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## **Effect of Terpenes Fraction of Iraqi Cicer Arietinum in Experimentally Induced Hyperlipidemic Mice**

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**Abstract**--The aim of this study is to investigate the antihyperlipidemic effect of Terpenes fraction of Iraqi *Cicer arietinum* in high-fat diet (HFD)-fed mice. Animals were divided into four groups (n =8). The total duration of the study was 56 days split into two intervals. During the first 28-days interval, mice were administered with HFD, whereas during the second 28-days interval they were co-administered HFD plus Terpenes (500mg/kg: p.o.) or the standard drug Atorvastatin (10mg/kg;p.o.). Terpenes treatment to HFD-induced hyperlipidemic mice caused a high significant decrement in the levels of total cholesterol, triglycerides, LDL-C and VLDL-C. Moreover, Terpenes resulted in significant increase in the levels of HDL-C, whereas it caused remarkable decreases in ALT, AST and ALP enzymatic activities also in total serum bilirubin levels among hyperlipidemic mice. Besides that, Terpenes treatment showed significant improvement in levels of tissue MDA and GSH in hyperlipidemic mice. Histopathological examination of hyperlipidemic mice showed a disorganized hepatic tissue, marked and diffused cytoplasmic fatty infiltration which was all ameliorated by Terpenes administration. The results revealed that Terpenes (500mg/kg;p.o.) possess potential ameliorating benefits against hyperlipidemia induced by HFD on lipid profile, liver function enzymes, oxidative stress parameters and hepatic histo-architecture. Further investigations are recommended and clinical trials are warranted to assess the efficacy and to fully dissect the mode of action underpinning the observed anti-hyperlipidemic effect of Terpenes.

**Keywords**---Hyperlipidemic, *Cicer areitinum*, Terpenes, lipid profile, Oxidative stress parameters.

## 1. Introduction

Hyperlipidemia is disorder of lipoprotein metabolism in which there is elevation of total serum cholesterol and/or triglycerides (TG), often accompanied by lowered levels of high-density lipoprotein (HDL) -cholesterol. They are caused by a combination of primary (familial) and secondary (acquired) factors such as (lifestyle, metabolic diseases, and drugs) [1]. Dyslipidemias have become a major public health problem around the world, as they are representing one of the paramount risk factors of the two main causes of morbidity and mortality in the world according to the WHO (World Health Organization), ischemic heart disease (IHD) and stroke. Although hyperlipidemia is considered more common among the western rich nations, the diet and some other environmental factors have extended this challenge worldwide [2- 4].

The choice of pharmaceutical treatment is based on the type of lipid abnormality. The drugs That Primarily Lower Atherogenic Cholesterol (LDL) are statins (the HMG-CoA reductase inhibitors), ezetimibe, resins, PCSK9 inhibitors, and niacin. The fibric acid derivatives (Fibrates), Niacin (nicotinic acid) and Omega-3 Polyunsaturated Fatty Acids (PUFA) are most effective at lowering TG and VLDL concentrations and increasing HDL cholesterol concentrations [5, 6]. Most of these drugs are linked with a number of adverse side effects including muscle-related complaints such as (myalgia, cramp, myopathy and rhabdomyolysis), flushing, dry skin, diarrhea, abnormal liver function, gall stone, gastric irritation and may provoke renal failure. Therefore, the development of promising new lipid-lowering agents alternatives to allopathic drugs is needed [7, 8]. **Chickpea** (*Cicer arietinum*), also called **garbanzo bean** or **Bengal gram**, annual plant of the pea family (Fabaceae), widely grown for its nutritious seeds and is one of the most consumed pulses worldwide. It is grown in north of Iraq, like Sulaimani, Kirkuk. Erbil, Duhok, and Ninawa provinces, and provides a good quality of spring genotypes [9]. Chickpea is a cholesterol free, good source of dietary fiber, polyunsaturated fatty acids such as linoleic and oleic acids, vitamins, and minerals, especially folate, calcium, magnesium, and potassium [10].

**Terpenes** are a large and diverse group of natural hydrocarbon secondary metabolites produced by a variety of plants such as tea, thyme, cannabis, conifers, and citrus fruits (e.g., lemon, orange, mandarin). They are responsible for the fragrance, taste, and pigment of plants. Terpenes are consisting of five-carbon isoprene units with chemical formula  $(C_5H_8)_n$ . [11]. Certain terpenes are largely used in natural folk medicine provide physical effect includes; anti-oxidant, anti-cancer, anti-inflammatory, anti-diabetic, anti-depressant, anti-plasmodial, anti-microbial, astringent, digestive, diuretic and many other properties [12].

