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# Effect of methanol extracts of three dietary plants growing in Egypt on mice fed with high fat diet

Hanan A El-Nahas[1], Salwa K El Nabarawy [2] , Afaf A Abdel-Hady[3], Ahmed M Abdel-Hady[4], Heba A Abdel Raouf[1*]

[1] Medicinal Chemistry Department, [3] Clinical Chemistry Department, [4] Pathology Department, Theodor Bilharz Research Institute, Giza, Egypt and [2] Zoology Department, Faculty of Science. Al-Azhar University, Girls-Branch, Cairo, Egypt.

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**ABSTRACT**

The objective of the present investigation was to evaluate the preventive effect of methanol extracts of three dietary plants growing in Egypt; *Cassia fistula* (Family Fabaceae), *Cynara scolymus* (Family Asteraceae) and *Glycine max* (Family fabaceae) on body weight gain, hepatic and kidney index, food intake, lipid profile and serum enzymes in mice fed high fat diet during different periods 4, 11 and 17 weeks. For this purpose, the mice were divided into groups of eight mice each group as follows; normal group, high fat diet (HFD) group , HFD + methanol extract of *C. fistula* group, HFD + Methanol extract of *C. scolymus* group and HFD + methanol extract of G. *max* group, the dose of plant extract in each treated group was 100 mg /Kg body weight. After 4 weeks to 17 weeks the body weight, the relative liver weight, lipid profile and serum enzymes increased in the control group (HFD) compared to the normal. Treatment with each plant plant extract led to reduction in body weight and improvement of lipid and serum enzymes of the treated mice compared to the control groups. Extract of *G.max* showed the highest activity. These results were supported by hepatic histology examination which showed improvement of fatty liver tissue.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) includes a wide spectrum of liver complications ranging from simple steatosis to steatohepatitis (NASH); advanced fibrosis and cirrhosis. The incidence of NAFLD is associated with obesity and has risen dramatically over the past several decades (Angulo, 2002; Federico *et al*., 2006; Ogden *et al*., 2006). Various studies have indicated that 58–74% of obese adults and 23–53% of obese children are afflicted with NAFLD (Luyckx *et al*., 1998; Nomura *et al*., 1998; Bellentani *et al*., 2000; Clark 2006; Papandreou *et al*., 2007). Although, hepatic steatosis is a simple phenomenon and has no complications but the effect of the oxidative stress may led to steatohepatitis which is associated with necrosis, inflammation, fibrosis and cirrhosis (Chitturi and Farrell, 2001; Mehta *et al*., 2002; Angulo, 2002; Adams and Angulo 2005; Angulo *et al*., 2007; Bruno *et al*., 2008; Younossi 2008; Sharma *et al*., 2010).

** Corresponding Author*
*Dr. Heba A Abdel Raouf, Medicinal Chemistry Department, Theodor Bilharz Research Institute, Kornish El-Nile Street, Warrak El-Hadar, Imbaba, Giza, Egypt. Fax: 02-0235408125. Email: h_hady10@yahoo.com*

Some drugs have been tested for the treatment of NAFLD, but generally no accepted conclusions have been reached (Loguercio *et al*., 2007). In recent years, the benefits of plant extracts on NAFLD progression have received increasing attention due to their advantages whereas plants are widely available around the world, their extracts have low or minimal side effects and they showed good antioxidant properties (Pan *et al*., 2009; Angulo, 2002; Bruno *et al*., 2008; Loguercio *et al*., 2007). The three plants under investigation are the leaves and small stems of *Cassia fistula* (Family Fabaceae), leaves of *Cynara scolymus* (Family Asteraceae) and seeds of *Glycine max* (Family Fabaceae). The three investigated plants showed several pharmacological activities as anti-inflammatory, antioxidant, anti-tumor and anti-pyretic activities (Neelam *et al*., 2011; Rajagopal *et al*., 2013; Ali *et al*., 2012; Kim *et al*., 2008).

In the present study, the methanol extracts of three plants growing in Egypt (*Cassia fistula*, *Cynara scolymus* and *Glycine max*) were prepared and the effect of these extracts on body weight, food intake, liver and kidney index, lipid profile and serum enzymes on mice fed with high fat diet were evaluated.

## MATERIAL AND METHODS

### Collection and identification of plant materials

The plants were collected during 2009 *Cassia fistula* was collected from El-Orman Botanical Garden while *Cynara scolymus* from Horticulture Research Center and *Glycine max* from Field Crops Research Institute. The plants were kindly identified by Prof. Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University. Voucher specimens of the plants were kept in Medicinal Chemistry Department, Theodor Bilharz Research Institute.

