

## Protective Effect of Olive Oil and Nigella Seed Oil on some Physiological Parameters in Rabbits Administered D-galactosamine

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### Summary:

This study was to evaluate some physiological parameters for the protective effect of olive oil and nigella seed oil in rabbits administered D-galactosamine (D-GaIN) (600 mg/kg) a single dose with concentrations of body weight of rabbits on some parameters of blood (84) of the male rabbits weighted (400-550g) and (10) weeks of age were randomly divided into six groups, six rabbits in each group, given all the animals normal food during the experimental period of 1,2 and 3 weeks, explained to the animals at the end of each period of experience and took blood samples to measure (Hb,RBC.s, WBC.s,WBC.d,N, WBC.d,L, WBC.d,M, WBC.d,E) was reached the following conclusions: D-GaIN injection caused gradually increase in Hemoglobin level (Hb) and WBC.s and reached the peak level in three weeks post treatment compared to control group. The joint administration of olive oil with D-GaIN to experimental animals improving of effect D-GaIN on oxidative stress with varying degree, there was gradually decreased in mean value of Hb and WBC.s at three-week compared to D-GaIN group. However, the elevation in the

mean value of WBC.d.L, WBC.d.N and WBC.d.E in two weeks compared to control. The treatment with Nigella oil after injecting D-GaIN to experimental animals influencing on hematological parameters with varying degree, treatment with Nigella oil after injecting D-GaIN to experimental animals influencing on hematological parameters with varying degree, there was gradually increase in mean value of Hb and WBC.d.L in two weeks compared to control group. Similarly, the mean value of WBC.d.N, WBC.s ( $9.30 \pm 2.03 \times 10^3$  cell/cu.mm. blood) counts were recorded in one week compared to control and D-GaIN groups.

*Keywords: (D-GaIN) D-galactosamine, olive oil, nigella oil*

## 1. Introduction

D-Galactosamine (D-GaIN) is an amino sugar that causes oxidative stress in various organisms "oxidative stress is associated with increasing of lipid peroxidation (LPO) products and development of many pathological alterations" especially liver diseases <sup>[1]</sup>. Liver injuries induced by D-galactosamine are the best characterized system of xenobiotics induced hepatotoxicity and commonly used models for the screening of antihepatotoxic and (or) hepatoprotective activities of drugs. Hence D-galactosamine mediated hepatotoxicity was chosen as the experimental model <sup>[2]</sup>.

Black cumin (*Nigella sativa* L.) is an annual herbaceous plant belonging to the Ranunculaceae family. The plant is indigenous to Mediterranean areas, through it grown in other parts of the world as well. The seeds of the *Nigella sativa* plant are black in color and look

like sesame seeds. Its seed contains 21% protein, 35 % carbohydrate, 35-38 % lipid <sup>[3]</sup>. *Nigella sativa* has been shown to produce multiple beneficial actions including hypoglycemic, hypocholesterolemic and antioxidant effects <sup>[4]</sup>.

The olive tree, *Olea europaea*, is native to the Mediterranean basin and parts of Asia. The fruit and compression extracted oil have a wide range of therapeutic and culinary applications. Olive oil also constitutes a major component of the “Mediterranean diet”. The chief active components of olive oil include oleic acid, phenolic constituents, and squalene <sup>[5]</sup>. The haematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status for both animals and humans <sup>[6]</sup>. This current study aims to investigate the protective effect of olive oil and nigella seed oil on some parameters of the liver, blood, heredity and oxidative stress against the toxicity of D-Galactosamine (D-GalN).

## 2. Materials and Methods

### Materials

#### Study area and experimental animals

The experimental animals consisted of eighty four local healthy male rabbits with body average weight (400-550g). With age average of (10) week were used in the present study. All animals were in standard environmental conditions and were provided with a standard commercial diet and water ad libitum. Animals were exposed to dark/light cycle of 12:12 hours. The study was carried out in Zoology laboratory, Department of Biology, Faculty of Applied Sciences,

Thamar University. All the procedures of testes and analyzed were performed in the Jamil Medical Laboratory, during the period from March 2012 to April 2013.

## Studied Materials

- a- Nigella seed oil was local from Al Ahlam Co. Sana'a -Yemen.
- b- Virgin olive oil was obtained from Al Shaimaa Co. Syria- Der Al Zor.

