**.

**Fresh broccoli:**

Fresh samples were cleaned by hand from the damaged leaves, then washed with tap water followed by distilled water, and cutting into very small sliced and mixed with the diet.

**Experimental design and animal groups:**

Thirty adult Sprague-Dawley strain male healthy albino rats weighting (80±5g) were used as experimental animals and divided into 5 groups (6 rats each). The experimental animals were fed on basal diet for one week to adapt with the basal diet. After that, rats were divided into two main groups. The first group (6 rats) was fed on basal diet for 7 weeks to be as a negative control group. The second group (24 rats) was divided into 4 sub-groups (6 rats each). The first sub-group (B1) were fed on hypercholesterolemic diet (diet containing 10% animal fat+1% cholesterol) for 7 weeks and were left as a positive control group (untreated group) as concluded by **Abdel maksoud *et al.* (1996)**.

The other sub-groups were fed on hypercholestrolemic diet for 3 weeks to be as hypercholesterolemic rats. After that animals (3 sub-groups) were fed different levels (10 & 20 & 30%) respectively, of fresh broccoli for 4 weeks.

The composition of standard basal diet [12.5% casein (82%protein), 10%corn oil, 5% cellulose, 4%mineral mixture, 1%vitamin mixture, 0.3% methionine, 0.2% choline chloride, up to 100% corn starch] according to **AIN (1993).** The composition of hypercholesterolemic diet (Table 1) as concluded by **Abdel maksoud *et al.* (1996).**

At the end of the experimental period animals were fasted for 12 hr. and blood samples were collected from the portal vein in heparinized centrifuge tubes. Plasma was separated by centrifugation at 3000 r. p. m. for 10 minutes at room temperature and kept in plastic vials then stored in the deep freeze at (-20°C) until analyzed.

**Biochemical analysis of serum:**

Determination of serum total cholesterol was determined according to **Allain (1974).** Serum triglyceride was conducted according to the method of **Fossati and Princie (1982).** High density lipoprotein cholesterol (HDL-c) and cholesterol bound to this fraction were determined according to the method of **Burstein (1970).** Low density lipoprotein cholesterol (LDL-c) was determined according to the method of **Friendewald *et al.* (1972).** Serum uric acid was determined according to the method described by **Haisman and Muller (1977).** Serum createnine was determined according to the method described by **Bohomer (1971)**. Serum urea nitrogen was determined according to the method described by **Patton and Crouch (1977).** Aspartate amino transferees (AST) activities were measured according to the method described by **Hafkenscheid (1979).** Alanine amino transferees (ALT) activities were measured according to the method described by **Clinica Chimica Acta (1980).** Alkaline phosphatase (ALP) activities were measured according to the method described by **Moss, (1982)**. Total protein was determined according to the method described by **Doumas *et al.* (1981).** Albumin was determined according to the method described by **Doumas *et al.* (1971).**

**Table (1): Composition of the experimental diet.**

| Ingredients | Sub Groups B | | | |
|---|---|---|---|---|
| | B1 | B2 | B3 | B4 |
| | Positive group (untreated) | 10% Fresh Broccoli | 20% Fresh Broccoli | 30% Fresh Broccoli |
| Casein (82% protein) | 12.5 | 12.5 | 12.5 | 12.5 |
| Fiber | 5 | 5 | 5 | 5 |
| Mineral mix | 4 | 4 | 4 | 4 |
| Vit. mixture | 1 | 1 | 1 | 1 |
| Methionine | 0.3 | 0.3 | 0.3 | 0.3 |
| Cholin chloride | 0.2 | 0.2 | 0.2 | 0.2 |
| Broccoli | _ | 10 | 20 | 30 |
| Corn starch | 66 | 56 | 46 | 36 |
| Cholesterol | 1 | 1 | 1 | 1 |
| Animal fat | 10 | 10 | 10 | 10 |

Statistical analyses were calculated using one way classification. Analysis of variance (ANOVA) and least significant difference (LSD) according to **Sndecor and Cochran (1972).**

## RESULTS AND DISCUSSION

### Chemical composition of broccoli:

Table (2) Show the chemical composition of fresh broccoli percentage. These results are in agreement with the data of fresh broccoli witch reported by **Karmas and Harris (1988) & Diksha- Dogra and Awasthi (2003).**

**Bio-active compounds in aqueous extract of broccoli:**

Table (3) show the phytochemical compounds, which present in aqueous extract of fresh broccoli. It could be used standard /official methods (ISO). These results are in agreement with those reported by **Edeoga *et al.* (2005) and Song *et al.* (2007).**

**Biochemical evaluation:**

Table (4) illustrated the effect of diet supplement with 10, 20 and 30% fresh broccoli on liver functions of hypercholesterolemic rats.