The present study was planned to investigate the antihyperlipidemic effect of Terpenes fraction of Iraqi *Cicer arietinum* in mice.

## 2. Materials and Methods

**2.1. Plant material:** The whole plant of *Cicer arietinum* of the Family (Fabaceae) was collected from north of Erbil, during the month of November, 2021, flowering time. The collected plant cleaned, dried at room temperature in a shade area, then pulverized by mechanical mills and weighted.

**2.2. Plant experiment work:** It was done in the phototherapy laboratory of pharmacological Department/ Pharmacy College of Baghdad University. The upper part of the plant *Cicer arietinum* were collected and treated as follows:

### 2.2.1. Extraction and fractionation of different active constituents:

A (400gm) of shade-dried coarsely powdered plant materials were defatted with hexane for 24 hours then allowed to dry at room temperature. The defatted plant materials were extracted with (2 L) of 85% ethanol in soxhlet apparatus until complete exhaustion. The alcoholic extract was evaporated to dryness; under reduced pressure at a temperature not exceeding 40 °C to give a dark greenish-yellow residue designated as a crude fraction. Crude fraction then acidified with 300ml of 5% hydrochloric acid to pH 2 and partitioned (three times) with equal volume of ethyl acetate to get two layers (aqueous acidic and ethyl acetate layer), this step is necessary to get rid from any basic compound found in the crude extract, fraction (1) which was represented (Alkaloid). The ethyl acetate layer of the original alcoholic extract (crude fraction) was evaporated to dryness under reduced pressure and basify with 300ml of 5% sodium hydroxide to pH 10 and extracted with chloroform in the separator funnel to get two layers, the aqueous basic layer which was separated, evaporated to dryness and acidify with 5% HCL to pH 2 then extracted with ethyl acetate to get fraction designated as fraction (2) which was represented (Flavonoids) and chloroform layer which was also separated and evaporated to dryness under reduced pressure then partitioned with methanol and petroleum ether to get two layers petroleum ether fraction (3) which was represented (Sterols) and methanol fraction which designated as fraction (4) which was represented (Terpenes) [13,14].

### 2.2.2. Preliminary qualitative phytochemical analysis:

Chemical tests were carried out using the ethanol extract from plant and its methanol fraction; we used standard procedures to identify the active constituents [15, 16]. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids and steroids in ethanol extract of *Cicer arietinum* while methanol fraction revealed the presence of Terpenoid.

**Test for Terpenoids:** Two ml of the organic extract and fractions were dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A grayish color was considered an indication for the presence of Terpenoid.

### 2.2.3. Identification and characterization of the isolated Terpenes fraction by using HPLC:

High performance liquid chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture. The mixture is separated using the basic principle of column **chromatography** and then identified and quantified by spectroscopy [17].

**2.3. Animal experiment work:** The study was conducted from December 2021 through April 2022 at the department of pharmacology–College of Medicine /AL Nahrain University. The experiments were approved by the Ethical Committee at the College of Medicine /AL Nahrain University. Thirty-two apparently healthy, albino male mice 2-3 months age, weight about 20-30g, were obtained from the Higher Institute for Diagnosis of Infertility and Assisted Reproduction Techniques/AL Nahrain University. The animals were acclimatized in standard environmental conditions and fed with food and water *ad libitum* for a week before commencement of the experiment.

**2.3.1. Induction of hyperlipidemia:** High Fat Diet (2% cholesterol and 1% peanut butter) was added to the standard diet (Seeds as sunflower and groundnut, Cereals, Fruits as grapes and apple, Vegetables, Vitamin as A, E and D<sub>3</sub>) in order to induce hyperlipidemia for 28 days [18]. Body weights were measured weekly for all groups.

**2.3.2. Extract administrant:** Terpenes fraction of Iraqi *cicer arietinum* was administered via intragastric tube for 28 days [18].

**2.3.3. Experimental design:** The mice were divided into 4 groups, 8 mice each group [19]:

- **Group 1 (normal):** standard diet for 28 days.
- **Group 2 (induced):** HFD for 28 days.
- **Group 3 (treated):** HFD for 28 days then atorvastatin 10 mg/kg for further 28 days.
- **Group 4:** HFD for 28 days then Terpenes fraction of *Cicer arietinum* at dose of 500 mg/kg for further 28 days.

**2.4. Collection of blood:** Mice were kept fasting for 24 hrs and blood samples were extracted via heart puncture. This was followed by centrifugation at 2500 rpm for 15 min. The obtained serum was used for the biochemical analysis of lipid profile indices and liver function enzymes.