## Hepatic- Digressive

### D-galactosamin (D-GaIN) (Inducer Agent)

D-GaIN was obtained from SRL Co. India. (600 mg) of (D-GaIN) was dissolved in (3 ml) distilled water. Then each one of animals was injected in (I.P) with (600 mg/ kg).

## Hematological Tests

Microscope -Improved Neubauer Chamber -WBC,RBC pipette -  
WBC,RBC diluting fluid -Geimsa stain -Slides glasses -Ethanol  
96%

## Methods

### Experimental Design

Animals were randomly divided into (6) groups as follows:

**Group I:** Negative control group which contains (9) animals and were injected intra peritoneal (I.P) with normal saline (3 ml/kg) as a daily dose for a period of (1, 2, 3) weeks. **Group II:** Positive control group which contains (15) animals, all were injected by D-galactosamine in only one dose (600 mg/ kg) for a period of (1,2,3) weeks, then all were injected by intra peritoneal (I.P) with normal saline (3 ml/kg) as a daily

dose until the end of experiment. **Group III:** This experimental group contains (15) animals which were given olive oil orally in a dose of (2 ml/ kg) once daily, for a period of (1, 2, 3) weeks. **Group IV:** This experimental group contains (15) animals which had injection (IP) of D-galactosamine in only one dose of (600 mg/kg), then all were given olive oil orally in a dose of (2 ml/kg) once daily, for a period of (1, 2, 3) weeks. **Group V:** This experimental group contains (15) animals which were given Nigella seed oil orally in a dose of (1 ml/kg) once daily, for a period of (1, 2, 3) weeks. **Group VI:** This experimental group contains (15) animals which had injection (IP) of D-galactosamine in only one dose of (600 mg/kg) , then all were given Nigella seed oil orally in a dose of (1 ml/kg) once daily, for a period of (1, 2, 3) weeks.

## **Hematological Tests**

### **Hemoglobin**

Hemoglobin was determined according to the method of <sup>[7,8]</sup>.

### **The count of red blood cell (RBCs)**

The R.B.C. count was determined using method described by <sup>[9]</sup>.

### **The total count of white blood cell (WBCs)**

The W.B.C count was determined using method described by <sup>[9]</sup>.

### **The differential count of white blood cell**

The W.B.C count was determined using method described by <sup>[9]</sup>.

## **Statistical Analysis:**

Results were analyzed and entering data in the statistical tables to the statistical program known as SPSS version 15.5 and scheduling

for the purpose of the work of statistical analysis appropriate to it, and by following test Statistical named analyses of variation unidirectional (One Way ANOVA) and to find out less significant difference was followed by post-test for this test (LSD). All data in this study were expressed as mean  $\pm$  standard deviation (S.D.). P values less than or equal to (0.05), (0.01), (0.001) was considered significant.

### 3- Results and Discussion

#### Hemoglobin (Hb)

As shown the higher mean value was recorded in experiment group of D-GaIN ( $13.80 \pm 0.37$  g/dl) in three weeks, whereas, the lower mean value with experiment of olive oil ( $9.22 \pm 2.80$ g/dl) in two weeks compared to control group. There were significant differences in Hb values among the experiment and control groups ( $P < 0.01$ ).

In the current study, the results revealed that administration of D-GaIN to experimental animals influencing on hematological parameters with varying degree. As shown, there was gradually increase in mean value of hemoglobin and reached the peak level in three weeks post treatment compared to control group. Similarly, the mean value of WBCs and WBC.d.L counts were recorded in two and three weeks compared to control group. However, D-GaIN was influenced adversely on mean value of differential count of WBC. The result of this study is not in accordance with findings of <sup>[10]</sup>.

**Table: - (1) showed the Hemoglobin level in control groups and experimental groups**

Parameter Groups	N	Hemoglobin level g/dL.		
		1.W Mean ± S.D.	2.W Mean ± S.D.	3.W Mean ± S.D.
Control	3	10.16 ±1.92	10.90± 1.15	11.93±0.70 <sup>c</sup>
D-GalN	5	10.10±0.45	11.18± 1.26	13.80± 0.37 <sup>c</sup>
Olive oil	5	9.22± 2.80 <sup>A</sup>	11.88±0.55	10.54 ±1.17 <sup>c</sup>
Nigella oil	5	10.66± 1.40	10.94± 1.43	12.18± 0.34 <sup>c</sup>
Olive oil+ D-GalN	5	11.42 ±2.11	11.00±1.20	12.00±0.54 <sup>c</sup>
Nigella oil+ D-GalN	5	10.64 ±1.27	13.14±0.68 <sup>Bb</sup>	12.04± 0.16 <sup>c</sup>

