The Potential Effect of the Infection by Downy Mildew (*Bremia Lactucae*) in Lettuce Plants (*Lactuca Sativa,L*) on the Experimental Rats

Prof. Dr,Mohamed Mostafa El-Sayed
prof. of Nutrition and Food Science
Ex- Dean Faculty of Home Economics
Menoufia University

Assistant.Prof.Dr. Abeer Nazeih Ahmed Abd- El-Rahman
Assistant prof. of Nutrition and Food Science
Faculty of Home Economics
Menoufia University

Donia Fathi Muhammad Al-Sahiti
Master's researcher
Department of Nutrition and Food Science
Faculty of Home Economics
Menoufia University

Abstract:

This study aimed to find out the possible effect of downy mildew infection of lettuce plants in powder form on experimental rats. Thirty adult white male rats , each weighing (110-140) g , were used and divided into 5 equal groups (sex rats in each group). The first group is the negative control group. As for the other groups, healthy and infected lettuce leaves were added, used at a rate of 5% and 10% each of the main meal in crushed form. At the end of the experiment, they were anesthetized and blood samples were collected and body weight gain , feed intake, Feed Efficiency Ratio, liver functions (GPT - GOT - ALP), kidney functions (urea - creatinine - uric acid), total protein, (albumin - globulin), blood lipid level (total cholesterol - triglycerides), lipoproteins (HDL-c - LDL-c - VLDL-c) and complete blood count (hemoglobin - hematocrit - platelets - RBCs - WBCs) were evaluated. Histopathological examination of the liver and kidney was also performed. The obtained results concluded that feeding on lettuce leaves led to a significant increase in (hemoglobin, hematocrit, platelets and red blood cells) and a decrease in (albumin, uric acid, urea, creatinine, white blood cells and liver and kidney weight) compared to the negative control group. Also feeding on 5% of infected lettuce leaves gave the highest value in (hemoglobin, hematocrit, platelets and red blood cells) and the lowest value in (albumin, uric acid, creatinine and white blood cells).

Keywords: Biological evaluation – Lipid profile – Complete blood count – Liver and kidney functions - Histopathological Changes.

التأثير المحتمل للإصابة بالبياض الزغبي
(Bremia Lactucae) Downy Mildew
لنباتات الخس (*Lactuca Sativa, L*) علي فئران التجارب

المستخلص

هدفت هذه الدراسة إلي معرفة التأثيرالمحتمل للإصابة بمرض البياض الزغبي لنباتات الخس في صورة مسحوق علي فئران التجارب. تم استخدام ٣٠ فأر ذكر أبيض بالغ يتراوح وزن كل منهما (١١٠– ١٤٠) جم وتم تقسيمهم إلي ٥ مجموعات متساوية (ست فئران في كل مجموعة). المجموعة الأولي هي المجموعة الضابطة السالبة أما المجموعات الأخري أضيفت أوراق الخس السليمة والمصابة المستخدمة بنسبة ٥% و ١٠% لكل منهما من الوجبة الأساسية علي هيئة مطحون. وفي نهاية التجربة تم تخديرهم وتجميع عينات الدم ثم تم تقييم وزن الجسم المكتسب ، الغذاء المتناول ، نسبة كفاءة الغذاء ، وظائف الكبد(GPT – GOT– ALP) ، وظائف الكلي (اليوريا – الكرياتينين – حمض اليوريك) ، البروتين الكلي ، (الألبيومين – الجلوبيولين) ، مستوي دهون الدم (الكوليسترول الكلي – الجليسريدات الثلاثية) ، الليبوبروتينات (HDL–c – VLDL–c – LDL–c) و فحص الدم الشامل (الهيموجلوبين– الهيماتوكريت – الصفائح الدموية – WBCs – RBCs) كذلك تم إجراء الفحص الهستوباثولوجي لكل من الكبد والكلي . وقد خلصت النتائج المتحصل عليها إلي أن التغذية علي أوراق الخس أدت إلي زيادة معنوية في (الهيموجلوبين ،الهيماتوكريت ، الصفائح الدموية وكرات الدم الحمراء) وانخفاض في(الألبيومين، حمض اليوريك ، اليوريا ، الكرياتينين ، كرات الدم البيضاء ووزن الكبد والكلي) مقارنة بالمجموعة الضابطة السالبة و أيضا التغذية علي ٥% من أوراق الخس المصابة أعطت أعلي قيمة في(الهيموجلوبين ، الهيماتوكريت، الصفائح الدومية وكرات الدم الحمراء) و أقل قيمة في (الألبيومين ، حمض اليوريك ، الكرياتينين وكرات الدم البيضاء).

الكلمات الافتتاحية : التقييم البيولوجي – دهون الدم – صورة الدم – وظائف الكلي والكبد– التغيرات الهستوباثولوجية .

Introduction:

Lettuce (*Lactuca sativa, L*) belongs to the Asteraceae family and originates in the Mediterranean. Due to the minimal processing procedures, such as cutting, washing, drying, packing, and low-temperature distribution and storage, fresh-cut lettuce maintains its nutritional value and bioactivity. Additionally, the findings demonstrated that the consumption of lettuce preserved in modified-atmosphere packaging did not alter the plasma redox state of healthy participants **(Serafini *et al* ., 2002**).