## **2.5. Biochemical analysis:**

### **2.5.1. The biochemical analysis of lipid profile indices and liver function enzymes:**

The standard diagnostic kits were used for estimation of serum total cholesterol (TC), triglyceride (TG), LDL, VLDL, HDL, ALT, AST, ALP, and total serum bilirubin (TSB) levels by using autoanalyzer [20].

### **2.5.2. The biochemical analysis of Oxidative stress parameters:**

At the end of the experimental period and after blood sampling, animals were sacrificed and the liver organ was removed for analysis and divided into two parts for assay. For oxidative stress measurement, the samples of MDA and glutathione were prepared from homogenization of first part of liver tissue, after that, centrifuged homogenates for 15 minutes 5000 rpm. The standard diagnostic kits were used for assay of tissue MDA and GSH level. This assay utilizes the competitive inhibition enzyme immunoassay technique [21, 22].

### 2.5.3. Histopathological examination:

The hepatic tissue samples were first rinsed in phosphate-buffered saline (PBS), then fixed in 10% neutral buffered formalin, standardized dehydration in ascending grades of alcohol, and paraffin embedding procedures were conducted. Microtomy of paraffin embedded ultrathin sections (5 mm thickness) was carried out and slides were stained by hematoxylin-eosin (H & E) dyes. The sections were analyzed using an Olympus light microscope with an attached photograph machine [23].

**2.6. Statistical analysis:** The Statistical Analysis System-SAS (2018) program was used to detect the effect of difference factors in study parameters. Unpaired t-test and ANOVA test were used to significant compare between means in this study.

## 3. Results:

### 3.1. Comparison between apparently healthy group and induced (nontreated) group in relation to different parameters

The results of t-test showed highly significant increase (**p<0.001**) in the level of serum (TC, TG, LDL, VLDL, ALT, AST, ALP), tissue MDA and (Micro+ Macrovesicular) steatosis with significant increase (**p<0.05**) in TSB in induced (non-treated) groups. Also, the results showed statistical highly significant (**p<0.001**) reduction in HDL and tissue GSH in induced (non-treated) groups in comparison with apparently healthy group (**Table1**), (**Fig 2, 3**).

Table 1: Comparison of different parameters between healthy (normal) control and induced (non- treated) group by unpaired t-test

| Parameter                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Normal group<br>Mean±SD | Induced group<br>Mean±SD | P value          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------------------|------------------|
| T. Chol. (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                | 135.8±17.2              | 241.98±5.5               | <b>&lt;0.001</b> |
| TG (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 85.92±14.04             | 183.87±13.75             | <b>&lt;0.001</b> |
| VLDL (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 17.18±2.81              | 36.79±2.75               | <b>&lt;0.001</b> |
| LDL (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 67.58±19.83             | 185.03±10.03             | <b>&lt;0.001</b> |
| HDL (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 49.37±6.1               | 16.59±5.63               | <b>&lt;0.001</b> |
| ALT (U/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 17.73±3.69              | 95.77±26.0               | <b>&lt;0.001</b> |
| AST (U/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 15.33±3.83              | 196.39±53.4              | <b>&lt;0.001</b> |
| ALP (U/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 22.52±3.02              | 52.87±1.96               | <b>&lt;0.001</b> |
| TSB (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 1.29±0.19               | 4.87±1.1                 | <b>0.002</b>     |
| MDA (nmol/ml )                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.53±0.23               | 4.1±1.31                 | <b>&lt;0.001</b> |
| SGH (U/ml )                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 315.77±66.97            | 57.24±19.85              | <b>&lt;0.001</b> |
| Steatosis*                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 0.625±0.518             | 2.625± 0.517             | <b>&lt;0.001</b> |
| Highly statistical significant (p≤0.001), statistically significant(p≤0.05), TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein , VLDL: very low density lipoprotein, AST: Aspartate amino transferase, ALT: alanine aminotransferase, ALp : alkaline phosphatase, MDA: Malondialdehyde, GSH: glutathione, TSB: total serum bilirubin, SD; standard deviation, Steatosis*: (micro+ macrovesicular steatosis). |                         |                          |                  |

### 3.2. Comparison of Terpenes (500mg/kg) treated group with induced (non-treated) group and group treated with Atorvastatin (10mg/kg) in relation to different parameters by ANOVA test:

In comparison with induced (non-treated) group, both groups treated with Terpenes (500mg/kg; p.o.) and Atorvastatin (10mg/kg; p.o.) showed statistical highly significant ( $p<0.001$ ) decrease in serum level of TC, TG, LDL, VLDL, ALT, AST, ALP, TSB and tissue MDA. Also, the results showed highly significant ( $p<0.001$ ) increase in HDL and tissue GSH in both treated groups. Besides, the results showed highly significant ( $p<0.001$ ) decrease in steatosis in group treated with Terpenes (500mg/kg; p.o.) and significant ( $p<0.05$ ) decrease in steatosis in group treated with Atorvastatin (10mg/kg; p.o.) (Table 2), (Figures 3, 4, 5). In comparison with Atorvastatin (10mg/kg; p.o.) treated group, group treated with Terpenes (500mg/kg; p.o.) showed statistically significant ( $p<0.05$ ) increase in serum levels of TC and LDL and statistically no significant ( $p>0.05$ ) in other parameters; (Table 2), (Fig. 4, 5).

Table 2: Comparison of Terpenes (500mg/kg) treated group with induced (non-treated) group and group treated with Atorvastatin (10mg/kg) in relation to different parameters by ANOVA

| Parameter                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Induced (non-treated) group<br>Mean±SD | Atorvastatin (10mg/kg) group<br>Mean±SD | Terpenes (500 mg/kg) group<br>Mean±SD | P value          |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|-----------------------------------------|---------------------------------------|------------------|
| T.Chol.(mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | 241.98±5.5                             | 112.35±14.39 <sup>a**</sup>             | 142.4±14.43 <sup>a** b*</sup>         | <b>&lt;0.001</b> |
| TG (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 183.87±13.75                           | 83.48±5.9 <sup>a**</sup>                | 98.89±8.71 <sup>a**bNS</sup>          | <b>&lt;0.001</b> |
| VLDL (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 36.79±2.75                             | 16.73±1.12 <sup>a**</sup>               | 19.78±1.74 <sup>a**bNS</sup>          | <b>&lt;0.001</b> |
| LDL (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 185.03±10.03                           | 46.84±15.3 <sup>a**</sup>               | 79.43±15.14 <sup>a** b*</sup>         | <b>&lt;0.001</b> |
| HDL (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 16.59±5.63                             | 48.77±2.17 <sup>a**</sup>               | 43.21±3.46 <sup>a**bNS</sup>          | <b>&lt;0.001</b> |
| ALT (U/L )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 95.77±26.0                             | 19.31±1.84 <sup>a**</sup>               | 19.19±1.36 <sup>a**bNS</sup>          | <b>&lt;0.001</b> |
| AST (U/L )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 196.39±53.4                            | 12.84±2.97 <sup>a**</sup>               | 15.98±1.3 <sup>a**bNS</sup>           | <b>&lt;0.001</b> |
| ALP (U/L )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 52.87±1.96                             | 21.34±5.26 <sup>a**</sup>               | 20.99±4.43 <sup>a**bNS</sup>          | <b>&lt;0.001</b> |
| TSB (mg/dl )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 4.87±1.1                               | 1.5±0.15 <sup>a**</sup>                 | 1.6±0.12 <sup>a**bNS</sup>            | <b>&lt;0.001</b> |
| MDA(nmol/ml )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 4.1±1.31                               | 0.69±0.2 <sup>a**</sup>                 | 0.59±0.08 <sup>a**bNS</sup>           | <b>&lt;0.001</b> |
| SGH (U/ml )                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 57.24±19.85                            | 289.26±33.29 <sup>a**</sup>             | 298.35±38.19 <sup>a**bNS</sup>        | <b>&lt;0.001</b> |
| Steatosis*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 2.625± 0.517                           | 1.00 ± 0.00 <sup>a*</sup>               | 0.875 ± 0.354 <sup>a**bNS</sup>       | <b>0.002</b>     |
| a: comparison with induced (non-treated) group, b: comparison with Atorvastatin (10mg/kg) treated mice group, Highly statistical significant ( $p\leq 0.001$ ), statistically significant ( $p\leq 0.05$ ), NS: not statistical significant ( $p>0.05$ ), TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein, AST: Aspartate amino transferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, MDA: Malondialdehyde, GSH: glutathione, TSB: total serum bilirubin, SD; standard deviation, Steatosis*: (micro+ macrovesicular steatosis). |                                        |                                         |                                       |                  |