### Animals

A healthy albino male mice were obtained from Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute and housed in polypropyline cages (8 per cage) with stainless steel covers to acclimatize under the laboratory conditions (Temperature 25±2$^0$C, humidity 55±5 and 12 hour light /dark cycle) for at least one week before the experimental process. The mice were given standard normal diet and water *ad libitum*.

### Diets

#### *Normal diet*

Mice were fed with normal diet (60.6% carbohydrates, 10. 5% fat, 19% protein, 4.3% fiber, 5 % mineral mixture and 0.2% vitamin mixture).

#### *High fat diet (HFD)*

Mice were fed with high fat diet (60.6% carbohydrates, 40% fat, 19% protein, 4.3% fiber, 5 % mineral mixture and 0.2 % vitamin mixture).

### Kits

Kits for detecting triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (γ–GT) were purchased from Seppim, France; Stanbio Laboratory, USA and Quimica Clinica, Spain.

### Preparation of plant extracts

500 gm of each plant under investigation was shade dried at room temperature, grounded into fine powder using a mixer grinder and then extracted for several times using 70% methanol. The methanolic extract of each plant was combined and filtered through Whatmann filter paper then concentrated under reduced pressure using a rotatory evaporator at 40$^o$C till complete removal of the methanol. The dried methanolic extract of each plant was weighed and kept well from moisture in a plastic vials in desiccator for determination of their effects in the present study.

### Evaluation the effect of methanol extract of each plant on body weight, food intake, liver and kidney index, Lipid profile and serum enzymes in mice fed high fat diet

Evaluation of the effect of each plant extract on body weight, food intake, liver and kidney index, lipid profile and serum enzymes in mice fed high fat diet was carried out during different periods 4, 11 and 17 weeks. For this purpose, the mice after acclimatization for one week, were randomly divided into 15 groups (8 mice per group) as follows:

#### *Normal group*

Groups 1, 2 and 3 were fed normal diet for different periods (4 weeks for group 1, 11 weeks for group 2 and 17 weeks for group 3).

#### *Control groups (HFD groups)*

Groups 4, 5 and 6 were fed high fat diet for different periods (4 weeks for group 4, 11 weeks for group 5 and 17 weeks for group 6).

#### *Treated groups*

Groups 7, 8 and 9 were fed high fat diet + methanol extract of *C. fistula* (dose 100 mg/kg. body weight) for different periods (4 weeks for group 7, 11 weeks for group 8 and 17 weeks for group 9).

Groups 10, 11 and 12 were fed high fat diet + methanol extract of *C. scolymus* (dose 100 mg/kg. body weight) for different periods (4 weeks for group 10, 11 weeks for group 11 and 17 weeks for group 12).

Groups 13, 14 and 15 were fed high fat diet + methanol extract of *G. max* (dose 100 mg/kg. body weight) for different periods (4 weeks for group 13, 11 weeks for group 14 and 17 weeks for group 15). All groups received tap water and the dose was administrated by gavage using specially designed mice oral needle (Jalil *et al*, 2009).

The animals body weights were recorded weekly and the food intake were determinated on a per cage basis throughout the study over week. Food intake (gm/mice/week) was calculating by subtracting the remaining food weight from the initial food weight of the previous week and dividing by the number of mice housed in the cage. The body weight gain was calculated by the equation:- final body weight - initial body weight.

### Blood collection

At the end of each experimental period , the animals were fasted overnight then sacrificed by decapitation. Blood was collected into dry clean centrifuge tubes and serum was separated by centrifuging at 3000 rpm for 15 min. Serum samples were kept frozen for biochemical analyses.

### Organs Preparation

The animals were thereafter quickly dissected and the liver and kidneys were removed, dried on tissue and individually weighed for each mouse.

### Hepatic index and relative kidney weight

Throughout the experiment, body weight was recorded weekly, then at the end of the experiment the body weight and the liver and kidney weight from control and treated groups were measured and recorded. Hepatic index was estimated from the

ratio of liver weight to body weight and also relative kidney weight was calculated.

**Food intake**

The amount of food consumed weekly was measured for all control and experimental groups from the quantity of feed supply and the amount remaining by the end of each experimental week.

**Measurements of lipid profiles**

Among lipid profiles, concentration of triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) were measured by enzymatic colorimetric methods using commercial assay kits. Meanwhile, low density lipoprotein (LDL) was calculated from Friedevald,s equation (Sharma *et al*., 2010).

**Biochemical estimations (analysis)**

Alkaline phosphatase (ALP) activity, the activities of alanine aminotransferases (ALT) and gamma-glutamyl transpeptidase (γ-GT) were estimated according to the reported methods using assay kits.

