# Study the effect of some functional foods on the liver patients in experimental animals

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## ABSTRACT

This study targeted the effect of the proportion of 7.5% *Allium sativum* and 5% *Echinacea* on cirrhosis liver on rats fed on experimental meals after being injected by carbon tetrachloride ($Ccl_4$) mixed with paraffin oil. Twenty adult male albino rat, (215±10g), were divided into four groups a group of the negative control group and the positive control group, while the other groups were fed on meal contained 7.5% *Allium sativum* and 5% *Echinacea*. At the end of the experiment was determined the BWG%, FI and FER, were estimated liver functions, as well as lipid profiles by serum blood. The results referring to that the negative control group and the group fed on 5% *Echinacea* had high significant differences ($P < 0.01$) as compared to the positive control group; regarding to the BWG%. As for the TC, TG, HDL-c, LDL-c, VLDL-c, ALT and AST; the results showed that the negative control group and the groups fed on 7.5% *Allium sativum* and 5% *Echinacea* had high significant differences at ($P<0.01$) as compared to the positive control group. The study recommended that *Allium sativum* and *Echinacea* can lise as a dietary supplement to some foods for improvement the nutritive value and medical status of liver cirrhosis.

**Key words**: *Allium sativum* – *Milk thistle* – *Echinacea* – Liver cirrhosis – Treatment – experimental animals – biological evaluation – chemical analysis – statistical analysis – histological examination.

# دراسة تأثير بعض الأغذية الوظيفية على مرضى الكبد فى حيوانات التجارب

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**الملخص**

استهدف هذا البحث تقييم تأثير نسبة (٧.٥% من الثوم و٥% من حشيشة القنفد) على تليف الكبد في الفئران التي تغذت على وجبات تجريبية بعد حقنها برابع كلوريد الكربون مخلوطا بزيت البرافين ، وقد تم استخدام عشرين من ذكور فئران الألبينو البالغة وكان وزنها ٢١٥±١٠جم، حيث تم تقسيمها إلي أربع مجموعات كانت عبارة عن المجموعة الضابطة السالبة والمجموعة الضابطة الموجبة، أما المجموعتين الأخريين تم تغذيتهما علي الوجبة الأساسية القياسية مضافا إليها النسبة المتفق عليها من الثوم وحشيشة القنفد. وفي نهاية التجربة تم حساب النسبة المئوية للزيادة في وزن الجسم والزيادة فى الوزن ومعدل كفاءة الغذاء، كما تم تقدير وظائف الكبد، وكذا دلالات الدهون عن طريق سيرم الدم. وقد أوضحت النتائج المتحصل عليها أن المجموعة الضابطة السالبة والمجموعة التى تغذت على ٥% من حشيشة القنفد فروقا معنوية عند مستوى ($P<0.01$) مقارنة بالمجموعة الضابطة الموجبة فيما يتعلق بالنسبة المئوية لوزن الجسم المكتسب، فى حين أن المجموعة التى تغذت على ٧.٥% من الثوم أظهرت فروقا معنوية عند مستوى ($P<0.05$) بالمقارنة بالمجموعة الضابطة الموجبة. أما بالنسبة للكوليستيرول الكلي والدهون الثلاثية، البروتينات الدهنية عالية الكثافة، البروتينات الدهنية منخفضة الكثافة، البروتينات الدهنية منخفضة الكثافة جدا، ALT و AST أظهرت النتائج أن كلا من المجموعة الضابطة السالبة والمجموعات التي تغذت على ٧.٥٪ من الثوم و ٥٪ من حشيشة القنفد قد أشارت إلى فروق معنوية ذات دلالة إحصائية عالية ($P<0.01$) بالمقارنة بالمجموعة الضابطة الموجبة. وتوصى الدراسة بضرورة تناول الثوم وحشيشة القنفد حتى نتجنب تليف الكبد، مع إجراء أبحاث مستقبلية وهستوباثولوجية لمعرفة سلامة الأثر الناتج لإستعمال الثوم وحشيشة القنفد .

**الكلمات الكشافة:**

الثوم – القرطم – حشيشة القنفد – تليف الكبد – العلاج – حيوانات التجارب – التقييم البيولوجى – التحليل الكيميائى – التحليل الإحصائى – الإختبارات الهستوباثولوجية.