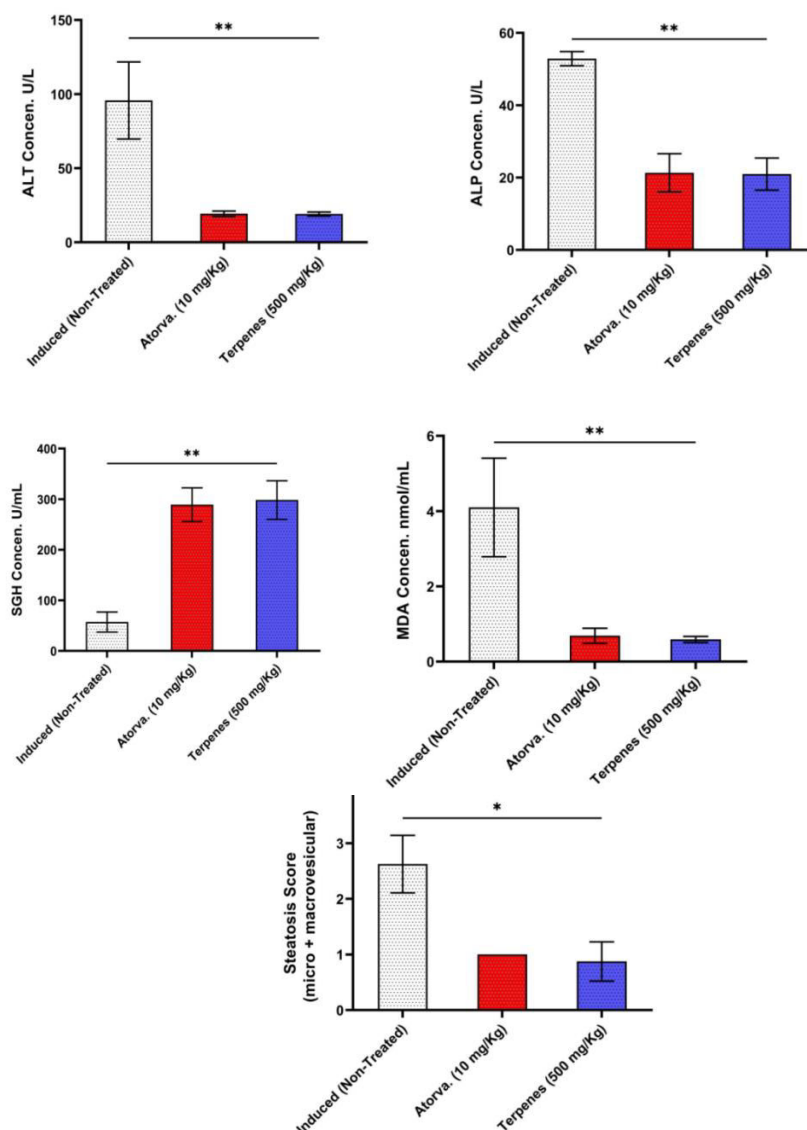
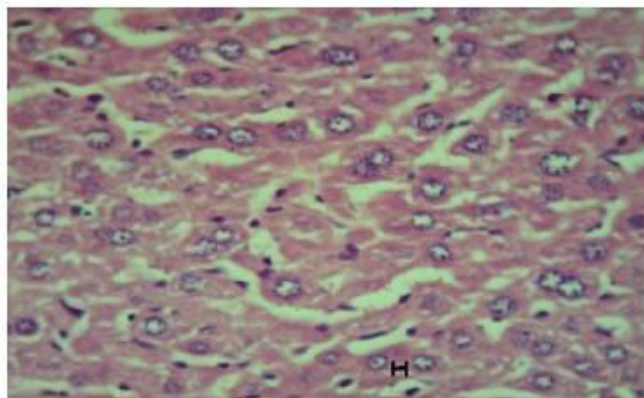


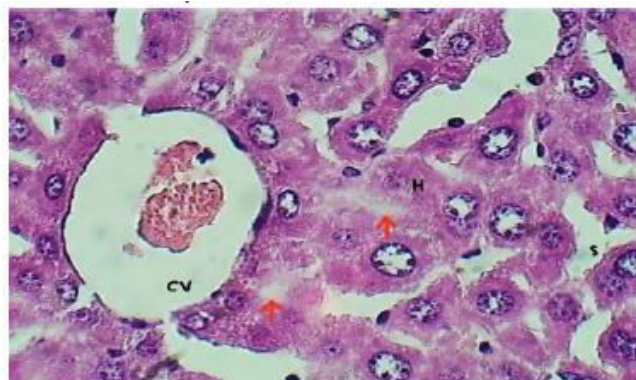
Figure 1: Comparison between induced (non-treated) mice group and groups treated with Atorvastatin (10mg/kg) and Terpenes (500mg/kg) in respect to different parameters. P\*\*are high significant at ( $\leq 0.001$ ) and P\* are significant at ( $\leq 0.05$ ). ALT: Alanine aminotransferase, ALP: Alanine aminotransferase, MDA: Malondialdehyde, GSH: Glutathione. Liver steatosis (micro+ macrovesicular) score of Terpenes (500mg/kg) group and Atorvastatin (10mg/kg) versus non-treated induced group are expressed as Mean  $\pm$  SD and were analyzed using ANOVA test. Semi-quantitative steatosis score ranged from 0 to 3 {0, normal steatosis of liver ( $< 5\%$ ); 1, mild steatosis of liver (5-33%); 2, moderate steatosis of liver (33-66%); 3, severe steatosis of liver ( $> 66\%$ )}. [24].