**Histopathological studies**

After removal from the mice, liver tissues were immediately fixed in 10 % buffered formaldehyde to avoid decomposition and processed for histological examination by conventional methods with hematoxylin and eosin (H&E) stain. They were embedded in paraffin after removal of water using alcohol dehydration. Serial of sections of the tissue (7 µm) were cut using a microtome and these were stained with hematoxylin and then stained with eosin. Then they were examined microscopically by a pathologist using light microscope and images were captured canon power shot A640 camera..

**Statistical analysis (of data)**

All experiments of the antioxidant activity were run in triplicate. All data were analyzed using Student's t-test and $p < 0.01$ was considered significant. Values are expressed as means ± SD. Regression analysis was used to evaluate associations between variables.

**RESULTS AND DISCUSSION**

Non alcoholic liver disease (NAFLD) is one of the most common causes of chronic liver injury in many countries around the world and it might lead to important public health problems (Clark *et al*., 2002; Elahi 2012). NAFLD comprises a spectrum of disorders which range from simple steatosis to inflammatory steatohepatitis (Pascos and palates 2009; Asari *et al.*, 2011). Several therapeutic agents are used in the treatment of NAFLD and associated disorders but recently more attention has been shifted to use of plants as a possible means of alleviating the symptoms associated with NAFLD (Xiao *et al.*, 2013; Tan *et al.*, 2013). Numerous studies have been reported on animal models that have mimic human NAFLD. Generally, animals fed with high-fat diet have been used to develop NAFLD models (Lieber *et al*.,2004 ). In this study, three plant extracts were chosen for treatment of obese mice during three periods 4 weeks (short term), 11( midterm) and 17 weeks (long term ) according to the previous studies by Bose *et al*., 2008 and Lion *et al*. 2012 .

**Effect of the extracts on body weight**

Body weight was measured once a week. Body weight increased significantly in mice fed high fat diet compared with normal group (fed normal diet) in all periods as shown in table (1). Body weight gain was high after 17 weeks (22.18±1.9) whereas it was small after 4 weeks (4.28±1.9) and after 11 and 17 weeks were 14.39 ± 1.24 and 22.18±1.9 compared to control. These results are in full agreement with other previous studies (Han *et al.*, 1999; Han *et al.*, 2000) which reported that mice fed high fat diet increased body weight and adipose tissue mass than those fed normal diet. Treatment of mice fed high fat diet (HFD) with the methanol extract of each plant (100mg/kg.b.wt.) was carried out. Data presented in table (1) revealed that, the extract of the three plants caused a reduction of body weight during all treatment periods. High reduction in body gain was recorded with *G. max* after 4, 11 and 17 weeks (1.5±1.6), (3.48±1.26) and (4.39±1.4) compared to the control (HFD) group. This finding agrees with many authors as (Han *et al*., 2002; Park *et al*., 2005,2006; Choi *et al.*, 2007; Bose *et al.*, 2008). They found that plant extracts greatly reduced body weight in dose dependent manner in high fat fed mice. These results mean that the tested plant extracts possess effect in normalizing body weight in HFD mice (Burno *et al.*, 2008).

**Food intake**

The food intake was determined by the difference between the initial weight of administrated food and the weight of food left at the end of each period. The results in table (2) revealed that mice fed HFD (control group) continued to show small increase in food intake compared with normal group until the end of the study. Treatment of group HFD with each plant extract showed small reduction in food intake as 4.45 gm with three plant extracts. Although there is small difference in food intake in control group and treated groups but no significant difference between all groups. This means that the high fat diet only or high fat diet + plant extract did not affect on the mice appetite as well as the energy intake for long term consumption and these results agree with (Park *et al*., 2005; Choi *et al*., 2007; Bruno *et al.*, 2008; Ramgobal *et al.*, 2010; Chandrasekaran *et al.*, 2012).

**Liver and kidney index**

The results in table (3) showed that the liver of mice in group (HFD) was significantly increased when compared with the normal group. This result agrees well with Zheng *et al.*, 2008 and Cui *et al.*, 2011. They reported that the liver weight increased in HFD induced obese animal. Treatment with the methanol extracts

of each plant led to decrease the liver index comparing with the control group (HFD) in all periods. Also, the three extracts led to reduction in liver index of mice but there is no significant difference in the liver index reduction in three plant extracts treatment. Zheng *et al.*, 2008 and Cui *et al.*, 2011 reported that the liver index in treated groups with plant extracts shows decreasing when compared with the control group (HFD). As regard to the effect of high fat diet on mice kidney and groups treated with plant extracts, the results in table (4) revealed that the high fat diet did not significantly change in kidney index, small reduction in kidney index observed in treated groups with plant extracts compared to the control group (HFD). No significant changes in color, texture and weight of the kidney in treated groups. The same results were obtained in other previous studies (Hong *et al*., 2006; Park *et al*., 2006).

