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# Hepatoprotective Effect of *Origanum elongatum* against Carbon Tetrachloride ($CCl_4$) Induced Toxicity in Rats

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author BD designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author JA provided technical advices and author AE identified and authenticated the studied plant. All authors read and approved the final manuscript.*

***Research Article***

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## ABSTRACT

**Aims:** This study was undertaken to investigate the potential effect of methanol extract of *Origanum elongatum* (OEME) given orally by gavage against intraperitoneal injection a single dose of Carbon tetrachloride ($CCl_4$)(0.6 ml/kg) induced hepatotoxicity in rats.
**Study Design:** Biochemical analysis, histological examination and in vivo study.
**Place and Duration of Study:** Laboratory of Biology and health (Faculty of Science), between June 2012 and August 2012.
**Methodology**: Hepatoprotective activity of OEME at four doses of 250 mg/kg, 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight. The degree of protection was estimated by biochemical analysis of serum liver biomarkers: AST, ALT, ALP and by liver histopathological examination.
**Results:** The total phenolic content of OEME (83.61 ± 0.19 mg AGE / g extract) and total flavonoid content (10.85 ± 0.05 mg QE / g extract) were found significantly high. The

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present study showed that OEME was dose dependently decreased hepatic histopathological changes and serum liver biomarkers levels in $CCl_4$ intoxicated rats.
**Conclusion:** Data from present results revealed the hepatoprotection of OEME against hepatotoxic products or drugs.

*Keywords: Origanum elongatum; silymarin; hepatoprotection; $CCl_4$; hepatotoxicity; serum liver biomarkers; histopathological examination.*

## 1. INTRODUCTION

The liver is the most complex organ in the body. It plays a vital role in regulating metabolism processes, performing many essential functions in order to maintain life, such as glycogen storage, production of necessary biochemicals for digestion, plasma protein synthesis and detoxification [1,2].

These functions are carried out generally by hepatocytes especially for the process of blood filtration, for chemical digestion of medications, but also against environmental pollution toxins, and alcohol intoxication which can have largely damaging effects over long periods of exposure or abuse [3].

Yet, the remarkable progress in phytotherapy remains insufficient. Only a small number of medicinal plants, in our country, that are used in folklore medicine -for curing ailments related to liver - are scientifically evaluated for its activities [4]. In our search for new natural hepatoprotective agents, we chose Origanum elongatum, a plant belonging to the Lamiaceae family (Emb. & Maire). It's 30- 80 centimeters tall and has dark green oval or elongated oval leaves. It grows in shale or limestone soils between 400 and 1,500 meters of altitude. It is known for its rarity and white inflorescence attached to vertical rods. It blooms from June to October [5]. This plant is an endemic herb originally from the north of Morocco which is widely used for its therapeutic virtues against various diseases such as diarrhea, respiratory infections and urinary tract infections, and as an aromatic plant for its flavor or as a food preservative [5]. The use of some halogenated alkanes such as carbon tetrachloride ($CCl_4$) classified as a potential human carcinogen, increases the frequency of liver tumors in experimental animals. It's widely used as a model for the study of agents that cause liver damage by formation of trichloromethyl and trichloromethyl peroxyl radicals by a free-radical mechanism [6]. Recently, many natural agents possessing antioxidative properties have been reported to prevent and treat liver damages caused by free radicals induced by $CCl_4$ in experimental animal's model [7].

However, the literature survey revealed that this plant has not been scientifically investigated. Thus, we take this opportunity to study the hepatoprotective activity of methanol extract of *Origanum Elongatum* leaves against $CCl_4$ induced liver damage in the Wistar albino rats.