## INTRODUCTION

The liver is the largest organ in the body. A human liver normally weighs 1.44 – 1.66 kg (3.2 – 3.7 lb.). It was found high in the upper abdomen, behind the ribs. It contains four sections called lobes. Within each lobe, lobules contain liver cells and passageways for blood circulation, called sinusoids. It is within the lobules that the specialized liver cells transform chemical substances into nutrients; the body can use or neutralizes potential toxins to protect the body from damage. The liver also has a remarkable power to regenerate itself. However, there are illnesses that can cause permanent and irreversible damage to the liver. The liver being the most active gland of the body secretes about 600 – 800 ml of bile daily. Liver diseases may have a number of causes: infectious agents, toxic agents, dietary agents, storage diseases and congenital disorders (**ADA, 2000**); (**Zakim *et al.,* 2002**) and (**Cotran *et al.,* 2005**). The liver is very complex organ and has many functions (**Jackson, 2002**). Cirrhosis is a result of advanced liver disease. It is characterized by replacement of liver tissue by fibrosis (scar tissue) and regenerative nodules (lumps that occur due to attempted repair of damaged tissue). These changes lead to loss of liver function. Cirrhosis is most commonly caused by alcoholism, hepatitis B and hepatitis C, and fatty liver disease, but has many other possible causes. Some cases are idiopathic (of unknown cause) (**Iredale, 2003**). Herbal medicines are now used by up to 50% of the Western population, in a substantial minority of instances for the treatment or prevention of liver cirrhosis. Herbal preparations contain many bioactive compounds with potentially deleterious as well as beneficial effects. There is clearly a need for greater education of patients, doctors and nutritionists about herbal therapy, for legislation to control the quality of herbal preparations, and in particular for further randomized controlled trials to establish the value and

safety of such preparations in digestive, other disorders and liver cirrhosis (**Langmead and Rampton, 2001**).

## MATERIALS AND METHODS

**Materials:**

1- *Echinacea* (raw) was purchased from Surour Company for herbs and species store, Tanta, Gharbia, Egypt, and *Echinacea* was grinding in the house; but garlic (*Allium sativum*) powder was purchased in ready.

2- Basal diet was installed after purchased its ingredients from AL-Gomhoriya Co., Tanta -Gharbia - Egypt.

3- Minerals and vitamins mixtures were purchased from AL-Gomhoriya Co., Tanta - Gharbia - Egypt.

**Animals:**

Twenty Sprague-Dawley white male albino rats, each (215±10g), were used in this investigation obtained from serum and vaccine center. All rats were housed in group cages under conditions of controlled temperature (22-24°C) and illumination (12-h light cycle starting at 6 AM) for at least six days before experiments.

**Animals and experimental design:**

Rats were housed individually in well aerated cages under hygienic laboratory conditions. Animals were housed of Institute of ophthalmology, Cairo University, and fed for one week on standard died for adaptation before the beginning of the experiment according to (**NRC, 1995**). Rats were divided into 4 groups, every 5 rats in each group fed on certain diet for 28 days as following:

**Group (1) (Negative or normal group) (-Ve):** Fed on basal diet only, as a negative control.

**Group (2) (Positive group) (+Ve):** Fed on basal diet after injection with ($CCl_4$), as a positive control and the infection continues throughout the period of the experiment.

**Group (3) (Treatment group):** Liver cirrhosis groups, fed on basal diet plus 7.5% *Allium sativum* and 5% *Echinacea*.

**Methods:**

**Biological evaluation:**

During the experimental period, the consumed diet was recorded everyday, feed intake (FI), and body weight was recorded every three day. Biological evaluation of the different diets was carried out by determination of body weight gain, percentage of body weight gain and feed efficiency ratio (**Chapman *et al.*, 1959**), using the following formulas:

**Body weight gain (BWG) (g)** = Final weight − Initial weigh

$$\text{Body weight gain (BWG \%)} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Body weight gain}}{\text{Feed intake}}$$

**Biochemical analysis:**

At the end of the experiment, the rats were fasted overnight and serially anesthetized with diethyl ether. Blood was collected in clean dry centrifuge tubes from hepatic portal vein, this tubes containing 3.1% sodium citrate solution (1:10v/v) to prepare serum and plasma, respectively (**Jacobs *et al.*, 2001**) and (**Malhotra, 2003**). Blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. Serum was carefully separated and transferred into dry clean ebendorf

tubes and kept frozen at 20 ċ until analysis (**Turkdogan *et al.*, 2003**). Serum total cholesterol, triglyceride (TG) and high density lipoprotein cholesterol (HDL-c) were determined using enzymatic colorimetric methods of **Richmond, (1973) and Allain, (1974); Fassati and Principe, (1982)** and **Burstein, (1970) and Lopez, (1977)** respectively. The determination of low density lipoprotein cholesterol (LDL-c) was calculated as mg/dl (**Castelli, 1977**) equation:

**LDL-c (mg/dl)** = Total cholesterol − [HDL-c + VLDL-c]

When very low density lipoprotein cholesterol (VLDL-c) was carried out according to **Srivastava *et al.*, (2002)** equation:

**VLDL-c (mg/dl)** = Triglycerides / 5

Serum aspartate and alanine amino transferase (AST and ALT) were determined using enzymatic colorimetric methods (**Reitman and Frankel, 1957**).