**Lipid profile**

The liver regulates systemic lipid homoesteatosis which is involved in the redistribution of lipoprotein triacylglycerol for storage and utilization by peripheral tissues (Donnelly *et al.*, 2005). In the present study, feeding mice with high fat diet for different periods (4,11 and 17 weeks) led to increasing the serum triglycerides (TG), total cholesterol (TC)and low density lipoprotein (LDL) levels were observed compared to those of the normal group (normal diet) whereas the HDL levels was decreased as shown in table (5). Treatment of the high fat diet (HFD) group with each plant extract improved the lipid profile by decreasing TG (44%), TC (37%) and LDL (27%) levels and increasing HDL (169%) levels as compared to the control. Similar results were obtained with other previous studies (Zheng *et al.*, 2008; Xia *et al.*, 2010). Then, it was evident that the high fat diet reduced serum HDL level and elevated LDL, TG and TC levels in the control group (HFD). Treatment with the methanol extract of each plant under investigation led to reduction of TG, TC and LDL and elevation of HDL level. Therefore, these extracts had a strong hypotriglyceridemic and hypocholesterolemic effects. These finding seem to be in accordance with the results of (Donnelly *et al.*, 2005; Yang *et al.*, 2006; Zheng *et al.*, 2008; Xia *et al.*, 2010; Cui *et al.*, 2011; Lion *et al.*, 2012). These results suggest that the methanol extract of each plant could be helpful in decreasing the incidence of several fatty liver disease through a reduction in TC, LDL and TG and an increase in HDL level. To the best of our knowledge, this is the first report of the hypolipidemic potentials of these plant extracts.

**Serum enzymes**

Clinical diagnosis of disease and damage to the structural integrity of the liver is commonly assessed by monitoring the status of serum ALT, ALP and γ-GT activities which are sensitive serological indicators of liver toxicity (Simon-Giavaritti *et al.*, 2002; Amin and Hamza 2005). Higher activities of these enzymes in serum have been in response to oxidative stress induced by high fat diets (Amin and Hamza 2005). In the present study these parameters as shown in table (6) were significantly enhanced by the high fat diet compared to the normal group. This suggest that excessive fat intake might cause critical injury to the liver due to over production of free radicals and ROS (reactive oxygen species) which extent deleterious effect on liver (Amin and Hamza 2005; Donnelly *et al.*, 2005).

Treatment of control group (HFD) with each plant extract led to reduction of serum ALT, ALP and γ-GT activities. As shown in table (6). This suggest that these plant extracts can reverse liver toxicity induced by high fat diet. Some previous studies showed that plant extracts with antioxidant properties can protect the liver from various toxicant (Zheng *et al.*, 2008; Elahi 2012; Ates *et al.*, 2012). Therefore, in this study, owing to the methanol extract of plant extracts have antioxidant properties, these extracts showed free radical scavenging activity and exhibited protection of the mice liver from toxicants.