## 2. MATERIALS AND METHODS

### 2.1 Material and Extraction

The *Origanum elongatum* (Emb. & Maire) used in this research was collected in September 2011, from the Banu Aammart area in the Rif Mountains, over a height of 1.240 meters. (al-Husaima, Morocco). The plant was identified and authenticated by Pr ENNABILI Abdesalam (National Institute of Medicinal and Aromatic Plants, Taounate, Morocco). Samples were

further transported to the laboratory. The leaves of the plant were air dried under the shade and then milled into powder using an electric grinder. The investigated dried powdered plant materials were extracted with methanol. The plant extracts were filtered by Whatman No. 1 filter paper and the combined filtrate was then dried under vacuum using a rotary evaporator (Heidolph Collegiate, LV28798826, New Jersey, USA) at a temperature not exceeding 45 °C. The dried plant extracts were stored in a dark bottle for investigation at 2 - 8°C.

## 2.2 Determination of Total Phenolic Content

The total phenolic content was determined by using the Folin-Ciocalteu method (Singleton et al.,) [8]. The extract was diluted to the concentration of 500 µg /ml, and aliquots of 100 µl were mixed with 500 µl of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 400 µl of $Na_2CO_3$ (7.5 %). After 60 min at room temperature, the absorbance was measured at 765 nm using a Spectro-photometer (J.P. Selecta, sa 4120007, Barcelona, Spain) against a blank sample. Results were expressed in mg of Gallic acid (5 - 100 µg/ml) dissolved in distilled water per g dry extract and the values are presented as means of triplicate analyses.

## 2.3 Determination of Flavonoid Content

Flavonoid content was determined according the Aluminum chloride ($AlCl_3$) colorimetric method of Brighente et al. [9]. A total of 750 µl of 2% $AlCl_3$ in methanol was mixed with the same volume of methanol solution of plant extract. After 15 min of staying at room temperature, the absorbance was measured at 430 nm in a spectrophotometer against the blank sample. Results were expressed in mg of quercetin (5 - 25 µg/ml) dissolved in methanol per g dry extract, and the values are presented as means of triplicate analyses.

## 2.4 Animals

Adult Wistar albino rats weighing 190 - 250 g were used for this study, obtained from the Animal House Colony of Faculty of Science, Abdelmalek Essaadi University Tetuan, Morocco. The animals were kept in polypropylene cages and maintained at 25 ± 3ºC under 12 h light/dark cycle. The animals were allowed free access to standard dry rodent diet and water *ad libitum*, implementing the European Communities Council Directive 86/609/EEC.

## 2.5 Chemicals and Drugs

All other organic solvents used for this study were of analytical grade. Silymarin was purchased from Swiss Herbal Remedies (Ltd, Canada) and $CCl_4$ from Sigma-Aldrich (Somaprol, Casablanca, Morocco).

## 2.6 Acute Toxicity Study

The acute toxicity of OEME was evaluated in rats using the method suggested by Seth et *al.* [10]. Thirty six rats were divided into six equal groups. Five groups received the extract at various doses of 250 mg, 500 mg, 1000 mg, 2000 mg, and 3000 mg per kg body weight orally by gavage. The animals were observed continuously for the first 4 h for any behavioural changes. Finally, the number of survivors was noted after 24 h and on the $7^{th}$ day of experimentation. Rats in all the groups were weighed the first and the last day of study

compared to those of the control group (received distilled water), and the body weight (bw) gain (g) was calculated using the following equation:

Body weight gain (g) = final bw – initial bw

## 2.7 Experimental Groups and Protocol

After one week of adaptation, rats were divided into seven groups of six rats each and treated orally by gavage according to Samaranayake et al. [11] as below for 7 days:

| | |
|---|---|
| Group 1: | Normal group served as negative control received only distilled water |
| Group 2: | $CCl_4$ group served as positive control received only distilled water. |
| Group 3: | As reference group received silymarin 50 mg/kg per *os* once per day for 7 days. |
| Group 4: | Received OEME 250mg/kg body weight (bw) per os for 7 days. |
| Group 5: | Received OEME 500 mg/kg bw per *os* once per day for 7 days. |
| Group 6: | Received OEME 1000 mg/kg bw per *os* once per day for 7 days. |
| Group 7: | Received OEME 2000 mg/kg bw per *os* once per day for 7 days. |

The animals were kept under observation up to 7 days after drug administration so as to find out changes or toxic symptoms. 7 days after the beginning of the experiment, $CCl_4$ (0.6 ml/kg bw) was administered to groups 2, 3, 4, 5, 6 and 7 by intraperitoneal injection which is clearly documented to induce hepatotoxicity in rats [12]. Body weights of rats in all groups were measured at the beginning and the end of the experimentation. In addition, the weight gains were calculated using this equation:

Weight gain (g) = final weight - initial weight

While the body weight changes (%) were calculated using the following equation:

Body weight change (%) = ((final bw - initial bw) / initial b w) x100.