**Table (2): Chemical composition percentage of fresh broccoli.**

| Sample | Moisture | Protein | Fat | Ash | Fiber | Carbohydrates |
|---|---|---|---|---|---|---|
| **F. Broccoli** | 85.97 | 3.17 | 0.49 | 0.99 | 1.10 | 8.28 |

**Table (3): Bio-active compounds in aqueous extract of fresh broccoli.**

| Bio-active compounds Flavonoids and Saponine | Fresh Broccoli |
|---|---|
| **Flavonoids:**(ppm) | -------- |
| Rutin | 37.00 |
| Quercitrin | 26.64 |
| Narenginin | 5.42 |
| Querctin | 10.55 |
| Kampferol | 9.68 |
| Luteolin | 5.29 |
| Apignin | 26.02 |
| **Saponine (%)** | 2.18 |

**Table (4): Effect of diet supplement with fresh broccoli on liver functions (serum AST, ALT and ALP enzymes) of hypercholesterolemic rats.**

| Group of animals | AST (µ /L) | ALT (µ /L) | ALP (µ /L) |
|---|---|---|---|
| **Negative control group** | 40.76 ± 0.45 d | 23.28 ± 0.61 d | 180.56 ± 17.04 e |
| **Positive group** | 80.22 ± 0.23 a | 40.67 ± 0.71 a | 340.12 ± 46.96 a |
| **Hypercholesterolemic group + 10% F. broccoli** | 69.20 ± 1.33 b | 32.12 ± 1.12 b | 300.37 ±45.66 b |
| **Hypercholesterolemic group + 20% F. broccoli** | 52.50 ± 0.87 c | 27.10 ± 0.83 c | 280.64 ± 30.64 c |
| **Hypercholesterolemic group + 30% F. broccoli** | 44.80 ± 1.13 d | 25.03 ± 0.41 d | 211.32 ± 73.44 d |

* Mean values of 6 rats± SD

a,b,c.d and e means significant difference between groups according to one-way ANOVA..

The same letters are not significantly different within groups at level ($p < 0.05$).

Data in table (4) show that, high significant differences ($P<0.05$) of serum AST values between positive group of animals compared with negative control group and rats fed on diet supplement with 10, 20 and 30% fresh broccoli. Also, there are significant differences ($P<0.05$) of serum AST levels within groups of rats fed on diet supplement with fresh broccoli. While there is no significant difference ($P<0.05$) of AST activity between rats fed on 30% fresh broccoli compared with negative control group. These result indicated that, 10, 20 and 30% levels of fresh broccoli improve serum AST activity of hypercholesterolemic rats. Thirty percent level of fresh broccoli is more effective than the other levels of fresh broccoli. These means 30% level of fresh broccoli improve activity of serum AST enzyme.

Data in table (4) show that, there are high significant differences

(P<0.05) of serum ALT activity between positive group of animals compared with negative control group and rats fed 10, 20 and 30% fresh broccoli. While there is no significant difference (P<0.05) of serum ALT activity between rats fed on 30% fresh broccoli compared with negative control group. These means that 30% level of fresh broccoli improve the activity of serum ALT enzyme. The data of serum alkaline phosphatase (ALP) activity show that there are high significant differences (P<0.05) of ALP activity between positive group of animals compared with negative control group, and rats fed fresh broccoli. While there is a slightly significant difference (P<0.05) of serum ALP activity between rats fed on 30% fresh broccoli compared with negative control group, these means that 30% level of fresh broccoli improve the activity of serum ALT enzyme.

Data in table (4) indicated that, diet supplemented with 10, 20 and 30% of fresh broccoli improve liver functions (serum AST, ALT and ALP enzymes) of hypercholesterolemic rats, but 30% level of fresh broccoli is more effective than other levels. These results are in agreement with the histopathological studies at photo 1 to 10.

Table (5) recorded the effect of diet containing different levels 10, 20 and 30% of fresh broccoli on kidney functions of hypercholesterolemic rats.

Data in table (5) show that there are high significant differences (P<0.05) of serum urea values between positive group of rats compared with other groups of animals. Also, there are significant differences (P<0.05) of serum urea values within groups of rats fed on fresh broccoli. While there is less significant difference (P<0.05) of urea value between rats fed on 30% fresh broccoli compared with the negative control group, these means that 30% level of fresh broccoli improve serum urea value.

**Table (5): Effect of diet supplement with fresh broccoli on kidney functions (serum urea, uric acid and createnine) of hypercholesterolemic rats.**

| Group of animals | Urea (mg/dl) | Uric acid (mg/dl) | Createnine (mg/dl) |
|---|---|---|---|
| **Negative control group** | 20.40±0.31 e | 3.50±0.15 e | 0.80±0.05 c |
| **Positive group** | 39.90±0.34 a | 8.68±0.14 a | 2.55±0.13 a |
| **Hypercholesterolemic group + 10% F. broccoli** | 31.83±0.48 b | 7.33±0.35 b | 2.01±0.07 b |
| **Hypercholesterolemic group + 20% F. broccoli** | 27.50±0.67 c | 5.80±0.25 c | 1.7o±0.22 b |
| **Hypercholesterolemic group + 30% F. broccoli** | 23.00±0.63 d | 4.60±0.07 d | 0.92±0.05 c |

* Mean values of 6 rats± SD

a,b,c,d. and e means significant difference between groups according to one-way ANOVA.

The same letters are not significantly different within groups at level ($p < 0.05$).

Serum uric acid values in table (5) indicate that, there are significant differences (P<0.05) between positive group of animal compared with other groups. Also, there are significant differences (P<0.05) of serum uric acid values within group of rats fed on fresh broccoli. While there is a slightly significant difference (P<0.05) of uric acid value between rats fed on 30% fresh broccoli compared with the negative control group, these means that 30% level of fresh broccoli improve serum uric acid value.

Serum createnine values in table (5) show that, there are high significant differences (P<0.05) of createnine values of untreated group and other groups. While there is no significant difference (P<0.05) of

serum createnine values within groups of rats fed on 10 and 20 % fresh broccoli. Also, there is no significant difference (P<0.05) of createnine value between rats fed on 30% fresh broccoli compared with negative control group, these means that 30% level of fresh broccoli improve serum createnine value than other levels.