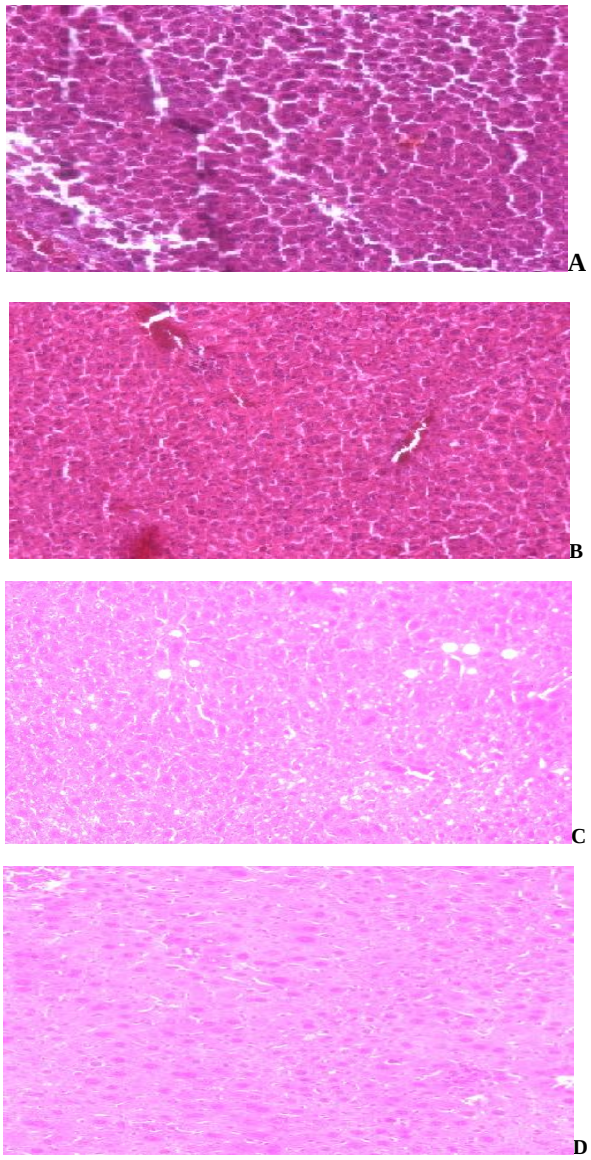


**Fig. 1a, b, c, d:** Histological studies of tested groups: (A) HFD group, (B) HFD+*C.fistula*, (C) HFD+*C.scolymus*, (D) HFD+*G.max*.

**Table. 1:** Effect of the methanol extracts of *C.fistula, C.Scolymus* and *G.max* (100mg/kg.b.wt) on body weight gain in mice fed with high fat diet compared to control group (HFD) at different periods (4, 11and 17 weeks) respectively.

| Weeks of treatment | Body weight of groups of mice | | | | |
|---|---|---|---|---|---|
| | Normal | HFD | HFD + C. fistula | HFD + C. scolymus | HFD+G. max |
| 1 | 22.45±1.91 | 22.34±1.92 | 22.43±1.76 | 23.59±1.59 | 21.51±1.73 |
| 2 | 22.63±1.52 | 23.33±1.21 | 22.89±1.20 | 24.38±1.31 | 21.71±0.63 |
| 3 | 23.17±0.91 | 24.97±2.78 | 23.36±0.70 | 24.44±0.66 | 22.39±1.17 |
| 4 | 24.15±1.44 | 26.62±1.05 | 23.55±1.36 | 25.94±1.11 | 22.66±0.47# |
| Body weight gain(g) | 1.7 ± 1.24 | 4.28 ±1.9 *↑ | 1.12 ±1.4 # ↑ | 2.35 ±1.7# ↑ | 1.15±1.6*# ↓ |
| 5 | 24.61±1.13 | 27.84±1.13* | 24.73±0.66# | 26.55±0.33 | 21.79±1.14# |
| 6 | 24.93±1.04 | 28.57±2.35* | 24.84±1.25# | 27.44±0.69 | 21.85±0.54#* |
| 7 | 25.28±0.08 | 29.03±1. 18* | 25.11±0.56# | 28.14±1.36 | 22.65±1.06#* |
| 8 | 25.36±1.76 | 30.66±0.96* | 26.63±1.03# | 28.82±0.89 | 22.78±1.13#* |
| 9 | 25.49±0.11 | 32.48±1.60* | 26.96±1.35# | 29.13±1.43 | 22.82±1.26#* |
| 10 | 26.22±1.56 | 34.69±3.50* | 27.01±1.03# | 29.28±0.99# | 23.52±1.16#* |
| 11 | 26.74±0.05 | 36.73±1.89* | 28.10±1.05# | 29.69±1.21# | 24.99±0.80#* |
| Body weight gain(g) | 4.29± 1.37 | 14.39±1.52*↑ | 5.67±1.24# ↑ | 6.1±2.45 #↑ | 3.48±1.26#↑ |
| 12 | 27.43±1.58 | 37.13±3.05* | 28.94±0.36# | 30.09±1.23# | 22.68±0.70#* |
| 13 | 28.62±1.36 | 38.19±4.60* | 29.19±1.19# | 31.18±0.60# | 22.98±0.63#* |
| 14 | 29.33±0.51 | 39.46±2.16* | 29.90±1.15# | 32.31±1.62# | 23.83±0.93#* |
| 15 | 29.52±1.10 | 40.67±0.74* | 30.45±1.57# | 32.73±0.67# | 24.17±0.28#* |
| 16 | 29.98±0.90 | 42.19±2.43* | 30.61±1.29# | 33.14±0.70# | 24.70±0.60#* |
| 17 | 30.20±0.85 | 44.52±2.75* | 31.13±0.80# | 33.81±0.68# | 25.90±0.83#* |
| Body weight gain(g) | 7.75±2.4 ↑ | 22.18±1.9*↑ | 8.7±1.7# ↑ | 10.22±1.9# ↑ | 4.39±1.4*# ↑ |

**Table. 2:**Effect of the methanol extracts of *C.fistula, C.Scolymus* and *G.max* (100mg/kg.b.wt) on food intake (gm/animal/week) in mice fed with high fat diet compared to control group (HFD) at different periods (4, 11, 17 weeks) respectively.