*A,B,C: indicate to comparison with normal control group at one, two, three weeks respectively. a,b,c: indicate to comparison with D-GalN group at one, two, three weeks respectively. D-GalN: D-Galactosamine, M±S.D.: Mean ± Stander Deviation.1,2,3W: one, two, three weeks, N: number of animals.*

### The count of Red Blood Cell (RBCs)

As shown the higher mean value was recorded in experiment group of nigella oil ( $4.90 \pm 1.22 \times 10^6$  cell/cu.mm.b.) at one week; whereas, the lower mean value with experiment of olive oil ( $2.08 \pm 0.62 \times 10^6$  cell/cu.mm.b.) at one week compared to control and D-GalN groups. There were significant differences in RBC.s values among the experiment and control groups ( $P < 0.01$ ).

**Table: - (2) Showed the count of Red blood cells (R.B.C.s) in control groups and experimental groups**

Parameter Groups	N	The total count of Red blood cells ( $\times 10^6$ cell/cu.mm. blood)		
		1.W Mean ± S.D.	2.W Mean ± S.D.	3.W Mean ± S.D.
Control	3	4.36± 0.37	4.26 ±0.15	4.23±0.11
D-GalN	5	4.58± 0.25	4.30±0.12	4.62±0.08
Olive oil	5	2.08 ±0.62 <sup>Aa</sup>	4.20± .02	4.00± 0.10 <sup>c</sup>
Nigella oil	5	4.90 ±1.22 <sup>A</sup>	4.14±0.15	4.10± 0.12 <sup>c</sup>
Olive oil+ D-GalN	5	4.60 ±0.36	4.20 ±0.18	4.18 ±0.08
Nigella oil+ D-GalN	5	4.30± 0.33	3.72±0.48 <sup>b</sup>	4.30± 0.12

## Count of White Blood Cells (WBCs)

As shown the higher mean value was recorded in experiment group of nigella oil+D-GaIN ( $9.30 \pm 2.03 \times 10^3$  cell/cu.mm. b) at one week, whereas, the lower mean value with experiment of nigella oil ( $3.12 \pm 0.35 \times 10^3$  cell/cu.mm. b.) in three week compared to control and D-GaIN groups. There were significant differences in WBCs values among the experiment and control groups ( $P < 0.01$ ).

In present study, the result showed that treatment of olive oil with D-GaIN to experimental animals improving of effect D-GaIN on hematological parameters with varying degree. As shown, there was gradually decrease in mean value of Hb, and WBC.s were reached the close level of mean value of control in three weeks compared to D-GaIN group. However, the elevation in the mean value of WBC.d.L, WBC.d.N in two weeks compared to control and the elevation in the mean value of WBC.d.E were recorded on one and two weeks compared to control and D-GaIN groups. The result of this study is agreement with findings of <sup>[11]</sup> who found that olive oil improvement of the decreased in Hb, RBCs. and modulates the elevation in WBC.s. In this respect the present study showed marked reduction in Hb, WBCs., total leukocytes and elevation in monocytes, and lymphocytes. The outcome of these effects in all is indicative of lowered inflammatory tendency in response to olive oil intake, which in turn may decrease the risk of D-GaIN intoxicant. Moreover, olive oil with different phenolic contents were found to reduce fibrinogen levels and increase RBCs.

Count <sup>[12,13]</sup>, as well as Hb concentration <sup>[14]</sup>. Similar results were also observed with the present study, where injecting rabbits by D-GalN supplemented with olive oil showed lowering effect on Hb level, WBCs. and WBC.d.N, concomitant with elevations in WBC.d.L and other hematological indices (WBC.d.M and WBC.d.E). Other studies in humans and animals evidenced that phenolics (Phcs) from olive oil have demonstrated anti-inflammatory effects, through inhibiting a number of inflammatory mediators released by endothelial cells (ECs)<sup>[15]</sup>.