Data in table (5) indicated that, diet supplement with 10, 20 and 30% levels of fresh broccoli improved kidney functions of hypercholesterolemic rats**.** Thirty percent level of fresh broccoli is more effective than the other levels. These results are in agreement with the histopathological studies at photo 1 to 10. Also, these results were in agreement with those reported by **Park *et al.* (2008)**.

Data in table (6) show the effect of feeding different levels 10, 20 and 30% of fresh broccoli on biochemical changes in the lipid profile (serum total cholesterol, triglyceride, LDL-c and HDL-c).

Serum total cholesterol (TC) levels in table (6) indicate that, there are high significant differences (P<0.05) of serum total cholesterol levels of positive control group compared with the other group of animals. Also, there are significant differences (P<0.05) of serum total cholesterol values within groups of rats fed on fresh broccoli. While there is slightly significant difference (P<0.05) of serum total cholesterol value between group of rats fed on 30% fresh broccoli compared with the negative control group. These means that, 30% level of fresh broccoli improved serum TC value than other levels of fresh broccoli.

Serum triglyceride (TG) levels show that, there are high significant differences (P<0.05) between untreated group of rats and the other groups. Also, there are significant differences (P<0.05) of serum TG values within groups of rats fed on fresh broccoli. While there is low significant difference (P<0.05) of serum TG value between animals fed on 30% fresh broccoli compared with the negative control group of rats. These means that 30% level fresh broccoli improve serum TG value.

Serum high density lipoprotein cholesterol (HDL-c) values in Table (6) indicate that, there are high significant difference (P<0.05) between the positive control group compared with the rats fed on 20 and 30% of fresh broccoli. While there is no significant difference (P<0.05) of serum (HDL-c) values between rats fed on 10% fresh broccoli compared with

the positive control group. Also, there are significant differences (P<0.05) of serum (HDL-c) value within groups of rats fed on fresh broccoli. While there is slightly significant difference (P<0.05) of serum (HDL-c) value between animals fed on 30% fresh broccoli compared with negative control group. These means that (30%) level of fresh broccoli improve serum (HDL-c) value.

**Table (6): Effect of diet supplement with fresh broccoli on lipid profile (TC, TG, HDL-c and LDL-c) of hypercholesterolemic rats.**

| Group of animals | TC (mg/dl) | TG (mg/dl) | HDL-c (mg/dl) | LDL-c (mg/dl) |
|---|---|---|---|---|
| **Negative control group** | 169.80±2.23 e | 140.20±1.25 e | 22.14±0.56 a | 120.78±0.88 e |
| **Positive group** | 280.33±0.88 a | 187.00±1.06 a | 16.08±0.45 d | 182.85±0.86 a |
| **Hypercholesterolemic group + 10% F. broccoli** | 260.50±1.65 b | 172.50±1.09 b | 16.01±0.46 d | 177.63±0.71 b |
| **Hypercholesterolemic group + 20% F. broccoli** | 220.40±0.66 c | 158.83±1.60 c | 18.65±0.27 c | 168.40±0.73 c |
| **Hypercholesterolemic group + 30% F. broccoli** | 198.83±0.48 d | 151.00±0.63 d | 20.86±0.47 b | 146.10±0.55 d |

* Mean values of 6 rats± SD

a,b,c,d. and e means significant difference between groups according to one-way ANOVA..

The same letters are not significantly different within groups at level ($p < 0.05$).

Serum low density lipoprotein cholesterol (LDL-c) levels in table (6) indicate that, there are high significant differences ($P<0.05$) of serum (LDL-c) levels of positive control group compared with other groups of animals. Also, there are significant differences ($P<0.05$) of serum (LDL-c) value within groups of rats fed on fresh broccoli. While there is slightly significant difference ($P<0.05$) of serum (LDL-c) value between animals fed on (30%) fresh broccoli compared with the negative control group of rats, these means (30%) level of fresh broccoli improve serum LDL-c.

Data in table (6) indicated that, 10, 20 and 30% levels of fresh broccoli improve serum TC, TG, HDL-c and LDL-c values of hypercholesterolemic rats. Thirty percentage level of fresh broccoli is more effective than the other levels. These results are in agreement with the histopathological studies at photo 1 to 10.

These results are consistent of those reported by **Nakashima *et al.* (2003)** they found that, the daily intake of the tested beverage containing broccoli and cabbage are useful for lowering serum LDL-c and increasing serum HDL-c levels of hypercholesterolemic subjects. Also, these results were inagreement with **Rahmat *et al.* (2004)** they reported that, the consumption of green vegetables or guava and papaya leaves reduces oxidative stress alter lipid profile; also it could reduce the risk of disease caused by free radical activities and high cholesterol in blood. Also, inagreement with the data of **Tanaka *et al.* (2003)** they reported that, the broccoli and cabbage mixture showed cholesterol lowering effects in hypercholesterolemic rats, raising a possibility that daily consumption of these vegetables may be useful in lowering serum TC and LDL-c levels of hypercholesterolemic patients. **Park *et al.* (2008) and Yasmeen and Prabhu (2012)** noticed that, hypercholesterolemic rats fed on leaves of papaya showed improving in lipid profile.