After 24h, the animals were anaesthetized under Chloral hydrate 4% (1 ml/ 100 g bw) intraperitoneally and their blood samples were collected by cardiac puncture aseptically using sterile 5 ml syringe into tubes without anticoagulant and allowed to clot. Then sacrificed, their liver was separated, dried and weighed, then tacked for histopathological examination. Serum was separated by centrifuging at 4000 rpm for 5 min and analyzed for various biochemical parameters. The wet weight of the liver was defined as the absolute liver weight (g). The relative liver weight was calculated using the final bw and absolute liver weight according to the following equation to reduce the individual body weight differences:

Relative liver weight %= (Absolute liver weight (g) / Final bw (g)) x 100

## 2.8 Assessment of Liver Function

Biochemical parameters: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), were analyzed using the method recommended by the International Federation of Clinical Chemistry (IFCC) [13,14].

## 2.9 Histopathological Examination

Livers of the sacrificed rats were dissected, washed with distillated water, blotted with filter papers, weighed, then immersed in 10 % formalin solution, fixed in paraffin and sectioned using a microtome. Light microscopy was performed after slides had routinely been stained with Hematoxylin and Eosin for histopathological examination according to the method of Bancroft [15].

## 2.10 Statistical Analysis of Data

Data were statistically analyzed by Graphpad prism 5 (Graphpad Software, Inc., USA). Differences between control and treated groups were determined using one-way ANOVA followed by Dunnett's test for multiple comparisons with a $P < 0.05$ considered significant. The body weight, liver weight data were calculated as mean values ± SEM, and the significance of differences was assessed by means of one-way ANOVA, followed by Dunnett's multiple comparisons test.

# 3. RESULTS

## 3.1 Determination of antioxidant compounds

The phytochemical analysis conducted on OEME revealed the presence of total phenol and flavonoid. The total phenolic content was 83.61 ± 0.19 ($P$= 0.0004) mg AGE / g extract. The flavonoid content of the plant was 10.85 ± 0.05 ($P$= 0.0004) mg QE / g extract.

## 3.2 Acute Toxicity Study

In acute toxicity study, we found that the extract induced sedation and temporary loss of appetite by reducing feed intake, especially for doses equal or up to 2000 mg/kg, but this effect was eliminated 24 hours later. There were no signs of changes in rats' skin and fur, eyes and mucus membrane and neurological behavior. Furthermore, there was no mortality at any of the tested doses after 24 h of observation, neither after 7 days study period. The LD50 was greater than 3000 mg/kg bw.

The body weights of the treated rats increased gradually compared to those of the control rats. The groups which received 250 and 500 mg/kg of OEME had a body weight gain 12.33 g ± 1.60 ($P$= 0.08) and 12.67 g ± 1.63 ($P$= 0.20) respectively. These results are not significant compared with those in the control group, while the groups which received 1000 and 2000 mg/kg of OEME had a body weight gain 5.67 g ± 0.67 ($P$= 0.0002) and 1.67 g ± 0.67 ($P$< 0.0001) respectively. These results are significant compared with those in the control group. Except the group which received 3000 mg/kg of OEME, their weight was slightly decreased by -2.50 g ± 3.50 ($P$< 0.0001) at the end of experimentation (Table 1). Generally, the body weight gain (g) of all groups decreased gradually by increasing the dose of the extract.