| Weeks of treatment | Groups of mice | | | | |
|---|---|---|---|---|---|
| | Normal | HFD | HFD + *C. fistula* | HFD+*C. scolymus* | HFD+*G. max* |
| **1** | 95.35 | 88.05 | 57.98 | 62.08 | 56.83 |
| **2** | 97.50 | 92.34 | 66.67 | 75.50 | 50.36 |
| **3** | 81.75 | 85.65 | 60.83 | 62.66 | 45.46 |
| **4** | 77.00 | 97.27 | 77.50 | 84.16 | 51.45 |
| **5** | 74.00 | 102.50 | 82.96 | 79.18 | 66.40 |
| **6** | 94.50 | 101.79 | 77.25 | 80.00 | 56.30 |
| **7** | 87.32 | 107.50 | 87.55 | 86.05 | 77.66 |
| **8** | 79.35 | 109.35 | 82.50 | 83.33 | 80.66 |
| **9** | 84.02 | 106.66 | 75.63 | 80.45 | 76.45 |
| **10** | 91.32 | 112.50 | 69.88 | 78.00 | 73.35 |
| **11** | 86.25 | 110.05 | 85.16 | 93.05 | 75.89 |
| **12** | 97.25 | 105.54 | 77.66 | 76.50 | 62.44 |
| **13** | 100.00 | 109.50 | 62.05 | 70.00 | 70.00 |
| **14** | 95.26 | 102.50 | 76.00 | 85.06 | 55.00 |
| **15** | 90.95 | 105.00 | 75.59 | 71.00 | 65.00 |
| **16** | 85.49 | 103.45 | 70.50 | 76.50 | 60.55 |
| **17** | 82.35 | 100.61 | 65.79 | 72.89 | 62.35 |
| **Total** | 1406.66 | 1755.26 | 1244.45 | 1352.41 | 1085.15 |

No significant variation observed between any group.

**Table. 3 :** Effect of the methanol extracts of *C.fistula, C.Scolymus* and *G.max* (100mg/kg.b.wt) on hepatic index in mice fed with high fat diet compared to control group (HFD) at different periods (4, 11 and 17 weeks) respectively.

| Treatment group | Hepatic index of mice scarified after: | | |
|---|---|---|---|
| | 17 weeks | 11 weeks | 4 weeks |
| Normal | 4.83 ± 0.43 | 4.59 ± 1.01 | 3.99 ± 0.09 |
| High – fat diet | 5.99 ± 0.19 * | 4.99 ± 0.34 * | 5.05 ± 1.66 * |
| Change ratio (%) vs. normal | 19% ↑ | 8% ↑ | 21%↑ |
| HFD + *C. fistula* | 4.54 ± 0.06 | 4.47 ± 1.02 | 4.72 ± 1.42 |
| Change ratio (%) vs. normal | 6%↓ | 2% ↓ | 18% ↑ |
| Change ratio (%) vs. HFD | 24%↓ | 10% ↓ | 7% ↓ |
| HFD + *C. scolymus* | 5.15 ± 0.64 | 4.67 ± 0.26 | 4.72 ± 0.00 |
| Change ratio (%) vs. normal | 7% ↑ | 2% ↑ | 18% ↑ |
| Change ratio (%) vs. HFD | 14% ↓ | 6% ↓ | 6% ↓ |
| HFD + *G. max* | 4.41 ± 0.21 | 4.98 ± 1.20 | 4.55 ± 0.00 |
| Change ratio (%) vs. normal | 9% ↓ | 8% ↑ | 14% ↑ |
| Change ratio (%) vs. HFD | 26% ↓ | 0% | 10% ↓ |

Mean value of 8 animals ± SD, * $p < 0.05$

**Table. 4**: Effect of the methanol extracts of C.fistula, C.Scolymus and G.max (100mg/kg.b.wt) on kidney index in mice fed with high fat diet compared to control group (HFD) at different periods (4, 11 and 17 weeks) respectively.