Table (7) recorded that, there are high significant differences ($P<0.05$) of serum glucose level between untreated group of animals compared with the other groups. There are significant differences ($P<0.05$) of serum glucose levels within groups of rats fed on fresh broccoli. While there is no significant difference ($P<0.05$) of serum glucose value between rats fed on (30%) fresh broccoli compared with negative control group, these means (30%) level of fresh broccoli improve serum glucose value.

**Table (7): Effect of diet supplement with fresh broccoli on serum glucose level of hypercholesterolemic rats.**

| Group of animals | Glucose (mg/dl) |
|---|---|
| **Negative control group** | 82.50±1.78 e |
| **Positive group** | 106.00±0.58 a |
| **Hypercholesterolemic group + 10% F. broccoli** | 98.33±1.82 b |
| **Hypercholesterolemic group + 20% F. broccoli** | 91.00±0.58 c |
| **Hypercholesterolemic group + 30% F. broccoli** | 86.00±0.86 d |

* Mean values of 6 rats± SD

a,b,c,d and e means significant difference between groups according to one-way

ANOVA.. The same letters are not significantly different within groups at level ($p < 0.05$).

Data in Table (7) indicated that, 10, 20 and 30% levels of fresh broccoli improve serum glucose value of hypercholesterolemic rats. Thirty percentage level of fresh broccoli is more effective than other levels. These results are in agreement with those reported by **Park *et al.* (2008)** & **Yasmeen and Prabhu (2012)** they noticed that, hypercholesterolemic rats fed on leaves of papaya (containing flavonoids) showed improving in serum glucose value.

Data in table (8) explained the effect of diet supplemented with 10, 20 and 30% of fresh broccoli on serum total protein and albumin of hypercholesterolemic rats.

Serum total protein (TP) levels in table (8) indicate that, there are high significant differences ($P<0.05$) of TP values between untreated group of animals compared with the other groups. Also, there are significant differences ($P<0.05$) of TP values within groups of rats fed on different levels of fresh broccoli. While there is a slightly significant difference ($P<0.05$) of serum TP level between rats fed on (30%) fresh broccoli compared with negative control group. Diet supplemented with 10, 20 and 30% levels fresh broccoli improve serum TP value of

hypercholesterolemic rats, but 30% level of fresh broccoli was more effective than the other levels. These results are in agreement with the histopathological studies at photo 1 to 10.

Serum albumin (ALB) values in table (8) indicate that, there are high significant differences (P<0.05) of albumin values between positive group of animals compared with the other group of rats. Also, there are significant differences (P<0.05) of serum ALB level within groups of rats fed on fresh broccoli. However there is no significant difference (P<0.05) of serum albumin value between rats fed on 30% fresh broccoli compared with the negative control group of rats. These means 30% level of fresh broccoli is more effective on serum albumin value of hypercholesterolemic rats than the other levels.

Data in table (8) indicated that, 10, 20 and 30% levels of fresh broccoli improved serum total protein and albumin levels of hypercholesterolemic rats. Thirty percentage level of fresh broccoli is more effective than other levels.

**Table (8): Effect of diet supplement with fresh broccoli on serum level of total protein and albumin of hypercholesterolemic rats.**

| Group of animals | TP (g/dl) | ALB (g/dl) |
|---|---|---|
| **Negative control group** | 6.66 ± 0.28 e | 3.90 ± 0.11 d |
| **Positive group** | 15.75 ± 0.40 a | 7.77 ± 0. 16 a |
| **Hypercholesterolemic group + 10% F. broccoli** | 10.65 ± 0.37 b | 6.54 ± 0.37 b |
| **Hypercholesterolemic group + 20% F. broccoli** | 8.64 ± 0.29 c | 5.56 ± 0.19 c |
| **Hypercholesterolemic group + 30% F. broccoli** | 7.52 ± 0.17 d | 4.21 ± 0.18 d |

* Mean values of 6 rats± SD

a,b,c,d. and e means significant difference between groups according to

one-way ANOVA..

The same letters are not significantly different within groups at level ($p < 0.05$).

**Histopathological Results**

**Regarding normal control group:**

Microscopic examination of liver revealed slightly congested central vein and blood sinusoids (photo. 1). While kidneys of the same group showed degenerative changes of some renal tubules (photo. 2).