**Table 1. Initial, final weights (g) and Body weight gain (g) of rats administered different doses of OEME during 7 days**

| Groups | Initial weights (g) | Final weights (g) | Body weight gain (g) |
|---|---|---|---|
| Control | 197.00 ± 2.22 | 216.00 ± 1.29 | 19.00 ± 3.09 |
| 250 mg/kg | 207.20 ± 4.44$^{ns}$ | 219,50 ± 3,04$^{ns}$ | 12.33 ± 1.60$^{ns}$ |
| 500 mg/kg | 201.70 ± 1.02$^{ns}$ | 214,30 ± 1,05$^{ns}$ | 12.67 ± 1.63$^{ns}$ |
| 1000 mg/kg | 238.80 ± 1.90 * | 244.50 ± 2.05 * | 5.67 ± 0.67 ** |
| 2000 mg/kg | 245.30 ± 2.25 * | 247.00 ± 2.24 * | 1.67 ± 0.67 * |
| 3000 mg/kg | 227.20 ± 7.09 * | 224.70 ± 6.86$^{ns}$ | - 2.50 ± 3.50 * |

*Values are expressed as means ± standard error of means (SEM) of six rats treated for 7 days. * P < 0.0001 and ** P < 0.001 were statistically significant. ns: non-significant (P= 0.59) for initial weight and (P= 0.71) for final weight compared with control as determined by one way ANOVA followed by Dunnett's multiple comparisons test.*

## 3.3 Changes in Body and Liver Weight

Rats gavaged with OEME exhibited significantly reduced Body weight change (*P*= 0.03) and increase in both absolute liver weight (*P*= 0.008) and relative liver weight (*P*= 0.05) compared with the values obtained with negative control (C-) (Table 2).

But compared to positive control (C+), all groups showed a significantly decrease in terms of body weight changes in rats) (*P*= 0.03). It decreased both absolute liver weight (*P*= 0.008) and relative liver weight (*P*= 0.05) dose dependent (Table 2) except group 5 which showed a tenuous increase in body weight change which was non-significant (*P*=0.99). Compared to reference group, groups 4 and 5 showed an increase in rats body weight change (*P*= 0.1) and (*P*= 0.02), and in both absolute liver weight (*P*=0.0001) and relative liver weight (*P*= 0.0001), while the Groups 6 and 7 showed a slight decrease in rats body weight change (*P*= 0.002) and in relative liver weight (*P*= 0.01). This remains close to the results of this group and to the normal group.

## 3.4 Biochemical Results

In this study, we demonstrated that rats administrated with a single dose of $CCl_4$ exhibited a significant elevation in the levels of serum AST, ALT and ALP (*P*< 0,0001) compared them with normal group (C-) (Table:3), translating acute hepatotoxicity, as previously reported by Bansal AK [16] and Etuk EU [17]. But the group of rats that were gavaged with OEME (250, 500, 1000 and 2000 mg/kg) then injected with $CCl_4$, and the group which received silymarin and $CCl_4$ showed significant (*P*< 0.0001) decrease in the levels of serum aminotransferase and canalicular enzyme ALP (Table 3)

Especially at dose 2000 mg/kg/d which were very effective as the values were brought to the reference group. According to the results, we note that the rates of ALT are higher than the rates of AST in all groups of rats (Table 3).

**Table 2. Body weight, body weight change, absolute and relative liver weights of the rats in different groups during 7 days**