**Statistical analysis:**

Statistical analysis was performed by using computer program, Statistical Package for Social Science and compared with each other using the suitable tests (**SPSS, 1998**).

**RESULTS AND DISCUSSION**

Table (1) point to the effect of 7.5% *Allium sativum* and 5% *Echinacea* on percentage body weight gain (BWG %); feed intake (FI) and feed efficiency ratio (FER) in rats pretreated with $CCl_4$. As shown that the negative control group and group fed on 5% *Echinacea* showed high significant differences($P < 0.01$), when compared with positive control. However, group fed on 7.5% of *Allium sativum* pointed to significant difference ($P < 0.05$), when compared with positive control. The same table illustrated for feed intake (FI) that the negative control showed a significant differences ($P < 0.05$), when compared with positive control.

However, the groups fed on 7.5% *Allium sativum* and 5% *Echinacea* were showed a high significant differences (P < 0.01), when compared with positive control. Regarding feed efficiency ratio (FER), it was found from data of the same table that the negative control showed a high significant differences(P < 0.01), when compared with positive control; while the group was fed on 5% *Echinacea* was refer to a significant differences (P < 0.05), when compared with positive control. These results were agreeable with **Zaina**, (2004) were reported that many patients with liver cirrhosis are suffering from also lose weight. As well as, **Feranchek**, (2005) were showed that the liver cirrhosis can lead to malnutrition, and the major causes of malnutrition in patients with liver cirrhosis are poor dietary intake. Mal-digestion, mal-absorption, and abnormalities in the metabolism and storage of micro and macro nutrients are too leading to malnutrition in patients with liver cirrhosis. This report was supported my study based on the results.

**Table (1): Effects of 7.5% *Allium sativum* and 5% *Echinacea* on BWG %; FI and FER in rats pretreated with $CCl_4$**

| Groups | BWG % | FI g/ day | FER |
|---|---|---|---|
| **-Ve** | 3.14 ±0.86[b] | 225.3 ±8.1[a] | 0.03 ±0.01[b] |
| **+Ve** | -21.44 ±1.09[c] | 203.2 ±2.3[c] | -0.04 ±0.01[c] |
| 7.5% ***Allium sativum*** | 4.18 ±0.74[a] | 210.4 ±3.7[b] | 0.04 ±0.009[bc] |
| ***5%Echinacea*** | 3.72 ±0.90[b] | 216.1 ±3.5[b] | 0.06 ±0.02[a] |

[a] Differences are significant at 5% (P < 0.05)

[b] Differences are high significant at 1% (P < 0.01)

[c] Differences are very high significant at 0.1% (P < 0.001)

**Control –ve**: Rats were fed on basal diet only

**Control +ve**: Rats were pretreated with $CCl_4$ and fed on basal diet

***Allium sativum***: Fed on basal diet plus 7.5% *Allium sativum*

***Echinacea***: Fed on basal diet plus 5.0% *Echinacea*

Table (2) revealed the effect of 7.5% *Allium sativum* and 5% *Echinacea* on total cholesterol (TC) and triglycerides (TG) in rats pretreated with $CCl_4$. The negative control; group fed on 7.5% *Allium sativum* and 5% *Echinacea* showed to a high significant differences ($P < 0.01$), comparing to positive control for total cholesterol (TC). As for serum triglycerides (TG) from the same table, the control negative; group fed on 7.5% *Allium sativum* and 5% of *Echinacea* were referred to a high significant differences $P < 0.01$, comparing to control positive. **Rombeau and Rolandelli, (2001)** reported that the liver cirrhosis lead to increase total cholesterol (TC) and triglycerides (TG), and fat mal-digestion, mal-absorption. From this results, it was be observe that all diets were had favorable effects on serum total cholesterol and triglycerides. These results were supported with **Blumenthal *et al.*, (2000); Sheppard, (2005)** and **Singh and Panda, (2005)** and agreeable with our results.