Additionally, phytochemical compositions and contents are different in various types. Compared with green lettuce and red lettuce, some studies have reported that red lettuce has comparatively higher phenolic levels than green. Thus, red lettuce is a good source of antioxidants in the daily diet **(Wilson *et al*., 2004**).

Lettuce is a successful and diverse plant distributed worldwide .The first cultivated lettuce appears in several primitive writings in early 2680 BCE as a medicinal herb. The Asteraceae can be considered the family of plants with a large number of species, around 23,000 to 30,000 **(Funk *et al.,* 2005)** . In Asian countries, lettuce and asparagus lettuce are traditionally cooked rather than consumed raw **(Mou , 2009).** Consuming lettuce is beneficial for human health related to the bioactive compounds in vegetables (**Coria- Cayupán *et al*.,2009**).

Natural antioxidant compounds extracted and identified from plant sources such as lettuce have become a popular trend because of the general awareness of the importance of a healthy diet and limitations on the use of hazardous synthetic antioxidants **(Vázquez *et al.,* 2012**).

The neurological properties of lettuce are another health aspect of lettuce and detailed research in recent times has proved the anxiolytic properties of lettuce in vivo. In an experimental animal model, for lettuce extract fed animals, locomotive activity was found to be reduced, suggesting significant anxiolysis **(Komaki *et al.,* 2014).**

Formally, there are seven different types in the lettuce group, including Cos (Romaine), Butterhead, Leaf , Stalk (or Asparagus), Crisphead (Iceberg) , Latin, and Oilseed. The whole heads of lettuce and the fresh- cut form are the common products in the market. The colors, shapes, and nutrients are important factors to affect consumer purchase choice **(Mampholo *et al*., 2016**).

Lettuce, a low-calories, low-fat, and low-sodium salad vegetable, is rich in fiber, folate, and vitamin C, as well as essential minerals such as iron . It is also an abundant source of other natural health- promoting phytochemicals and vitamins, including glycosylated flavonoids, hydroxycinnamic acids, sesquiterpene lactones (lactucin and lactucopicrin), carotenoids, the group B vitamins, ascorbic acid, and tocopherols. Lettuce secondary metabolites are potentially associated with many health-beneficial properties, including antifree radical, anti-inflammatory, antidiabetic, anticancer, and anti cardiovascular diseases (CVDs) effects **(Kim *et al.*, 2016).**

Lettuce (*Lactuca sativa, L*) is one of the most frequently consumed vegetable crop species globally. The modern types of cultivated lettuce are categorized based on their huge diversity in morphological features and are mainly classified as crisphead, butterhead, romaine, looseleaf, or stem lettuce **(Zhang *et al.*, 2016).**

Lettuce is commercially accessible year-round and grown in open fields, greenhouses, or the plant factory with artificial light systems (PFALs). The annual global production of lettuce (and chicory) is 27.2 million tons; China, the United States of America, India, Spain, and Italy are the top five producers **(USDA, 2012).**

Lettuce is an important natural source of phytochemicals. These molecules, which include glycosylated flavonoids, phenolic acids, carotenoids, vitamin B groups, ascorbic acid, tocopherols, and sesquiterpene lactones, are vital bioactive nutritional components **(Uddin *et al*.**, **2020** and **Mitra *et al*.**,**2021**). It was found that lettuce contains a high amount of Vitamin C **(Medina- Lozano *et al*.**, **2021**).

Furthermore, several researchers found that hydroponic *Lactuca sativa* can heal diseases such as oxidative damage, cancer, Alzheimer’s disease, and diabetes **(Bahbah *et al.*, 2021** and **Naseem and Ismail , 2022**).

Lettuce is rich in water with 94–95% content and low in calories. It is also an excellent source of vitamins, minerals, and bioactive compounds such as polyphenols, carotenoids, and chlorophyll with related health benefits **(Yang *et al*.**, **2022)**.

The lettuce downy mildew, caused by the biotrophic oomycete *Bremia lactucae*, is one of the main diseases of lettuce crop, because it reduces the quality of the leaves - the marketable part of the crop and is often accentuated by post-harvest losses, which occur during transport and storage (**Kunjeti *et al.*, 2016** and **Parra *et al.*, 2016).**

Symptoms of lettuce downy mildew usually occur as pale-yellow, angular patches delineated by the veins of the leaves. On the abaxial side of the leaves, the presence of white sporangia, consisting of sporangiophores and sporangia is observed (**Mieslerová *et al.*, 2013).**

The disease management of lettuce downy mildew is mainly done through fungicide application and use of resistant cultivars, carrying Dm genes or resistance factors R, which express hypersensitivity reaction **(Castoldi *et al.*, 2014** and **Tobar-Tosse *et al.*, 2017)**.

Materials and Methods:

Materials:

The used plants:

Lettuce Leaves (*Lactuca sativa* , *L*) were obtained from local market in shebin El-kom.

Rats:

Thirty adult male albino rats, weighing 110-140g from Medical Insects Research Institute, Doki, Cairo, were used in this study.

Rats were housed in wire cages under the normal laboratory condition and were fed on standard diet for a week as an adaptation period. Diet was offered to rats in special food cups to avoid looser conditions of food, water was provided to the rats by glass tubes supported to one side of the cage, food and water provided adlabium and checked daily.