| Groups | Treatment | Body weight (g) | | Body weight change (%) | Absolute liver weight (g) | Relative liver weight (%) |
|---|---|---|---|---|---|---|
| | | Initial | Final | | | |
| 1 | Normal (C-) | 198.70 ± 1.43 | 217.70 ± 1.22 | 9.61 ± 1.26 | 7.46 ± 0.16 | 3.45 ± 0.07 |
| 2 | $CCl_4$ (C+) | 222.20 ± 3.79 $^{a}$ | 237.00 ± 5.48 $^{a}$ | 6.64 ± 1.15 $^{c}$ | 11.87 ± 0.21 $^{a}$ | 5.01 ± 0.05 $^{a}$ |
| 3 | Silymarin + $CCl_4$ | 202.30 ± 1.96 $^{ns,d}$ | 209.7 ± 3.37 $^{ns,d}$ | 3.59 ± 0.79 $^{b, f}$ | 7.86 ± 0.12 $^{ns,d}$ | 3.75 ± 0.06 $^{c,d}$ |
| 4 | OE (250mg/kg) + $CCl_4$ | 206.00 ± 4.76 $^{ns,e}$ | 218.33 ± 3.38 $^{ns,e}$ | 6.08 ± 0.87 $^{c, NS}$ | 10.92 ± 0.18 $^{a,NS}$ | 5.00 ± 0.06 $^{a,NS}$ |
| 5 | OE (500mg/kg) + $CCl_4$ | 200.20 ± 0.70 $^{ns,d}$ | 213.70 ± 1.90 $^{ns,d}$ | 6.74 ± 0.84 $^{ns, NS}$ | 9.25 ± 0.22 $^{a,d}$ | 4.32 ± 0.09 $^{be}$ |
| 6 | OE (1000mg/kg) + $CCl_4$ | 240.20 ± 2.58 $^{a,d}$ | 245.83 ± 2.07 $^{a,ns}$ | 2.37 ± 0.34 $^{a, e}$ | 8.61 ± 0.26 $^{b,d}$ | 3.50 ± 0.09 $^{ns,d}$ |
| 7 | OE (2000mg/kg) + $CCl_4$ | 247.00 ± 2.06 $^{a,d}$ | 248.83 ± 2.05 $^{a,f}$ | 0.74 ± 0.19 $^{a, d}$ | 8.63 ± 0.12 $^{ns, d}$ | 3.47 ± 0.04 $^{ns,d}$ |

*Each value was the mean ± SEM of six animals per group. Data were analyzed by using one way ANOVA followed by Dunnett's multiple comparisons test. a P< 0.0001; b P< 0.001; c P< 0.05 Vs negative control group. d P< 0.0005; e P< 0.005; f P< 0.05 Vs positive control group. ns: non-significant compared with negative control group. NS: non-significant compared with positive control group.*

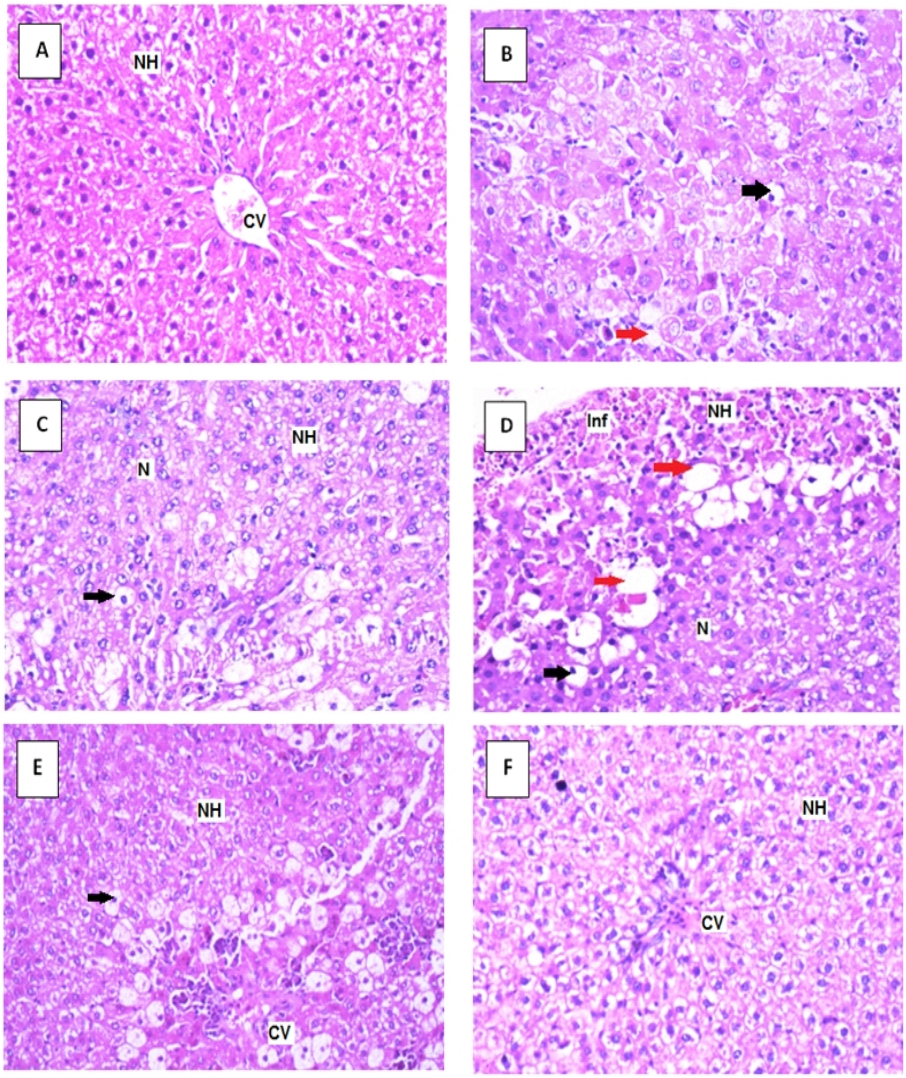
**Table 3. Effect of OEME and silymarin on biochemical parameters (AST, ALT and ALP) on CCl4 induced liver damage in rats**