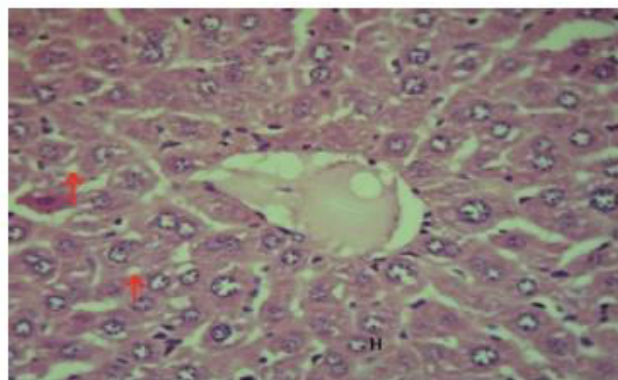
**Figure 2:** Histopathological examination of mice tissue of normal control group showed normal Hepatocyte (H). (H&E stain, 40X).



**Figure3:** Histopathological examination of mice liver of induced (non-treated) group showed marked & diffuse Fatty steatosis of hepatocyte (Red arrows) & sinusoidal dilation with central vein (cv) congestion (Black arrows). (H&E stain, 40X).



**Figure 4:** Histopathological examination of mice Liver tissue treated with Atorvastatin (10 mg/kg) Showed mild central vein congestion (CV) & Normal hepatocytes (H) with mild fatty steatosis (red arrows). (H&E stain, 40X).



**Figure 5:** Histopathological examination of mice liver treated with terpenes (500mg/kg) showed normal hepatocyte (H) with few scatter changes (red arrow). (H & E stain, 40X ).

#### 4. Discussion

Hyperlipidemia is characterized by the rising in serum lipid profile namely total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) and thus is considered one of the primary risk factors leading to myocardial

infarction and CVDs. Hyperlipidemia is directly correlated with a prominent metabolic dysregulation in the affected patients [25]. It could also result in serious cardiac pathologies such as atherosclerosis, due to the fact that hyperlipidemia is associated with elevated serum TC and TG; thus, it has been documented as a prevalent susceptibility marker of atherosclerotic heart disease [26].

The above-mentioned changes in association with declining (HDL-C) serum level eventually result in hyperlipidemia, causing advanced cardiac pathological conditions [27]. Moreover, the interfering harmful effect of HFD with the process of lipid metabolism in the liver is the primary factor responsible for the development of nonalcoholic fatty liver disease (NAFLD) [28]. Practical strategies to treat hyperlipidemia include the decrease lipids synthesis and their gut absorption using synthetic therapeutic agents like statins, Fibrates and bile acid sequestrates. However, the use of these agents might be manifested with series of side effects most notably rhabdomyolysis, myopathy and increase risk of gallstone formation. Hence, developing a novel and effective anti-hyperlipidemic therapeutic agents with minimal adverse side effects is urgently required [29]. The opportunity of treating this variety of pathological conditions using natural products and medicinal plants is emerging as new rising trend in recent years as they are safer, well-tolerated therapeutic strategy with minimal side effects in comparison to synthetic drugs [30].

#### **4.1. Extraction of *Cicer arietinum* in process:**

The process of extraction of active ingredients from plant materials is the first step in the exploitation of phyto-chemicals toward the preparation of pharmaceuticals, dietary supplements, cosmetic products and food ingredients [31]. In this study, the extraction process of the plant done sequentially according to solubility and polarity of the active constituents have been extracted and isolated to be ready for use in the experiments [32] to assess their hypolipidemic activity.

The total amount of *Cicer arietinum* upper part used in this study were 400 gm., hexane was used for defatted oil from the plant in order to exclude the surplus fatty components [33] that interfere with extraction process by adhering on the flask and biologic effect inside the body [34]. The alcoholic method considered the initial step in the extraction of many active constituents from plant material. The use of aqueous alcohol (alcohol and water mixing) allows the extraction of both polar and nonpolar compounds [35]. The alcoholic solution (85% ethanol) that was used in this study is valuable for extraction of carotenoids and flavonoids [36], saponins [37], amino acids and trigonelline [38], thiamine and riboflavin [39], and tocopherol [40] from different plants. After filtration, the ethanolic (crude) extract was subjected to evaporation to get extract free from alcohol to avoid the harmful effect of ethanol on the hepatic indicators of present study through using of rotary vacuum evaporator to avoid heat degradation of the bioactive constituents during evaporation. Methanol is a well-known solvent possessing the ability to extract semi-polar bio active compounds [41], so it used to extract Terpenes in the current study.