Methods:

Preparation of materials:

All healthy lettuce leaves and infected with downy mildew were ground to give a powder after being sun-dried separetely **(Rojano-Aguilar *et al.*, 2017)** and kept in dark glass vials in a cool, dry and dark place to reduce oxidation of their contents until they are used.

Biological experiments:

Basal diet composition of tested rats:

Table (A): The composition of standard (basal) diet.

Compounds	Amount
Protein	10%
Corn oil	10%
Mineral Mixture	4%
Vitamin Mixture	1%
Cellulose	5%
Choline chloride	0.2%
Methionine	0.3%
Corn starch	Up to 100% (79.5%)

AIN., (1993).

Experimental design:

The experiment was done in the Faculty of Home Economics, Menoufia University, Shebin El-kom. Rats were housed in wire cages in a room temperature 25 C^{o} and kept under normal healthy conditions.

Rats were divided into the following groups:

Group (1): Negative control group- normal group- (6 RATS), In this group rats were kept on standard diet and tap water.

Group (2): (6 RATS) Normal Rats were fed on healthy lettuce leaves powder at a concentration of 5% of the basal diet.

Group (3): (6 RATS) Normal Rats were fed on healthy lettuce leaves powder at a concentration of 10% of the basal diet.

Group (4): (6 RATS) Normal Rats were fed on infected lettuce leaves powder at a concentration of 5% of the basal diet.

Group (5): (6 RATS) Normal Rats were fed on infected lettuce leaves powder at a concentration of 10% of the basal diet.

Blood Sampling And Organs:

Blood samples were collected after 12 hours fasting at the end of the experiment using the abdominal aorta in which the rats were scarified under ether anetheized. Blood samples were received in to clean dry centerfuge tubes and left to clot at room temperature, then centerfuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully aspirate, transferred in to clean cuvet tubes, and stored frozen at-20^{o}C for analysis. All serum samples were analyzed for determination the following parameters:

Lipid profile / cholesterol, total cholesterol **(T.C)**, triglycerides **(T.G),** high density lipoprotein **(H.D.L-C),** low density lipoprotein **(L.D.L-C),** very low density lipoprotein **(V.L.D.L-C),** urea, creatinin, uric acid, glotamic oxaloacetic transaminas **(G.O.T),** glotamic pyruvic transaminas **(G.P.T),** and alkaline phsphatase **(A.L.P).**

At the same time, the organs: kidney and liver were removed, washed in salin solution, dried by filter paper, weighted, and stored frozen in formalin solution 10% for histopathological testing according to method mentioned by **Drury and Wallington (1980).**

Analytical Methods:

The following techniques were used for determination of different parameters in serum.

Estimation of serum lipid:

Triglycerides:

Enzymatic calorimetric determination of triglycerides was carried out according to **Fassati and prencipe (1982).**

Total cholesterol:

The principle use of total cholesterol determination according to **Allen (1974).**

HDL-cholesterol:

HDL can be determined by the same method used for total cholesterol, according to **Lopez (1977).**

VLDL and LDL- cholesterol:

The determination of VLDL (very low density lipoproteins) and LDL were carried out according to the method of **Lee and Nieman (1996)** as follows:

VLDL-c (mg/dl) = Triglycerdes/5

LDL-c (mg/dl) = (Total cholesterol – HDL) – VLDL

Determination of renal functions:

Estimation of urea:

Urea was determinated according to the enzymatic method of **Patton and Crouch (1977)**.

Estimation of creatinine in serum:

Creatinine was determinated according to kinetic method of **Henry (1974)**.

Determination of Uric acid:

The intensity of this red color formed is proportional to the uric acid concentration in the sample **(Schultz, 1984).**

Determination of liver functions:

Determination of serum alkaline phosphates (Alp):

Enzymatic calorimetric determination of alkaline phosphates was carried out according to **Belfield and Goldberg (1971).**

Estimation of serum glotamic oxaloacetic transaminases (GOT) and glotamic pyruvic transaminase (GPT):

GOT and GPT activities were measured according to method described by **Yound (1975)** and **Tietz (1976).**

<u>Histopathological Investigation:</u>

Small specimens from liver and kidney were collected from all experimental groups, fixed in 15% neutral buffered formalin, dehydrated in ascending concentration of ethanol (**70, 80, and 90%**), cleared in xylene and embedded in paraffin. Sections of (**4 - 6**) μm thickness were prepared and stained with Hematoxylin and Eosin according to **Bancroft *et al.*, (1996).**

<u>Statically analysis:</u>

The data were statistically analyzed using a computerized costat program by one way ANOVA. The results are presented as mean ± SD. Differences between treatments at ($P < 0.05$) were considered significant **(Snedecor and Cochran ,1967)**.

<u>Results and discussion:</u>

Changes of total cholesterol (TC) and triglycerides (TG) level of normal rats fed on diet supplemented with lettuce leaves:

Data tabulated in Table (1) show the changes of total cholesterol level of normal rats fed on diet supplemented with lettuce leaves. It is clear to mention that the total cholesterol level of control negative group recorded the highest value when comparing with other groups. The mean values were 140.35 , 85.24 , 88.58 , 73.52 and 92.49 mg/dl , respectively. There is non significant difference among the groups 2 , 3 and 5 but there is significant difference with the groups 1 and 4.