| Group | Treatment | AST (U/l) | ALT (U/l) | ALP (U/l) |
|---|---|---|---|---|
| Group 1 | Normal (C-) | 58.00 ± 2.22 | 126.333 ± 3.08 | 159.70 ± 9.29 |
| Group 2 | $CCl_4$ (C+) | 1038.00 ± 39.68 * | 2231.00 ± 4.42 * | 759.20 ± 8.02 * |
| Group 3 | silymarin+$CCl_4$ | 209.50 ± 3.05 *# | 319.500 ± 4.71 *# | 199.70 ± 2.02 *# |
| Group 4 | OE (250mg/kg) + $CCl_4$ | 933.50 ± 25.06 *# | 1518.00 ± 6.84 *# | 610.00 ± 8.77 *# |
| Group 5 | OE (500mg/kg) + $CCl_4$ | 854.83 ± 18.65 *# | 991.70 ± 3.25 *# | 518.00 ± 33.57 *# |
| Group 6 | OE (1000mg/kg) + $CCl_4$ | 488.70 ± 14.14 *# | 723.70 ± 27.37 *# | 498.20 ± 23.07 *# |
| Group7 | OE (2000mg/kg) + $CCl_4$ | 262.50 ± 5.408 *# | 324.33 ± 6.941 *# | 360.50 ± 9.82 *# |

*Values are the mean ± SEM of six rats. Symbols represent statistical significance * P < 0.0001 as compared to Normal (C-) group and # P < 0.0001 as compared to CCl4 (C+) group.*

### 3.5 Histological Results

Histopathological studies of the liver sections of $CCl_4$ treated animals showed hepatic cells with severe toxicity characterized by a marked portal inflammation, with cytoplasmic vacuolization of the hepatocytes. A marked piecemeal necrosis, presence of ballooning degeneration and marked focal centrilobular necrosis (N), associated with marked polymorphic inflammatory foci. This inflammation (Inf) is composed of lymphocytes, plasma cells, neutrophils and essentially eosinophils, in addition to Congestion of central veins (CV) (Fig. 1(B)).