#### **4.2. Comparison between induced (non-treated) group and apparently healthy group in relation to different parameters:**

In the present study, feeding the mice high fat diet (HFD) for 28 days led to highly significant increase in serum total TC, TG, LDL and VLDL in induced (hyperlipidemic) mice as compared to normal (control) group fed normal standard diet (Table 1). These changes observed in hyperlipidemic group may be due to disturbance lipid metabolism mainly by decreasing  $\beta$ -oxidation, increasing cholesterol synthesis and oxidative stress (OS) by decreasing free radical scavenger enzyme gene expression [42]. Also, decreased lipoprotein lipase (LPL) activity accompanied by a depressed antioxidant defense system [43]. Oxidative stress (OS) has been documented to play a pivotal role in the patho-physiology and progression of diverse human diseases including CVD, CVI and DM [44]. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were highly significant increase among induced (non-treated) group than in normal group.

These consequences were may due to the disturbance of lipid metabolism because of high fat intake resulting in accumulation of TG in liver and an increased increment of the liver index and hepatic steatosis occurred [45] since the liver has a crucial role in regulating blood lipid level through LDL clearance and HDL-Crecruitment [46]. Moreover, the increase in liver enzymes may also due to excess reactive oxygen species (ROS) production in the mitochondria as a result of lipid overload. The ROS cause hepatic cells inflammation by activation cytokines [47]. Consequently, the excess fat infiltration, ROS and inflammatory cytokines explain a condition of hepatic toxicity [48]. Hence, the liver function markers (ALT, AST and ALP) as well as total serum bilirubin (TSB) showed noteworthy leakage in the serum and indicated the membrane damage of the hepatocytes

Malondialdehyde (MDA) which is a product of lipid peroxidation or reaction of oxygen with unsaturated lipids [49], were highly significant increase in induced (non-treated) mice. The elevated levels of MDA in induced (non-treated) mice suggest increased lipid peroxidation in fat deposits that could be released and have detrimental effects on hepatic cells and other hepatocytes. In hepatocytes, ROS and lipid peroxidation products further impair the respiratory chain either directly or indirectly through oxidative damage to the mitochondrial genome [29]. These features, in turn, lead to the generation of more ROS and a vicious cycle ensues. In addition, the results were supported by histological examination which revealed degenerative changes in the liver (**Fig. 3**).

Glutathione (GSH) is an intracellular hydrophilic antioxidant [50]. It is the most important endogenous defense mechanism against oxidative stress in body. It plays an essential role in maintenance of membrane protein -SH groups in the reduced form, the oxidation of which can cause altered cellular function and structure [51].

#### **4.3. Comparison of Terpenes (500mg/kg) treated group with induced (non-treated) group and group treated with Atorvastatin (10mg/kg) in relation to different parameters by ANOVA test**

According to HPLC analysis, the principle active constituents of Terpenes are pinene, camphene, limonene and thujene. The diversity in active constituents of Terpenes made them possess different pharmacology effects as antihyperlipidemia, anti-inflammatory, antioxidant, antidiabetes, anticancer and antiviral, through different mechanisms. In the present study, the serum levels of lipid profile TC, TG, HDL-C, LDL-C and VLDL-C were monitored and the results were depicted in (Tables 2). Highly significant decrement in the serum levels of lipid profile noticed among Terpenes (500 mg/kg; p.o.) treated mice as compared to hyperlipidemia mice group. These results were further supported by a significant improvement in HDL-C serum level as compared to hyperlipidemia in mice.

Highly Significant reduction in serum levels of liver enzymes (ALT, AST and ALP) have been noticed with the administration of Terpenes (500mg/kg; p.o.) as compared to hyperlipidemia mice group. They protect cells from damage induced by oxidative stress which is generally considered to be a cause of degenerative diseases [52]. Also, the reduction in serum level of (TSB) support liver ameliorates effect of Terpenes. The antioxidant effects of Terpenes through elevate catalase, superoxide dismutase, peroxidase activities, and lowered glutathione content as well as restore the mitochondrial membrane [53]. Besides, significant reduction of tissue MDA and increased defense mechanism through increase glutathione level were showed with Terpenes (500mg/kg; p.o.) fraction treated groups in comparison with induced (non-treated) group.