**Table: - (3) Showed the count of white blood cells in control groups and experimental groups**

Parameter Groups	N	The total count of white blood cells ( $\times 10^3$ cell/cu.mm. blood)		
		1.W Mean $\pm$ S.D.	2.W Mean $\pm$ S.D.	3.W Mean $\pm$ S.D.
Control	3	3.06 $\pm$ 1.43	4.93 $\pm$ 1.10	3.70 $\pm$ 0.55 <sup>c</sup>
D-GalN	5	3.86 $\pm$ 1.60	5.22 $\pm$ 0.32	5.26 $\pm$ 0.16 <sup>c</sup>
Olive oil	5	4.44 $\pm$ 0.24	3.54 $\pm$ 1.50 <sup>b</sup>	4.06 $\pm$ 1.40
Nigella oil	5	4.38 $\pm$ 0.47	4.02 $\pm$ 0.75	3.12 $\pm$ 0.35 <sup>c</sup>
Olive oil+ D-GalN	5	4.00 $\pm$ 1.00	3.80 $\pm$ 0.12 <sup>b</sup>	4.48 $\pm$ 0.76 <sup>c</sup>
Nigella oil+ D-GalN	5	9.30 $\pm$ 2.03 <sup>Aa</sup>	4.46 $\pm$ 0.13	5.06 $\pm$ 0.51

### Differential Count of White Blood Cells (WBC.d.)

#### Neutrophil (WBC.d.N)

As shown the higher mean value was recorded in experiment group of nigella oil+D-GalN (59.80  $\pm$  7.75%) in one week; whereas, the lower mean value with experiment of nigella oil (34.00  $\pm$  3.00%) in three weeks compared to control and D-GalN groups. There were

significant differences in WBC.d.N values among the experiment and control groups ( $P<0.01$ ).

**Table: - (4) Showed the differential count of white blood cells (Neutrophils) in control groups and experimental groups.**

Parameter Groups	N	The differential count of White blood cells (Neutrophil %)		
		1.W Mean $\pm$ S.D.	2.W Mean $\pm$ S.D.	3.W Mean $\pm$ S.D.
Control	3	51.00 $\pm$ 2.00	51.00 $\pm$ 1.00	40.67 $\pm$ 1.15
D-GalN	5	46.80 $\pm$ 0.83	47.00 $\pm$ 1.00	42.80 $\pm$ 2.04
Olive oil	5	44.60 $\pm$ 3.20 <sup>A</sup>	39.00 $\pm$ 3.00 <sup>Bb</sup>	38.60 $\pm$ 7.05
Nigella oil	5	47.40 $\pm$ 3.50	40.60 $\pm$ 1.34 <sup>Bb</sup>	34.00 $\pm$ 3.00 <sup>Cc</sup>
Olive oil+ D-GalN	5	46.20 $\pm$ 2.04	40.80 $\pm$ 0.83 <sup>Bb</sup>	43.20 $\pm$ 3.03
Nigella oil+ D-GalN	5	59.80 $\pm$ 7.75 <sup>Aa</sup>	35.00 $\pm$ 3.00 <sup>Bb</sup>	45.40 $\pm$ 2.51

### Lymphocytes (WBC.d.L)

As shown the higher mean value was recorded in experiment group of nigella oil+D-GalN (60.20  $\pm$  2.04%) at three week; whereas, the lower mean value with experiment of nigella oil (35.20  $\pm$  7.53%) at one week compared to control and D-GalN groups. There were significant differences in WBC.d.L values among the experiment and control groups ( $P<0.01$ ).

**Table: - (5) Showed the differential count of white blood cells (Lymphocytes) in control groups and experimental groups**

Groups	Parameter	N	The differential count of white blood cells (Lymphocyte) %		
			W.1 Mean ± S.D.	W.2 Mean ± S.D.	W.3 Mean ± S.D.
Control		3	43.00± 3.00	42.33 ±1.52 <sup>b</sup>	53.33± 1.52
D-GalN		5	45.20 ±5.71	47.60 ±2.19 <sup>B</sup>	50.00± 1.00
Olive oil		5	49.00± 3.74 <sup>A</sup>	53.40 ±2.51 <sup>Bb</sup>	56.00± 5.61 <sup>c</sup>
Nigella oil		5	47.00 ±4.58	53.40 ±1.67 <sup>Bb</sup>	60.20 ±2.04 <sup>c</sup>
Olive oil+ D-GalN		5	45.20 ±2.68	51.60 ±1.34 <sup>B</sup>	50.80± 1.09
Nigella oil+ D-GalN		5	35.20± 7.53 <sup>Aa</sup>	59.20 ±3.49 <sup>Bb</sup>	47.60 ±2.51 <sup>C</sup>

### Monocytes (WBC.d.M)

As shown the higher mean value was recorded in experiment group of olive oil+D-GaIN (5.00 ±1.00%) at two week; whereas, the lower mean value with experiment of nigella oil+D-GaIN (2.80± 0.83%) at one week compared to control and D-GaIN groups. There were significant differences in WBC.d.M values among the experiment and control groups (P<0.01).