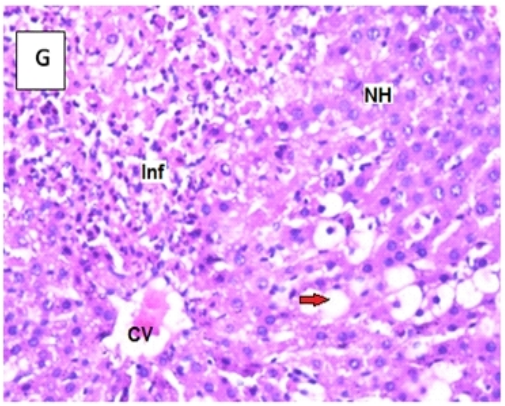



**Fig. 1. Histopathological studies of the liver sections by light microscopy**

*(A) A liver section of the (C-) showing normal histology architecture (Hematoxylin-eosin (B) (H and E), × 40).*
*(B) Liver section of the (C+) shows the $CCl_4$-induced destruction of architecture in hepatic cells showing fat vacuole ( ) and ballooning degeneration ( ) (H and E, × 40).*
*(C; D; E; F) Liver section shows the recovery of $CCl_4$-induced damaged by OEME of different doses 250, 500, 1000 and 2000 mg/kg, respectively showing normal arrangement of hepatocytes (NH), necrosis (N), inflammation (inf) and moderate accumulation of fatty vacuoles ( ), (× 40).*
*(G) Liver section shows the recovery of $CCl_4$-induced damaged by silymarin showing normal arrangement of hepatocytes absence of necrosis and few fatty vacuoles (H and E, × 40).*

Histological section of liver from (C-) (Figure: 1 (A)) showed normal hepatic (NH) cells with well-preserved cytoplasm, prominent nucleus and well brought out central vein (preserved liver architecture). Treatment with different doses of OEME produced a slight polymorphic portal inflammation containing some eosinophils, but without epithelial lesions of the bile ducts. Some various gradation of change in fat comprising of moderate to tiny-sized vacuoles (hepatic steatosis). Portal fibrosis was not observed. Area of patchy necrosis was reduced or absent. The life spans of hepatocytes were regular. Lobular necrosis was minimal or absent, but we noted a lobular inflammation with mild clusters of polymorphs. The veins were slightly to moderately dilated. But generally, architecture was well preserved (Fig. 1 (C, D, E, F)). Treatment with silymarin (Figure: 1 (G)) appeared to significantly prevent against $CCl_4$ toxicity as revealed by the hepatic cells with well-preserved cytoplasm with prominent nucleus, marked decrease in inflammatory cells and normal central vein. Comparing these results of the histopathological scoring of the liver tissues intoxicated with the $CCl_4$ showed that the presence of destruction of architecture in hepatic cells by ballooning degeneration, necrosis, inflammation ect was reduced remarkably when pretreated with the OEME or silymarin.

## 4. DISCUSSION

In the case of acute toxicity study, and during the seven days study period, there was no death record, nor signs of changes in the rats in any of the treated groups. The animals exhibited slight changes in behaviour (temporary sedation and loss of appetite by reduction in feed intake) but did not expressed changes in their physiopatological activities or in neurological behaviour. These changes are maybe a result of a set of adaptive biochemical and physiological changes that reduce metabolism in response to a lack of food. In this study, food intake decreased with increase of OEME doses treatment. These results are very

similar to those reported by Cho S et al. [18]. These signs were seen rarely at 250 mg/kg bw dose group but progressed and became increasingly pronounced as the dose increased towards 3000 mg/kg bw. The LD50, being greater than 3000 mg/kg bw, is not considered to be safe, as suggested by Lork [19]. There must be more than 5000 mg / kg body weight, in order to consider it as safe and nontoxic. Although there was a decrease in the weight gain in the treated groups of rats compared with control, we simply conclude, that the body responds to periods of low energy intake levels. During this short period of 7 days, it seems that the body is burning primarily free fatty acids, from body fat stores, causing a decrease in adipose tissue and decrease in weight gain.