In respect to histopathological examination of the liver (Fig.3, 5) the results showed significant improvement in hepatocyte among Terpenes (500mg/kg; p.o.) treated mice group in comparison with induce (non-treated) mice group and these support antihyperlipidemic and anti-inflammatory effect of Terpenes which may be exert through inhibited pro-inflammatory cytokines such that occur with limonene pre-treatment [54]. Also, the lipid metabolism effect may be due to presence of Camphene, is a bicyclic monoterpene (C<sub>10</sub>H<sub>16</sub>). It is reported to prevent hepatic steatosis and exerts Hypolipidemic effect in high fat diet (HFD) mice [55].

Statins have strong lipid lowering effect which exerted through inhibition of hepatic (HMG-CoA reductase inhibitor) which consider the rate-limiting step of cholesterol synthesis [56]. Atorvastatin as a standard cholesterol lowering drug is used in present study. Atorvastatin treatment has been associated with a high significant reduction in lipid profile (TC, TG, LDL and VLDL). Furthermore, plasma HDL-C level was found to be increased in comparison with induce (non-treated) mice group. Also, treatment with Atorvastatin (10 mg/kg; p.o.) has been associated with hepatoprotective effect through significant reduction in liver enzymes (ALT, AST, and ALP). In other study, Atorvastatin (10 mg/kg; p.o.) treatment has been associated with a broad spectrum of hepatic adverse effects. The most common is usually transient elevation of serum aminotransferase levels [57]. Although the underlying mechanism remains unclear, it may result from changes in the lipid components of the hepatocyte membrane, leading to an

increase in its permeability with a subsequent leakage of liver enzymes [58]. A high significant reduction serum levels of TSB and tissue MDA are another indicator for hepatoprotective effect of Atorvastatin (10mg/kg;p.o.) throughout antioxidant action of increase the function of the endogenous antioxidants and reduce lipid peroxidation by significant increase GSH level. Furthermore, the histopathology examination of induced mice groups treated with Atorvastatin (10mg/kg; p.o.) revealed a mild diffused micro vesicular steatosis while the induced (non-treated) group showed marked and diffuse cytoplasmic fatty infiltration (microvesicular and macrovesicular steatosis) and granular degeneration of hepatic cells; (Fig 3, 4).

In the present study, the effects of Terpenes (500mg/kg; p.o.) and Atorvastatin (10mg/kg; p.o.) on serum levels of TC, TG, LDL, HDL and VLDL was comparable although there is no significant difference between them but Atorvastatin seems to be more effective in certain lipid profile parameters (Table2). In comparison with Atorvastatin (10mg/kg; p.o.) treated group, treatment with Terpenes (500mg/kg) showed an evident reduction in the activities of the liver enzymes (ALT and ALP) with prominent effect on tissue MDA and GSH. Also, significant reduction in liver steatosis have been noticed with Terpenes (500mg/kg) treated groups in comparison with Atorvastatin (10mg/kg; p.o.) treated group, confirming a protective potential of Terpenes against hyperlipidemia. However, 500 mg/kg of Terpenes fraction was shown to be the perfect dose against hyperlipidemia. The diversity in active constituents of Terpenes made them possess antihyperlipidemic and antiinflammatory effects as pinene which present in great amount in extract [59]. Terpenes possess good oxygen radical scavenging potential [60]. Antioxidants interfere with the oxidative processes by scavenging free radicals, suppressing reactive oxygen species formation either by inhibition of enzymes or chelating trace elements involved in free radical production and by upregulating or protecting antioxidant defenses [61]. Mice treated with Atorvastatin (10mg/kg; p.o.) revealed improvement in cellular histo-architectural morphology, while Terpenes (500 mg/kg; p.o.) treated mice showed distinct quasi normal histopathology profile.

## Conclusions

1. Terpenes fractions of Iraqi *Cicer areitinumin* upper part demonstrated a significant anti-hyperlipidemic effect and this effect was more obvious in dose of (500mg/kg; p.o.).
2. Terpenes fraction (500mg/kg; p.o.) demonstrated a significant anti-inflammatory and antioxidant effect through free radical scavenging activity. The diversity in presence of phytochemical compounds like pinene, camphene, limonene and thujene are probably responsible for inhibition lipid peroxidation.
3. Effects of Terpenes (500mg/kg; p.o.) and Atorvastatin (10mg/kg; p.o.) were comparable in relation to different parameters although Terpenes (500mg/kg; p.o.) showed more hepatoprotective effect according to histopathology outcomes and a significant improvement of liver enzymes serum levels.

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