**Table (2): Effects of 7.5% *Allium sativum* and 5% *Echinacea* on TC and TG in rats pretreated with $CCl_4$**

| Groups | TC mg/dl | TG mg/dl |
|---|---|---|
| **-Ve** | $129.50 \pm 2.00^{b}$ | $96.10 \pm 1.20^{b}$ |
| **+Ve** | $224.60 \pm 3.20^{a}$ | $154.40 \pm 3.40^{a}$ |
| 7.5% ***Allium sativum*** | $127.90 \pm 0.78^{b}$ | $95.00 \pm 1.70^{b}$ |
| ***5% Echinacea*** | $128.50 \pm 0.69^{b}$ | $95.30 \pm 1.50^{b}$ |

[a] Differences are significant at 5% ($P < 0.05$)

[b] Differences are high significant at 1% ($P < 0.01$)

[c] Differences are very high significant at 0.1% ($P < 0.001$)

**Control –ve:** Rats were fed on basal diet only

**Control +ve:** Rats were pretreated with $CCl_4$ and fed on basal diet

***Allium sativum***: Fed on basal diet plus 7.5% *Allium sativum*

***Echinacea***: Fed on basal diet plus 5.0% *Echinacea*

Table (3) illustrated the effect of 7.5% *Allium sativum* and 5% *Echinacea* on serum lipoproteins [HDL-c; LDL-c and VLDL-c] in rats pretreated with $CCl_4$. From the outcomes in table (3) the negative control group and groups fed on 7.5% *llium sativum* and 5% *Echinacea* had a high significant differences at (P <0.01) as compared to the positive control group. **Rombeau and Rolandelli, (2001)** reported that the liver cirrhosis lead to increase serum lipoproteins synthesis, and fat mal-digestion, mal-absorption. From this results, it was be observed that all diets had favorable effects on serum lipoproteins. These results were supported with **Blumenthal *et al.,* (2000); Sheppard, (2005)** and **Singh and Panda, (2005)** and agreeable with our results.

**Table (3): Effects of 7.5% *Allium sativum* and 5% *Echinacea* on serum lipoproteins [HDL-c; LDL-c and VLDL-c] in rats pretreated with $CCl_4$**

| Groups | HDL-c mg/dl | LDL-c mg/dl | V.LDL-c mg/dl |
|---|---|---|---|
| **-Ve** | 56.30 ±1.70[b] | 63.20 ± 0.89[b] | 18.30 ± 0.78[b] |
| **+Ve** | 80.40 ±4.10[a] | 90.60 ±4.03[a] | 39.80 ± 3.70[a] |
| 7.5% ***Allium sativum*** | 54.00 ±1.70[b] | 64.00 ±1.20[b] | 19.00 ± 1.70[b] |
| ***5%Echinacea*** | 55.30 ±2.30[b] | 64.50 ±1.60[b] | 19.50 ±0.99[b] |

[a] Differences are significant at 5% (P < 0.05)

[b] Differences are high significant at 1% (P < 0.01)

[c] Differences are very high significant at 0.1% (P < 0.001)

**Control –ve**: Rats were fed on basal diet only

**Control +ve**: Rats were pretreated with $CCl_4$ and fed on basal diet

***Allium sativum***: Fed on basal diet plus 7.5% *Allium sativum*

***Echinacea***: Fed on basal diet plus 5.0% *Echinacea*

Table (4) pointed to the effect of 7.5% *Allium sativum* and 5% *Echinacea* on serum liver enzymes GOT (AST) and GPT (ALT) in rats were pretreated with $CCl_4$. From this table, the results were illustrated that the negative control and all diets showed a high significant differences (P

< 0.01), when compared with positive control for GOT (AST) and GPT (ALT). **Gaw *et al.,* (2004)** were reported that the higher activities of aminotransferase enzymes S.GOT (AST) and S.GPT (ALT) were indicated to hepatocellular damage. From these results, it was observed that S.GOT (AST) level of positive control was higher than all diets and negative control. Whereas, S.GPT (ALT) level of all diets and negative control were higher than positive control. All diets had very clear effect on S.GOT (AST) and S.GPT (ALT).These results were agreeable with **Weiss and Fintelmann, (2000)** and **Singh and Panda, (2005)**.

**Table (4): Effects of** 7.5% *Allium sativum* **and 5%** *Echinacea* **on [S.GOT (AST)] and [S.GPT (ALT)] in rats were pretreated with $CCl_4$**

| Groups | S.GOT [AST] u/ml | S.GPT [ALT] u/ml |
|---|---|---|
| **–Ve** | 33.20 ±2.40[b] | 32.80 ± 2.60[b] |
| **+Ve** | 65.20 ±3.90[a] | 74.20 ± 2.80[a] |
| 7.5% ***Allium sativum*** | 35.20 ±4.30[b] | 33.60 ± 2.90[b] |
| ***5%Echinacea*** | 34.20 ±3.20[b] | 32.90 ± 2.10[b] |

[a] Differences are significant at 5% (P < 0.05)

[b] Differences are high significant at 1% (P < 0.01)

[c] Differences are very high significant at 0.1% (P < 0.001)

**Control –ve:** Rats were fed on basal diet only

**Control +ve:** Rats were pretreated with $CCl_4$ and fed on basal diet

***Allium sativum*:** Fed on basal diet plus 7.5% *Allium sativum*

***Echinacea*:** Fed on basal diet plus 5.0% *Echinacea*

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