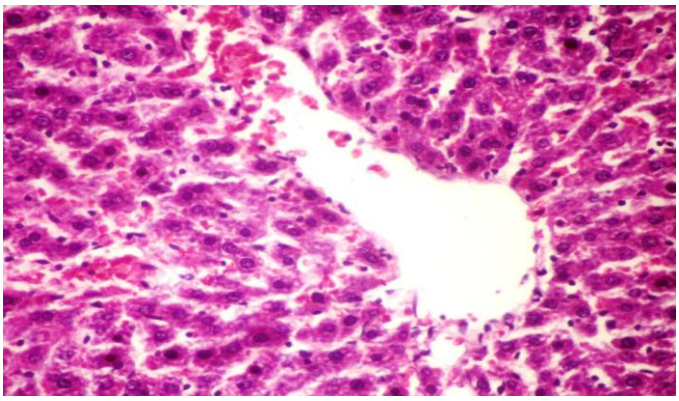

**Photo (1):Liver showing congested central vein (c), and blood sinusoids (arrows) (H4EX400).**

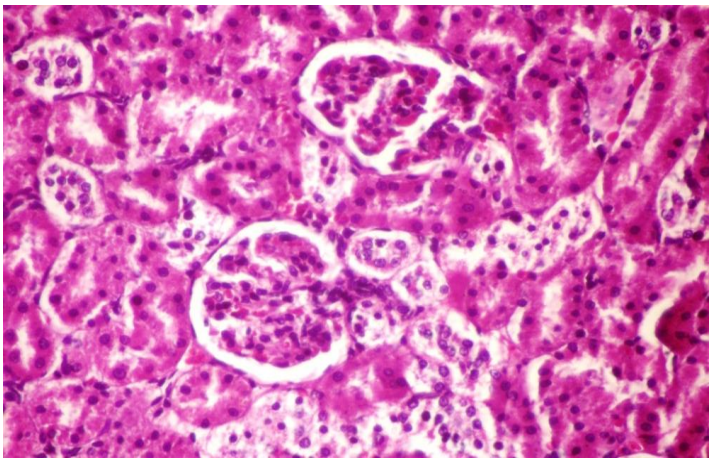

**Photo (2): Kidneys showing degeneration changes of some renal tubules (arrows) (H4E X 400).**

**Regarding positive control group:**

Microscopic examination of **liver** revealed congestion of the hepatoportal blood vessels **(photo. 3).** While **kidneys** of the same group showed interstitial blood vessel congestion and necrosis renal tubules **(photo. 4).**

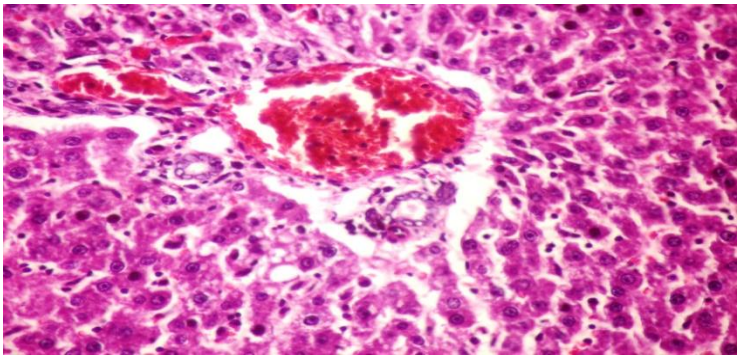

**Photo. (3): Liver showing congested of the hepatopatal blood vessel (c) (H4EX400).**

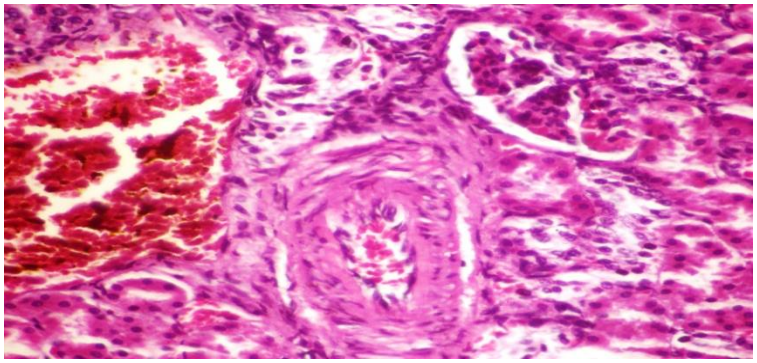

**Photo (4): Kidneys showing interstitial blood vessel cangestion (c) and necrosed renal tubules (n) (H4EX400)**

**Regarding group fed 10% fresh brocooli:**

Microscopic examination of **liver** revealed congested central vein and vacuolated hepatocytes **(photo 5).** While **kidneys** of the same group showed necrosed renal tubules **(photo 6).**

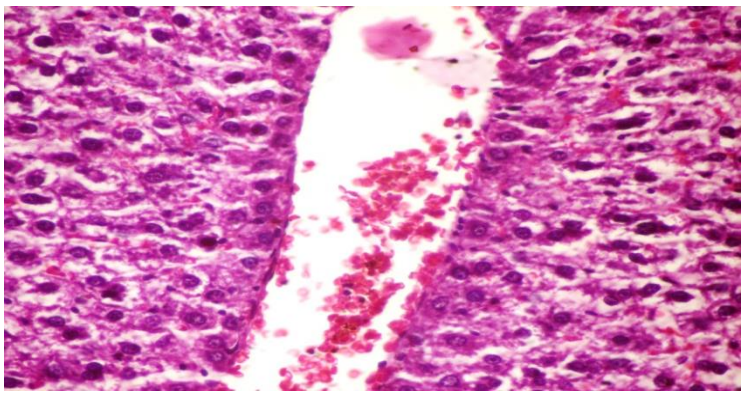

**Photo (5): Liver showing cangested central vein (c), and vacuolated hepatocytes (arrows) (H4EX400).**

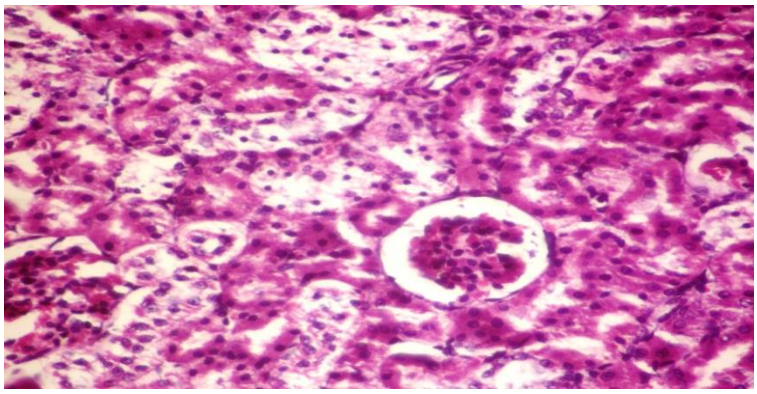

**Photo (6): Kidneys showing necrosed renal tubules (n) (H4EX400)**

**Regarding group fed 20% fresh brocooli:**

Microscopic examination of **liver** revealed congestion of the hepatoportal blood vessels **(photo. 7).** While **kidneys** of the same group showed necrosed renal tubules **(photo 8).**