Also, Data presented in Table (1) show the changes of triglycerides level of normal rats fed on diet supplemented with lettuce leaves. It could be observed that the total triglycerides level of control negative group recorded the highest value when comparing with groups 2, 3 and 5, the lowest value when comparing with group 4 with significant differences . The mean values were 77.75 , 65.29 , 59.45 , 54.7 and 85.81 mg/dl , respectively.

Table (1): Changes of total cholesterol (TC) and triglycerides (TG) level of normal rats fed on diet supplemented with lettuce leaves

Variables	(G1) Negative control	(G2) Healthy lettuce leaves 5%	(G3) Healthy lettuce leaves 10%	(G4) Infected lettuce leaves 5%	(G5) Infected lettuce leaves 10%	L.S.D ($P \leq 0.05$)
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean SD±	
TC level (mg/dl)	140.35[a] ± 7.85	85.24[b] ± 1.817	88.58[b] ± 1.945	73.52[c] ± 5.990	92.49[b] ± 2.81	8.629
%change of negative Control	–	-39.266	-36.886	-47.614	-34.100	–
TG level (mg/dl)	77.75[b] ± 2.257	65.29[c] ± 1.850	59.45[cd] ± 3.745	85.81[a] ± 4.797	54.7[d] ± 2.573	5.877
%change of negative control	–	-16.025	-23.529	10.374	-29.646	–

Means in the same row with different litters are significantly different.

Changes of high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) level of normal rats fed on diet supplemented with lettuce leaves:

Data tabulated in Table (2) show the changes of high density lipoprotein level (HDL-c) of normal rats fed on diet supplemented with lettuce leaves. It is clear to notice that the high density lipoprotein level of control negative group recorded the highest value when comparing with other groups. The mean values were 47.30 , 46.77 , 46.69 , 40.53 and 46.44 mg/dl , respectively. There is non significant difference between the groups 1, 2, 3 and 5 but there is significant difference with the group 4 when comparing with other groups . Data presented in Table (2) show the changes of low density lipoprotein level (LDL-c) of normal rats fed on diet supplemented with lettuce leaves. It could be observed that the low density lipoprotein level of control negative group recorded the highest value when comparing with other groups. The mean values were 77.50 , 25.41 , 29.99 , 15.82 and 35.11 mg/dl , respectively. There is non significant difference among the groups 2, 3 and 5 while there is significant difference with the groups 1 and 4. Also, Data given in Table (2) show the changes of very low density lipoprotein level (VLDL- c) of normal rats fed on diet supplemented with lettuce leaves. The obtained data indicated that the very low density lipoprotein level of control negative group recorded the highest value when comparing with groups 2, 3 and 5 , the lowest value when comparing with group 4 with significant differences . The mean values were 15.54 , 13.05 , 11.89 , 10.93 and 17.16 mg/dl , respectively.

Table (2):Changes of high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) level of normal rats fed on diet supplemented with lettuce leaves:

Variables	(G1) Negative control	(G2) Healthy lettuce leaves 5%	(G3) Healthy lettuce leaves 10%	(G4) Infected lettuce leaves 5%	(G5) Infected lettuce leaves 10%	L.S.D ($P \leq 0.05$)
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean SD±	
HDL-C level (mg/dl)	47.30[a] ± 1.190	46.77[a] ± 1.185	46.69[a] ± 2.998	40.53[b] ± 2.790	46.44[a] ± 1.502	3.8035
%change of negative control	_	-1.120	-1.283	-14.305	-1.824	_
LDL-C level (mg/dl)	77.50[a] ± 8.135	25.41[b] ± 0.779	29.99[b] ± 3.541	15.82[c] ± 3.833	35.11[b] ± 2.268	8.1026
%change of negative control	_	-67.214	-61.304	-79.580	-54.694	_
VLDL-C level (mg/dl)	15.54[b] ± 0.45	13.05[c] ± 0.37	11.89[cd] ± 0.75	17.16[a] ± 0.96	10.93[d] ± 0.517	1.1779
%change of negative control	_	-15.984	-23.487	10.424	-29.626	_

Means in the same row with different litters are significantly different.

Changes of GPT, GOT liver enzyme and ALP level of normal rats fed on diet supplemented with lettuce leaves:

Data given in Table (3) show the changes of GPT liver enzyme level of normal rats fed on diet supplemented with lettuce leaves. The obtained data indicated that the GPT liver enzyme level of control negative group recorded the highest value when comparing with groups 4 and 5, the lowest value when comparing with groups 2 and 3 with significant differences. The mean values were 84.02 , 74.63 , 61.80 , 98.71 and 90.40 U/L , respectively. Data given in Table (3) show the changes of GOT liver enzyme level of normal rats fed on diet supplemented with lettuce leaves. The obtained data indicated that the GOT liver enzyme level of control negative group recorded the lowest value when comparing with other groups. The mean values were 27.07 , 42.68 , 79.30 , 63.97 and 74.87 U/L , respectively. There is non significant difference among the groups 3 and 5 but there is significant difference between the groups1, 2 and 4. Also, Data given in Table (3) show the changes of Alkaline Phosphatase (ALP) level of normal rats fed on diet supplemented with lettuce leaves. It could be noticed that the Alkaline Phosphatase (ALP) level of control negative group recorded the highest value when comparing with other groups. The mean values were 154.66 , 124.13 , 77.35 , 92.69 and 78.18 U/L , respectively. There is non significant difference among the groups 3,4 and 5 but there is significant difference between with the groups 1 and 2.