| Treatment group | Kidney index of mice scarified after: | | |
|---|---|---|---|
| | 17 weeks | 11 weeks | 4 weeks |
| **Normal** | 1.59 ± 0.37 | 1.21 ± 0.54 | 1.38 ± 0.23 |
| **High – fat diet** | 1.42 ± 0.35 | 1.19 ± 0.06 | 1.42 ± 0.15 |
| **Change ratio (%) vs. normal** | 11% ↓ | 1%↓ | 3%↑ |
| **HFD + *C. fistula*** | 1.28 ± 0.11 | 0.98 ± 0.21 | 1.29 ± 0.19 |
| **Change ratio (%) vs. normal** | 19%↓ | 19% ↓ | 7% ↓ |
| **Change ratio (%) vs. HFD** | 10%↓ | 1% ↓ | 9% ↓ |
| **HFD + *C. scolymus*** | 1.67 ± 0.33 | 0.95 ± 0.23 | 1.22 ± 0.04 |
| **Change ratio (%) vs. normal** | 5% ↑ | 21%↓ | 11% ↓ |
| **Change ratio (%) vs. HFD** | 7% ↑ | 20%↓ | 14% ↓ |
| HFD + *G. max* | 1.52 ± 0.14 | 0.95 ± 0.2 | 1.41 ± 0.26 |
| Change ratio (%) vs. normal | 4% ↓ | 21% ↓ | 2%↑ |
| Change ratio (%) vs. HFD | 7% ↑ | 20% ↓ | 1%↓ |

Mean values of 8 animals ± SD, No significant difference was observed in any group.

**Table. 5:** Effect of the methanol extracts of *C. fistula*, *C.Scolymus* and *G.max* (100 mg/kg.b.wt) on lipid profile in mice fed with high fat diet compared to control group (HFD) at different periods (4, 11, 17 weeks) respectively.

| Lipid profile | Treatment groups | Treatment periods : 4 weeks | 11 weeks | 17 weeks |
|---|---|---|---|---|
| **Triglycerides (TG) (mg/dI)** | Normal | 176.58 ± 5.68 | 160.17 ± 1.50 | 170.91 ± 9.06 |
| | HFD | 219.66 ± 12.36 * | 236.92 ± 5.40 * | 275.90 ± 5.67 * |
| | HFD +*C. fistula* | 110.93 ± 5.62 * # | 99.39 ± 2.46 *# | 121 .8 ± 7.85 *# |
| | HFD +*C.scolymus* | 140.14 ± 14.42 *# | 132.33 ± 3.67 *# | 145.8 ± 11.74 *# |
| | HFD + *G. max* | 162.88 ± 3.19* # | 152.04 ± 3.11 # | 146.82 ± 13.26 # |
| **Total cholesterol (TC) (mg/dI)** | Normal | 83.30 ± 12.24 | 91.23 ± 6.63 | 101.56 ± 7.5 |
| | HFD | 167.58 ± 9.73 * | 184.37 ± 45.73 * | 192.28 ± 9.54 * |
| | HFD +*C. fistula* | 71.35 ± 10.55 # | 66.39 ± 4.95 *# | 72.53± 6.34 *# |
| | HFD +*C.scolymus* | 81.89 ± 8.08 # | 75.41 ± 5.32 # | 86.72 ±9.52 # |
| | HFD + *G. max* | 86.13 ± 4.10 # | 76.23 ± 7.26 # | 94.84± 9.36 # |
| **Low density lipoprotein (LDL) (mg/dI)** | Normal | 36.67 ± 5.06 | 32.44 ± 3.27 | 35.71 ± 4.50 |
| | HFD | 103.22 ± 11.88 * | 140.06 ± 4.74 * | 149.38 ± 4.87 * |
| | HFD +*C. fistula* | 69.22 ±10.07 *# | 49.54 ± 5.54 *# | 46.52 ± 7.83 *# |
| | HFD +*C.scolymus* | 60.52 ± 5.07 *# | 49.28 ± 8.40 *# | 42.61 ± 8.49 # |
| | HFD + *G. max* | 51.74 ± 3.06 # | 35.05 ± 6.64 # | 41.27 ± 7.36 # |
| **High density lipoprotein (HDL) (mg/dI)** | Normal | 38.31 ± 5.14 | 37.63 ± 3.12 | 38.55 ± 2.61 |
| | HDF | 14.81 ± 4.64 * | 15.32 ± 5.22 * | 17.64 ± 3.27 * |
| | HFD +*C. fistula* | 17.46 ± 8.16 * | 20.86 ± 2.12* | 22.51 ± 8.5* |
| | HFD +*C.scolymus* | 27.42 ± 2.50*# | 29.76 ± 3.06 *# | 26.73 ± 8.4* # |
| | HFD + *G. max* | 28.88 ± 5.79 # | 29.23 ± 3.59 # | 34.58 ±5.6 # |

Values represent the mean ± SD (n=8)
* $p < 0.01$ vs normal group , # $p < 0.01$ vs high fat diet group

**Table. 6 :**Effect of the methanol extracts of *C.fistula*, *C.Scolymus* and *G.max* (100mg/kg.b.wt) on some serum enzymes in mice fed with high fat diet compared to control group (HFD) at different periods (4, 11, 17 weeks) respectively.