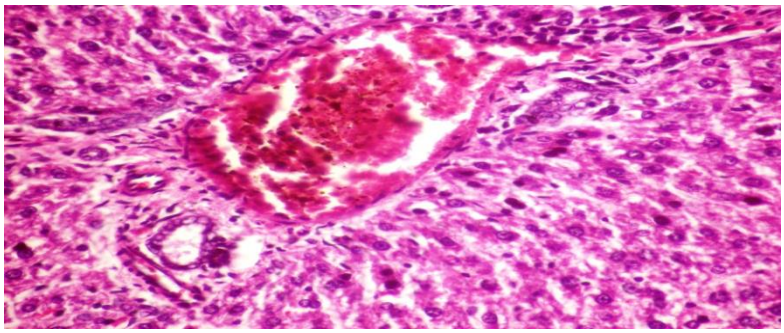

**Photo (7): Liver showing cangestion of the hepatopatal blood vessel (c) (H4EX400).**

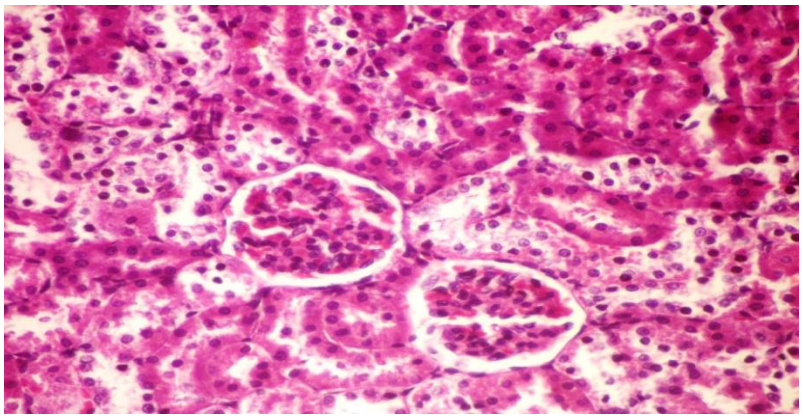

**Photo (8): Kidneys showing necrosed renal tubules (n) (H4EX400).**

**Regarding group fed 30% fresh brocooli:**

Microscopic examination of **liver** revealed area of necrosisin filtrated with mononuclear inflammatory cells **(photo. 9).** While **kidneys** of the same group showed blood vessel congestion and necrosed renal tubules **(photo. 10).**

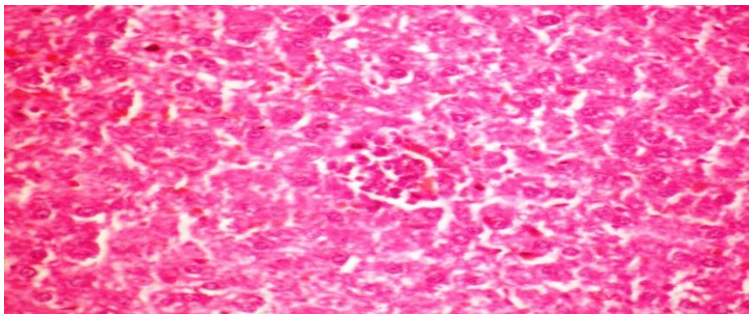

**Photo (9): Liver showing local area of necrosis infiltrated with manonuclear inflammatory cells (H4EX400)**

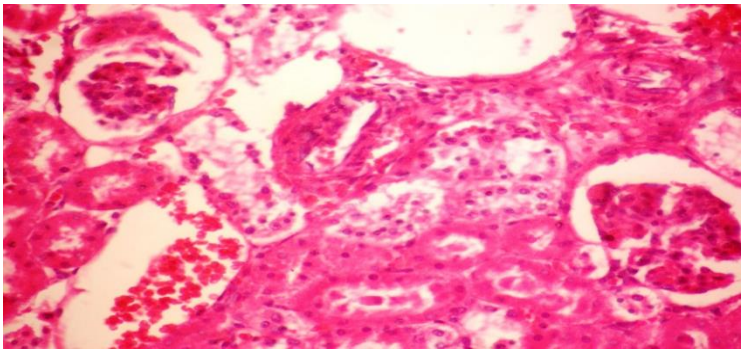

**Photo (10): Kidneys showing blood vessel congestion and necrosed renal tubules (H4EX400).**

The histopathological results of liver and kidney tissues showed that, liver of the negative and positive control revealed slightly congested central vein and blood sinusoids meanwhile, liver of rat from positive group showed congestion of the hepatoportal blood vessels. However, examined sections of liver of rat treated with 10%fresh revealed congested central vein and vacuolated hepatocytes. While liver of 20% revealed congestion of the hepatoportal blood vessels. Microscopic examination of rats liver fed 30% broccoli revealed portal tract leucocytic cell infiltration**.**

Concerning kidney of the negative control rat revealed degenerative changes of some renal tubules. While, kidney of rats from treated group,10% and 20% fresh broccoli showed interstitial blood vessel congestion and necrosed renal tubules**.** Some examined section from rat treated with 30% fresh broccoli revealed necrosed renal tubules and focal area of leucocytic cell infiltration.