Table (3): Changes of GPT, GOT liver enzyme and ALP level of normal rats fed on diet supplemented with lettuce leaves:

Variables	(G1) Negative control	(G2) Healthy lettuce leaves *5%*	(G3) Healthy lettuce leaves 10%	(G4) Infected lettuce leaves 5%	(G5) Infected lettuce leaves 10%	L.S.D (P ≤ 0.05)
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean SD±	
GPT Level (U/L)	84.02^{b} ± 3.1009	98.71^{a} ± 5.215	90.40^{b} ± 2.276	74.63^{c} ± 3.580	61.80^{d} ± 6.496	8.014
%change of negative Control	_	17.479	7.593	-11.175	-26.441	_
GOT	27.07^{d} ±	42.68^{c} ±	79.30^{a} ±	63.97^{b} ±	74.87^{a} ±	10.094

level (U/L)	3.494	6.936	2.242	9.102	2.391	
%change of negative Control	_	57.630	192.890	136.260	176.540	_
ALP Level (U/L)	154.66[a] ± 7.479	124.13[b] ± 11.00	77.35[c] ± 10.56	92.69[c] ± 9.24	78.18[c] ± 8.30	17.121
%change of negative control	_	-19.740	-49.987	-40.068	-49.450	_

Means in the same row with different litters are significantly different.

As regards to liver enzymes , results indicated that GPT levels (U/L) was significantly reduced when rats were feed on 5% or 10% infected lettuce leaves , whereas feeding rats on healthy lettuce leaves significantly caused an increase in GPT levels. However, feeding rats on 5% healthy lettuce leaves caused a significant reduction in GOT level in serum , whereas feeding rats on 5% infected lettuce leaves significantly reduced GOT level. Data revealed that feeding rats on 5% or 10% healthy leaves significantly caused a reduction in ALP level. Also, feeding rats on healthy lettuce leaves significantly decreased TC and TG levels when compared to control negative group, whereas feeding rats on 5% infected lettuce leaves significantly increased Triglycerides in blood serum. Results indicated that feeding rats on healthy lettuce leaves significantly caused an increase in HDL-c, whereas 5% infected lettuce leaves significantly reduced HDL-c in blood serum. In addition, feeding rats on 5% or 10% healthy lettuce leaves caused a depression in LDL-c levels when compared to negative control , also, feeding rats on 5% infected lettuce leaves significantly decreased LDL-c and gave the lowest value. As regards to VLDL-c , feeding rats on healthy lettuce leaves significantly reduced VLDL-c comparing to control negative group , whereas feeding rats on 5% infected leaves significantly increased the VLDL-c in blood serum.

Vázquez *et al.* (2012) and Komaki *et al.* (2014) reported that natural antioxidant compounds extracted from plant sources such as lettuce have become a popular trend because of the general awareness of the importance of a healthy of a healthy diet and limitation on the use of hazardous synthetic antioxidants. Also, **Kim *et al.* (2016)** indicated that lettuce a low - calories , low fat and low - sodium salad vegetable and is rich in fiber , folate and vitamin C as well as essential minerals such as iron. In addition , **Yang *et al.* (2022)** reported that lettuce is rich in water 94 - 95% content and low in calories and also an excellent source of vitamins , minerals , and bioactive compounds such as polyphenols , carotenoids and chlorophyll with related health benefits.

Changes of Uric Acid , urea and creatinine level of normal rats fed on diet supplemented with lettuce leaves:

Data tabulated in Table (4) show the changes of Uric acid level of normal rats fed on diet supplemented with lettuce leaves. It is clear to mention that the Uric acid level of control negative group recorded the highest value when comparing with other groups with significant differences. The mean values were 7.12, 4.28 , 6.73 , 3.99 and 5.25 mg/dl , respectively. Data tabulated in Table (4) show the changes of Urea level of normal rats fed on diet supplemented with lettuce leaves. It is clear to mention that the Urea level of control negative group recorded the highest value when comparing with other groups with significant differences. The mean values were 120.03, 104.40 , 85.16 , 53.84 and 42.45 mg/dl , respectively. Data given in Table (4) show the changes of creatinine level of normal rats fed on diet supplemented with lettuce leaves. The obtained data indicated that the creatinine level of control negative group recorded the highest value when comparing with other groups. The mean values were 0.98 , 0.68, 0.60, 0.54 and 0.63 mg/dl , respectively. There is non significant difference among the groups 2 and 5 while there is significant difference between the groups 1, 3 and 4.