**Table: - (6) Showed the differential count of white blood cells (Monocytes) in Control groups and experimental groups**

Groups	Parameter	N	The differential count of white blood cells (Monocyte %)		
			1.W Mean ± S.D.	2.W Mean ± S.D.	3.W Mean ± S.D.
Control		3	4.00± 1.00	4.00± 1.00	3.67 ±1.15
D-GalN		5	3.60 ±1.51	3.00 ±1.00	4.00 ±1.22
Olive oil		5	3.60 ±0.54	4.60 ±0.54 <sup>b</sup>	3.00 ±1.00
Nigella oil		5	3.60 ±1.51	3.60±0.54	3.60±0.54
Olive oil+ D-GalN		5	4.40 ±1.34	5.00 ±1.00 <sup>b</sup>	4.00 ±1.00
Nigella oil+ D-GalN		5	2.80±0.83	3.40±0.54	3.40 ±0.54

### Eosinophil (WBC.d.E)

As shown the higher mean value was recorded in experiment group of olive oil+D-GaIN ( $4.20 \pm 1.09\%$ ) at one week; whereas, the lower mean value with experiment of nigella oil ( $2.20 \pm 0.83\%$ ) at one week compared to control and D-GaIN groups. There were significant differences in WBC.d.E values among the experiment and control groups ( $P < 0.01$ ).

**Table: - (7) Showed the differential count of White blood cells (Eosinophil) in control groups and experimental groups**

Groups	Parameter	N	The differential count of white blood cells (Eosinophil %)		
			1.W Mean $\pm$ S.D.	2.W Mean $\pm$ S.D.	3.W Mean $\pm$ S.D.
Control		3	2.00 $\pm$ 1.00	2.67 $\pm$ 0.57	2.33 $\pm$ 0.57
D-GaIN		5	2.40 $\pm$ 0.54	2.20 $\pm$ 0.44	3.20 $\pm$ 0.83
Olive oil		5	2.80 $\pm$ 0.44	3.00 $\pm$ 0.03 <sup>b</sup>	2.40 $\pm$ 0.54
Nigella oil		5	2.00 $\pm$ 0.04	2.40 $\pm$ 0.54	2.20 $\pm$ 0.83 <sup>c</sup>
Olive oil+ D-GaIN		5	4.20 $\pm$ 1.09 <sup>Bb</sup>	2.60 $\pm$ 0.54	2.80 $\pm$ 0.44
Nigella oil+ D-GaIN		5	2.20 $\pm$ 0.44	2.40 $\pm$ 0.54	2.60 $\pm$ 0.54

In our study, the results revealed that administration of nigella oil with D-GaIN to experimental animals influencing on hematological parameters with varying degree. As shown, there was gradually increase in mean value of Hb at two week compared to control group. Similarly, the mean value of WBC.d.N and WBC.s counts were recorded on one week and the elevation in the mean value of WBC.d.L at two week compared to control and D-GaIN groups. However, nigella

oil with D-GaIN was not affected on mean value of differential count of WBC.M and WBC.E. The results of this study are not in accordance with findings of [10] who researched to different results. The reason could be attributed to different to dose administrated, equipment's and kits used. Previous research showed that use of nigella seed enhanced decreased levels of Hb, WBC and RBC in rabbits treated with carbon tetrachloride (the same oxidant) [16]. Aflatoxin can be causes hematological changes whereby using nigella seed makes resistance in rabbits under the aflatoxin diet [17]. This plant has been shown to modify established chaos in hematological parameters of diabetic rabbits and increase defensive mechanisms of body versus infection in these rabbits [18]. For instance, nigella seed improved anemia resulted from increased non-enzymatic glycosylation in membrane proteins of RBC in diabetics. This plant also decreases anemia via lipid peroxidation reduction in RBC hemolysis and nigella seed does not affect RBC levels and blood cells directly [19].

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