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## الملخص

**اختار الباحثون البروكلى ضمن اهم عشر أغذيه فى العالم لما له من تأثير ايجابي على الصحة، حيث يتميز بلونه الأخضر المبهج وطعمه المميز، فهو غذاء مفيد غنى بالمواد المضادة للأكسدة والتى تحمى الخلايا من التلف والإصابة بالسرطان. ويحتوى على كميات كبيرة من المعادن مثل الكالسيوم والمغنسيوم والحديد والفيتامينات الأساسية وأهمها فيتامين ج. حيث أكدت الأبحاث الطبية الحديثة ضرورة إدراج نبات البروكلى ضمن النظام الغذائي اليومي الذى يعمل على تقليل مستويات الكوليستيرول الضار فى الدم بنسبة 6% وفقا للتجارب العلمية الحديثة. (جريده اليوم السابع ،الخميس 9 ابريل 2015 ).**

**هدف الدراسة :**

تهدف هذه الدراسة لمعرفه تأثير التغذية بمستويات مختلفة من البروكلى الطازج على وظائف الكبد والكلى ودهون وسكر وبروتينات الدم وذلك باستخدام فئران الالبينو البالغة كحيوانات تجارب.

**قسمت هذه الدراسة الى اربعة أقسام:**

**اولآ: الدراسة الكيمائية**

تم اخذ عينة من نبات البروكلى الطازج لتقدير المكونات الكيميائية (البروتين، الدهن، الرطوبة، الألياف، الرماد، ثم الكربوهيدرات بالطرح من 100). وذلك بمركز التجارب والبحوث الزراعية بكلية الزراعة جامعة الزقازيق. وكذلك تم تقدير نسب المواد الفعالة مثل الفلافونيدات والصابونين بمركز البحوث الزراعية بالدقي .

**ثانيا: الدراسه البيولوجيه**

تم تجهيز ثلاثون من ذكور فئران الالبينو البالغين والتى تزن (80 ±5 جم) والتى تم شراؤها من المركز القومي للبحوث بالقاهرة. وتم تقسيم الفئران الى خمس مجموعات كل مجموعه ست فئران ووضعت فئران التجارب فى أقفاص سلكية تحت ظروف صحية مع تكييف الهواء ببيت الحيوان بكلية العلوم جامعه الزقازيق.

أجريت التجربة على مرحلتين. فى المرحله الاولى تم تغذية جميع الفئران على الوجبة القياسية لمدة أسبوع حتى تتأقلم على نوعية الغذاء. فى المرحلة الثانية، قسمت الفئران إلى مجموعتين. المجموعة الاولى مكونة من ستة فئران وأطلق عليها المجموعة الضابطة السالبة والتي تغذت على الوجبة القياسية لمدة سبعة أسابيع. اما المجموعة الثانية والمكونة من 24 فأر

قسمت إلى اربع مجموعات فرعية (كل مجموعة مكونة من ستة فئران) تغذت جميعها على غذاء يحتوى على 10% دهن حيوان+ 1% كوليستيرول وذلك لمدة ثلاثة أسابيع وذلك لأصابتها بارتفاع نسبة الكوليستيرول بالدم. واستمرت المجموعة الفرعية الاولى فى تناول تلك الوجبة لمدة اربعة اسابيع اخرى. اما المجموعات الفرعية الثلاث الاخرى فقد تغذت على الوجبة عالية الكوليستيرول مضافة اليها نسبة 10 &20&30% بروكلى طازج، على التوالى.

**ثالثا: الدراسه البيوكيميائية:**

فى نهاية التجربة البيولوجية تم تصويم الفئران 12 ساعة ووزنهم وذبحهم وتجميع عينات الدم من الوريد الكبدي البابى فى أنابيب الطرد المركزي لفصل السيرم وحفظه فى أنابيب محكمة الغلق ومعقمة ومرقمة وذلك لإجراء التحاليل البيوكيميائية التالية:

Total Cholesterol , Triglycerides , HDL-c , LDL-c , ALT , AST, ALP, TP. ,ALB. ,

Glucose , Urea , Uric acid and Createnine.

كما تم نزع الأعضاء الداخلية كل فأر (الكليتين والكبد) ثم وزنها بعد تجفيفها باستخدام ورق الترشيح وحفظها فى محلول الفورمالين 10% لكل فأر على حدا لإجراء الفحص الهستوباثولوجى لكل من الكبد والكلى.

تم جميع البيانات ثم تحليلها إحصائيا بواسطة برنامج (ANOVA) وعرضت النتائج فى صورة المتوسط الحسابي + الانحراف المعياري للمتوسط عند مستوى معنويه0,05%.