Table (4): Changes of Uric Acid , urea and creatinine level of normal rats fed on diet supplemented with lettuce leaves:

Variables	(G1) Negative control	(G2) Healthy lettuce leaves 5%	(G3) Healthy lettuce leaves 10%	(G4) Infected lettuce leaves 5%	(G5) Infected lettuce leaves 10%	L.S.D ($P \leq 0.05$)
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean SD±	
UA (mg/dl)	7.12^a ± 0.1006	4.28^d ± 0.285	6.73^b ± 0.075	3.99^e ± 0.1248	5.25^c ± 0.102	0.2856
%change of negative control	–	-39.870	-5.433	-43.984	-26.252	–

Urea (mg/dl)	**120.03[a] ± 3.87**	**104.40[b] ± 6.67**	**85.16[c] ± 6.75**	**53.84[d] ± 3.68**	**42.45[e] ± 3.39**	**9.286**
%change of negative control	**–**	**-13.019**	**-29.051**	**-55.144**	**-64.633**	**–**
creatinine (mg/dl)	**0.98[a] ± 0.035**	**0.68[b] ± 0.03**	**0.60[bc] ± 0.015**	**0.54[c] ± 0.055**	**0.63[b] ± 0.025**	**0.0635**
%change of negative control	**–**	**-30.824**	**-38.657**	**-45.066**	**-35.300**	**–**

Means in the same row with different litters are significantly different.

As regards to renal functions , creatinine showed with the highest level in basal diet group , whereas feeding rats on healthy lettuce leaves in both 5% or 10% level caused a reduction in uric acid, urea and creatinine levels in blood serum. However, feeding rats on 5% or 10% infected leaves caused a depression in urea level and the differences were significant as compared to the treatments.

The kidney and liver functions had effected by lettuce leaves in both healthy or infected leaves. These changes are agreement with those of **Uddin *et al.* (2020) and Mitra *et al.* (2021).** They reported that lettuce is an important natural source of phytochemicals. These molecules, which include glycosylated flavonoids, phenolic acids, Carotenoids, Vitamin B group, Ascorbic acid, tecopherols, and sesquiterpene lactones are vital bioactive nutritional components.

Histopathological Results:

Histopathological examination of liver:

Microscopically, liver of rats from group 1 revealed the normal histological architecture of hepatic lobule (Photos 1 and 2). In contrast, liver of rats from group 2 showed Kupffer cells activation, sinusoidal leukocytosis (Photo 3), focal hepatocellular necrosis associated with inflammatory cells infiltration (Photo 4) and infiltration of portal triad with inflammatory cells (Photo 5). Meanwhile, liver of rats from group 3 described slight Kupffer cells activation (Photos 6 and 7) and slight congestion of central vein (Photo 8). Furthermore, liver of rats from group 4 showed no histopathological alterations (Photo 9) except slight Kupffer cells activation (Photo 10) and slight vacuolation of sporadic hepatocytes (Photo 11) in some sections. Likewise, liver of rats from group 5 revealed apparent normal hepatic lobule (Photo 12).

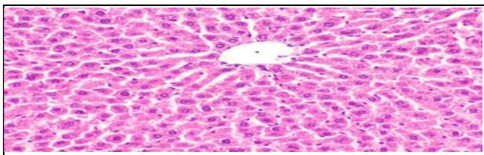

Photo (1): Photomicrograph of liver of rat from group 1 showing the normal histological architecture of hepatic lobule (H & E X 400).

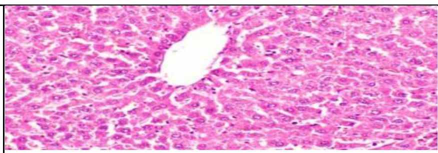

Photo (2): Photomicrograph of liver of rat from group 1 showing the normal histological architecture of hepatic lobule (H & E X 400).

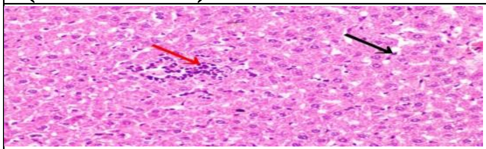

Photo (3): Photomicrograph of liver of rat from group 2 showing Kupffer cells activation (black arrow) and sinusoidal leukocytosis (red arrow) (H & E X 400).

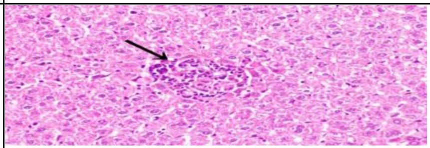

Photo (4): Photomicrograph of liver of rat from group 2 showing focal hepatocellular necrosis associated with inflammatory cells infiltration (arrow) (H & E X 400).

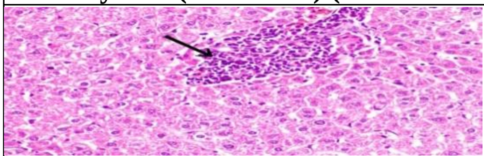

Photo (5): Photomicrograph of liver of rat from group 2 showing infiltration of portal triad with inflammatory cells (arrow) (H & E X 400).

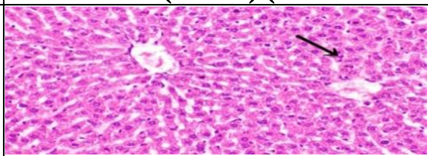

Photo (6): Photomicrograph of liver of rat from group 3 showing slight Kupffer cells activation (arrow) (H & E X 400).

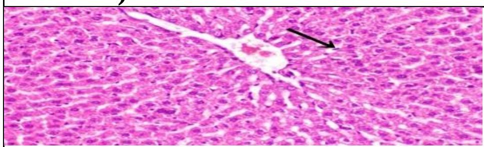

Photo (7): Photomicrograph of liver of rat from group 3 showing slight Kupffer cells activation (arrow) (H & E X 400).