| Biochemical parameters | Treatment groups | 4 weeks | 11 weeks | 17 weeks |
|---|---|---|---|---|
| **ALT ( U/l )** | Normal | 36.17 ± 12.30 | 41.45 ± 11.24 | 42.61 ± 4.00 |
| | HFD | 42.16 ± 3.58 | 98.88 ± 10.78* | 105.85 ± 7.01* |
| | HFD +*C. fistula* | 41.69 ± 10.08 | 37.68 ± 2.07 | 44.6 ± 5.6 |
| | HFD +*C.scolymus* | 40.05 ± 3.05 | 49.91 ± 0.58 | 59.9 ±15.3 |
| | HFD + *G. max* | 41.21 ± 7.50 | 36.66 ± 1.41 | 42.9 ±7.4 |
| **ALP (U/I)** | Normal | 124.87 ± 10.53 | 119.43 ± 8.91 | 124.88 ± 9.4 |
| | HFD | 127.10 ± 17.08 | 182.91 ± 9.96* | 198.12 ± 5.85* |
| | HFD +*C. fistula* | 119.34 ± 2.97 | 122.22 ± 1.25 | 143.70 ± 6.54 |
| | HFD +*C.scolymus* | 123.79 ± 13.90 | 137.62 ± 8.70 | 139.82 ± 11.51 |
| | HFD + *G. max* | 93.97 ± 26.90 | 103.44 ± 4.08 | 94.56 ± 14.64 |
| **γ – GT (U/I)** | Normal | 2.38 ± 0.52 | 2.06 ± 1.14 | 3.81 ± 1.53 |
| | HFD | 4.16 ± 3.63 | 5.53 ± 0.71* | 7.44 ± 1.27 * |
| | HFD +*C. fistula* | 3.81 ± 1.58 | 3.44 ± 0.77 | 4.42 ± 1.61 |
| | HFD +*C.scolymus* | 3.17 ± 1.03 | 4.91 ± 1.93 | 3.95 ± 1.48 |
| | HFD + *G. max* | 3.94 ± 0.91 | 3.95 ± 0.99 | 3.42 ± 1.54 |

Values are mean ± SD , n=8 , ALT : Alanine aminotransferase , ALP: Alkaline phosphatase , **γ – GT** : Gamma glutammyl transpeptidase , * $P < 0.05$ vs normal control

**Histopathological evaluation**

Histopathological examination is a good tool for evaluation the effect of high fat diet on experimental mice and also, the effect of treatment of the obese mice with plant extract.

Steatosis is the process describing the accumulation of triglyceride or neutral fats within the cell (Burt *et al*., 1998; Marques *et al*., 2010). Fatty liver is macrovesicular or microvesicular steatosis according to the size of the lipid vacuoles. The most common form, which is potentially reversible, is macrovesicular steatosis. Although it is thought to be benign condition, it may be associated with the development of necroinflammation and fibrosis (steatohepatitis). Microvesicular steatosis is generally the more severe disease (Burt *et al*., 1998; Marques *et al*., 2010).

From histopathological studies for control group (HFD) (Fig 1-A), it was noticed that macrovesicular steatosis are more abundant than the microvesicular. These histological assessments of hepatic injury are consistent with the elevated serum lipid concentrations. On the other hand, the human form of NAFLD is characterized by a mixed macro and microvesicular type of hepatic steatosis (Kleiner *et al*., 2005). The treated group with methanol extract with *G. max* fig (1-D) reduced or disappeared the micro and macrovesicular steatosis. Therefore, this extract seems to reverse fatty liver disease in obese mice This agrees well with the finding of Park *et al*., 2006 on *A. senticosus* extract. Also the other two extracts (*C. fist*ula and *C. scolymus*) fig (1-B, C) caused a marked reduction in the degree of steatosis as hepatic steatosis lower than obese mice. These results are in full agreements with the previously reported data on the potentialities of some food and herbal supplements to ameliorate or reverse hepatic steatosis in animal models or patients with non-alcoholic fatty liver disease (Capanni *et al*., 2006; Chou *et al*., 2006; Park *et al*., 2006; Bruno *et al*., 2008; Zhou *et al*., 2008; Takayama *et al*., 2009).

## CONCLUSION

It can finally be concluded that the three Egyptian plants are promising in improvement the symptoms of non-alcoholic fatty liver disease when used as prophylactic plants. Where, the methanolic extract of the three plants showed anti-obesity properties which appeared clearly in normalizing and decreasing body weight, hypolipidemic effect showed in improvement of lipid profile and serum enzymes and also, the three plants possess the ability to improve the histopathological study of the liver especially the methanol extract of *G. max*.

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