**رابعا: النتائج البيوكميائية:**

تأثير الوجبات عالية الكوليستيرول المضاف إليها مستويات مختلفة من البروكلى الطازج بنسب (10-20-30%) على مستوى دهون الدم:

تشير متوسطات قيم مستويات الكوليسترول الكلى والجليسريدات الثلاثية والكوليستيرول مرتفع الكثافة والكوليسترول منخفض الكثافة للحيوانات عالية الكوليستيرول والمغذاة على مستويات مختلفة من البروكلى الطازج أن هناك اختلافات معنوية عالية على مستوى ($p<0.05$) بين كل مجموعات الحيوانات المعالجة بمقارنتها بالمجموعة الضابطة الموجبة المصابة. بينما توجد اختلافات معنوية ضعيفة على مستوى ($p<0.05$) لهذه القيم للفئران المغذاة على 30% بروكلى طازج مقارنه بالمجموعة الضابطة السالبة (السليمة). هذا يعنى ان

تدعيم غذاء الفئران المصابة بارتفاع نسبة كوليستيرول الدم بـ30% بروكلى طازج ادى إلى تحسن فى مستويات الكوليستيرول الكلى والجليسريدات الثلاثية والكوليستيرول مرتفع الكثافة والكوليستيرول منخفض الكثافة عن باقي نسب التدعيم الأخرى.

تأثير الوجبات عالية الكوليستيرول المضاف إليها مستويات مختلفة من البروكلى الطازج بنسب (10-20-30%) على وظائف الكبد (ALP&AST & ALT):

تظهر متوسط قيم مستويات AST, ALP وALT أن هناك اختلافات معنوية عاليه على مستوى ($p<0.05$) بين قيم وAST, ALT ALP, لكل مجموعات الحيوانات المعالجة بمقارنتها بالمجموعة الضابطة الموجبة المصابة. بينما توجد اختلافات معنوية ضعيفة على نفس المستوى لهذه القيم للفئران المغذاة على 30% بروكلى طازج مقارنة بالمجموعة الضابطة السالبة (السليمة) .هذا يعنى ان 30% من البروكلى الطازج أفضل المستويات.

تأثير الوجبات عالية الكوليستيرول المضاف اليها مستويات مختلفة من البروكلى الطازج بنسب (10-20-30%) على وظائف الكلى (اليوريا، حامض اليوريك والكرياتينين):

تشير متوسط قيم وظائف الكلى للفئران المغذاة على علائق تجريبية مدعمة بمستويات مختلفة من البروكلى الطازج (10-20-30%). على وجود اختلافات معنوية عالية على مستوى ($p<0.05$) لكل من اليوريا وحامض اليوريك والكرياتينين بين كل مجموعات الحيوانات المعالجة بمقارنتها بالمجموعة الضابطة الموجبة (غير المعالجة). بينما توجد اختلافات معنوية ضعيفة على نفس المستوى من المعنوية لهذه القيم للفئران المغذاة على 30% بروكلى طازج مقارنة بالمجموعة الضابطة السالبة (السليمة). هذا يعنى ان 30% من البروكلى الطازج أفضل المستويات فى تحسين وظائف الكلى للفئران المصابة.

تأثير الوجبات عالية الكوليستيرول المضاف اليها مستويات مختلفة من البروكلى الطازج بنسب (10-20-30%) على مستوي سكر الدم (الجلوكوز):

يشير متوسط نسب سكر الدم (الجلوكوز) للفئران المغذاة على علائق تجريبية مدعمة بمستويات مختلفة من البروكلى الطازج (10-20-30%) ان هناك اختلافات معنوية كبيرة ($p<0.05$) لنسب سكر الدم (الجلوكوز) لكل من مجموعات الحيوانات تحت التجريب المعالجة عند مقارنتها بالمجموعة الضابطة الموجبة (غير المعالجة). بينما توجد اختلافات معنوية ضعيفة لتلك القيم على نفس المستوى من المعنوية فى الفئران المغذاة على 30% بروكلى طازج مقارنة بالمجموعة السالبة السليمة. هذا يعنى ان 30% من البروكلى الطازج افضل المستويات.

تأثير الوجبات عالية الكوليسترول المضاف اليها مستويات مختلفة من البروكلى الطازج بنسب (10-20-30%) على بروتينات الدم (Albumin &. TP.) فى فئران الألبينو:

تشير متوسط قيم البروتين الكلى والالبيومين للفئران المغذاه على علائق تجريبية مدعمة بمستويات مختلف من البروكلى الطازج (10-20-30%) ان هناك اختلافات معنوية كبيرة على مستوى ($p<0.05$) لكل من قيم الالبيومين والبروتين الكلى لمجموعات الحيوانات المعالجة عند مقارنتها بالمجموعة الضابطة الموجبة المصابة. بينما توجد اختلافات معنوية ضعيفة لتلك القيم على نفس المستوى من المعنوية فى الفئران المغذاة على 30% بروكلى طازج مقارنة بالمجموعة السالبة السليمة. حيث ان نسبة 30% بروكلى طازج سجلت افضل النتائج.

**خامسا: التوصيات:**

1- يوصى البحث بتناول نبات البروكلى بنسبة 30 % من كمية الوجبة حيث ان ذلك يحسن من وظائف الكبد والكلى.
2- يوصى البحث بتناول نبات البروكلى بنسبة 30 % من كمية الوجبة حيث ان ذلك يعمل على خفض نسبة سكر الدم.
3- كما يوصى البحث بتناول نبات البروكلى بنسبة 30 % من كمية الوجبة لكى يحسن من نسبة بروتين والبيومين سوائل الدم.