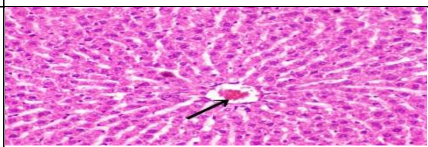

Photo (8): Photomicrograph of liver of rat from group 3 showing slight congestion of central vein (arrow) (H & E X 400).

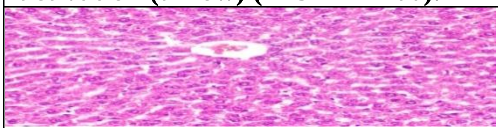

Photo (9): Photomicrograph of liver of rat from group 4 showing no histopathological alterations (H & E X 400).

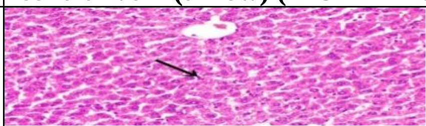

Photo (10): Photomicrograph of liver of rat from group 4 showing slight Kupffer cells activation (arrow) (H & E X 400).

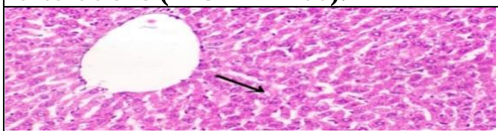

Photo (11): Photomicrograph of liver of rat from group 4 showing slight vacuolation of sporadic hepatocytes (arrow) (H & E X 400).

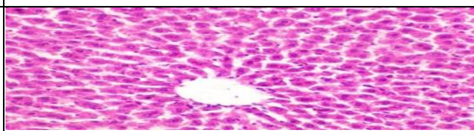

Photo (12): Photomicrograph of liver of rat from group 5 showing apparent normal hepatic lobule (H & E X 400).

References:

AIN. (American Institute of Nutrition), (1993): Purified diet for laboratory Rodent, Final report. J. Nutrition. 123:1939-1951. and O. compactume Benth. J. essential oil Res. 8 (6): 657-664.

Allain, C. C: (1974). Cholessterol enzymatic colorimetric method . J . of Clin. Chem.,20:470.

Bahbah, E.I.; Ghozy, S.; Attia, M.S.; Negida, A.; Emran, T.B.; Mitra, S.; Albadrani, G.M.; Abdel-Daim, M.M.; Uddin, M. and Simal- Gandara, J. (2021): Molecular mechanisms of astaxanthin as a potential neurotherapeutic agent. Mar. Drugs , 19, 201.

Bancroft, D.; Steven, A. and Tunner, R. (1996): Theory and practices of Histological Techniques, 4th Ed. Churchill Livingstone, Edinburg, London, Melbourne.

Belfied, A. and Goldberg, D.M. (1971): Alkaline phosphatase colorimetric method. J. of Enzyme, (12): 561.

Castoldi, R.; Charlo, HCO.; Melo, DM.; Candido, WS.; Vargas, PF.; Dalpian, T. and Braz, LT. (2014): Obtaining resistant lettuce progenies to downy mildew. Hortic Bras. 32:69-73.

Coria-Cayupán, Y.S.; Sánchez de Pinto, M.a.I. and Nazareno, M.n.A. (2009): Variations in bioactive substance contents and crop yields of lettuce (*Lactuca sativa L.)* cultivated in soils with different fertilization treatments. J. Agric. Food Chem. 57, 10122–10129.

Drury, R.A. and Wallington, E.A. (1980): Carlton's Histological Technique. 5th ed. Oxford University.

Fassati, P. and Prencipe, L. (1982): Triglyceride enzymatic colorimetric method. J. of Clin. Chem., (28): 2077.

Funk, V.A.; Bayer, R.J.; Keeley, S.; Chan, R.; Watson, L.; Gemeinholzer, B.; Schilling, E.; Panero, J.L.; Baldwin, B.G. and Garcia-Jacas, N. (2005): Everywhere but Antarctica: Using a supertree to understand the diversity and distribution of the Compositae. Biol. Skr. 55, 343–374.

Henry, R.J. (1974): Clinical Chemistry Principal and Techniques. 2nd ed. Harper and Publisher. New York.

Kim, M. J.; Moon, Y.; Tou, J. C.; Mou, B. and Waterland, N. L. (2016) : Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa L.*). Journal of Food Composition and Analysis, 49, 19–34.

Komaki, A.; Nasab, Z.K.; Shahidi, S.; Sarihi, A.; Salehi, I. and Ghaderi, A. (2014) : Anxiolytic effects of acute injection of hydro-alcoholic extract of lettuce in the elevated Plus-maze task in rats, Avicenna. J. Neuropsychol. Physiol. 1 (1), e 18695.

Kunjeti, SG.; Anchieta, A.; Martin, FN.; Choi, YJ.; Thines, M.; Michelmore, RW.; Koike, ST.; Tsuchida, C.; Mahaffee, W.; Subbarao, KV. and Klosterman, SJ. (2016): Detection and quantification of *Bremia lactucae* by spore trapping and quantitative PCR. Phytopathology. 106:1426-1437.

Lee, R. and Nieman, D. (1996): National Assessment. 2nd Ed., Mosby, Missouri, USA.

Lopez, M.F. (1977): HDL- cholesterol colorimetric method. J. of Clin. Chem., (230: 282

Mampholo, B. M.; Maboko, M. M.; Soundy, P. and Sivakumar, D. (2016): Phytochemicals and overall quality of leafy lettuce (*Lactuca sativa L.*) varieties grown in closed hydroponic system. Journal of Food Quality, 39(6), 805–815.

Medina-Lozano, I.; Bertolín, J. R. and Díaz, A. (2021): Nutritional value of commercial and traditional lettuce (*Lactuca sativa L.*) and wild relatives: Vitamin C and anthocyanin content. Food Chemistry, 359, 129864.

Mieslerová, B.; Lebeda, A.; Petrzelova, I. and Korbelova , P. (2013): Incicence of lettuce downy mildew (*Bremia lactucae*) and powdery mildew (*Golovinomyces cichoracearum*) in natural populations of prickly lettuce (*Lactuca serriola*). Plant Protect Sci. 49:24-32.

Mitra, S.; Rauf, A.; Tareq, A.M.; Jahan, S.; Emran, T.B.; Shahriar, T.G.; Dhama, K.; Alhumaydhi, F.A.; Aljohani, A.S. and Rebezov, M. (2021): Potential health benefits of carotenoid lutein: An updated review. Food Chem. Toxicol. 154, 112328.

Mou, B. (2009): Nutrient content of lettuce and its improvement. Curr.

Nutr. Food Sci. 5, 242–248.

Naseem, S. and Ismail, H. (2022): In vitro and in vivo evaluations of antioxidative, anti-Alzheimer, antidiabetic and anticancer potentials of hydroponically and soil grown *Lactuca sativa*. BMC Complementary Med. Ther. 22, 30.

Parra, L.; Maisonneuve, B.; Lebeda, A.; Schut, J.; Christopoulou, M.; Jeuken, M.; Mchale, L.; Truco, MJ. and Michelmore, R. (2016): Rationalization of genes for resistance to *Bremia lactucae* in lettuce. Euphytica. 210:309-326.

Patton. C.J. and Croush, S.R. (1977): Enzymatic Determination of Urea. J. Anal. Chem. 49: 464-469.

Rojano-Aguilar, A.; Santos, A.; Flores, J.; Salazar-Moreno, R. and Ruiz-García, A. (2017): Solar drying device tested with lettuce leaves. Acta Hortic. 1182, 105–110.

Schultz, A. (1984): Uric Kaplan A. Clin Chem. Mosby Co. St. Louis Toronto. Princeton; 1261-1266 and 418.

Serafini, M.; Bugianesi, R.; Salucci, M.; Azzini, E.; Raguzzini, A. and Maiani, G. (2002): Effect of acute ingestion of fresh and stored lettuce (*Lactuca sativa*) on plasma total antioxidant capacity and antioxidant levels in human subjects. Br. J. Nutr. 88, 615–623.

Snedecor, G.W. and Cochran, W.G. (1967): Statistical methods. 6th Edition, The Lowa State University, pub, U.S.A:841.

Tietz, N. W. (1976): Fundamentals of Clinical Chemistry. Philadelphia. B.W. Standers, P.243.

Tobar-Tosse, DE.; Candido, WS.; Marin, MV.; Panizi, RC.; Barbosa, JC. and Braz, LT. (2017): Resistance of green leaf lettuce lines to the *Bremia lactucae* races identified in São Paulo state. Summa Phytopathologica. 43:55-57.

Uddin, M.Z.; Rana, M.S.; Hossain, S.; Ferdous, S.; Dutta, E.; Dutta, M. and Emran, T.B. (2020): In vivo neuroprotective, antinociceptive, anti-inflammatory potential in Swiss albino mice and in vitro antioxidant and clot lysis activities of fractionated Holigarna longifolia Roxb. bark extract. J. Complement. Integr. Med. 17, 1-12.

USDA, (United States Department of Agriculture), 2012: "***Lactuca sativa,L***" (https://web.archive.org/web/20120608120552/http://plants.usda.gov/java/profile? symbol=LASA3) .

Vázquez, G.; Santos, J.; Freire, M.S.; Antorrena, G. and

González-Álvarez, J. (2012): Extraction of antioxidants from eucalyptus (Eucalyptus globulus) bark. Wood Sci. Technol. 46, 443–457.

Wilson, P.; Morrison, S.; Hedges, L.; Kerkhofs, N. and Lister, C. (2004): Phenolics contribute significantly to higher antioxidant activity of red lettuce compared to green lettuce. In Proceedings of the XXII International Conference on Polyphenols, Helsinki, Finland, pp. 273–274.

Yang, X.; Gil, M.I.; Yang, Q. and Tomás-Barberán, F.A. (2022): Bioactive compounds in lettuce: Highlighting the benefits to human health and impacts of preharvest and postharvest practices. Compr. Rev. Food Sci. Food Saf. 21, 4–45.

Yound , D.S. (1975): Determination of GOT. Clin. Chem., 22 (5): 21.

Zhang , F. H.; Yan, Y. L.; Wang, Y. and Liu, Z. (2016): Lactucin induces potent anti-cancer effects in HL-60 human leukemia cancer cells by inducing apoptosis and sub-G1 cell cycle arrest. Bangladesh Journal of Pharmacology, 11(2), 478–484.