$CCl_4$ as many halogenated alkanes has been widely used in our daily life but has been banned or restricted because of their distinct toxicity. His production has steeply declined since the 1980s; it was proved to be one of the most powerful hepatoxins capable of forming trichloromethyl and trichloromethylperoxyl radicals [20]. Yet it continues to provide an important service in scientific research as a model substance to demonstrate the mechanisms of action of hepatotoxic effects and carcinogenicity or to evaluate hepatoprotective agents [20, 21]. As we mentioned before, we evaluated body weight gain, absolute and relative liver weights ratio of the laboratory rats. In general, obtained data showed that the rats fed diet that contains OEME have seen a significant decrease in the body weight gain and a slight increase in the absolute and relative liver weight dose dependent, compared to both normal group and reference group of rats. But showed a decrease in the body weight gain and a decrease in the absolute and relative liver weights dose dependant, compared to group that received only $CCl_4$. We found a relationship between a decrease in the absolute and the relative liver weight, on the one hand, and body weight, on the other hand, in a dose dependent manner in intoxicated rats, which showed a higher relative liver weight but less lose of body weight changes than those who received OEME, especially at doses more or equal than 1000 mg / kg. The data obtained by Gnanaprakash K et *al*. [22] and Bulus T et *al*. [23] were in accordance with our results. It was reported that the body weight increase occurred gradually as the increase of adipose tissue, liver and other organs. Also, the opposite is relevant. The decrease in body weight occurred gradually as the increase in absolute liver weight happened. That is consistent with our results [24, 25, 26]. Cho S et *al.* [18] has demonstrated that in hepatotoxicity, liver weight increases generally as a consequence of fibrosis or hypertrophy of the liver and body weight decreases due to the modulation of adipogenesis. The alterations in the body weight and the liver weight in rats after $CCl_4$ injection were considered to be the result of the direct toxicity of the latter and the indirect toxicity related to liver damage [27]. Thereby, this change in relative liver weight is a valuable index of the extent of acute hepatic damage [28, 29, 30]. The results indicated that the OEME was protective against hepatotoxicity caused by $CCl_4$ in a dose dependent manner, where 2000 mg/kg was more effective than 1000 mg/kg and 1000 mg/kg was more effective than 500 mg/kg of dose. Using body weight and Liver weight for comparison in our previous analysis was reported by Bailey [28] who noted that liver weight is one of the best compared using organ-to-body weight ratios.

To diagnose hepatotoxicity, Serum enzymes AST, ALT and ALP are the most sensitive markers employed [31]. AST is normally found in different tissues as liver, kidney, heart, muscle and brain. It spills into systemic circulation when any one of these tissues is damaged. It's consequently not a specific indicator of hepatotoxicity [32]. ALT is most concentrated in the liver. It leaks out into the bloodstream as the result of liver injury. Therefore, it considered as a specific indicator of liver status [32, 33]. Alkaline phosphatase is the most frequently used test to detect obstruction in the biliary system. It is found both in the liver and the bile and it leaks into the bloodstream in a manner similar to that of the ALT

and AST [14]. It is also found in other organs, such as bone, placenta and intestine. Elevation of this enzyme may be found in a large number of disorders as drug-induced hepatitis in this case. The intoxication by $CCl_4$ and its metabolites ($CCl_3$ radical) causes acute hepatic failure. This radical, which is formed by a metabolic enzyme (cytochrome P450), induce peroxidation of the unsaturated fatty acids of cell membrane and leads to membrane injury and leakage of sensitive markers of hepatocellular injury, such as serum ALT and AST [34]. Our results are tangible, by the elevation of serum marker enzymes concentration AST, ALT and ALP beside histology study. This elevation results from mitochondrial damage, cell membrane damage in hepatocytes and the liver biliary obstruction (Cholestasis) respectively [34, 35]. But, the administration of OEME at doses range from 250 to 2000 mg/kg attenuated the increases in the activities of serum liver biomarkers, and and this has been demonstrated by Janakat, Al- Merie [36] and Janbaz [37] study of hepatoprotective activity of some plants against $CCl_4$ induced hepatic damage in rats. Reduction of AST by premedication of OEME suggests that it has a possible effect on liver mitochondria because 80% of the total AST is released from mitochondria liver tissue. It remains a hypothesis. Even though is based on observations obtained from the results, it needs to be demonstrated by further studies. However, the elevation of ALT and AST with ALT> AST is in favor of a drug-induced hepatitis [38], and these results join our study and conclusions. For using silymarin as reference [39], its use as a pretreatment has significantly reduced the elevation in the serum ALT, AST and ALP activities induced by administration of $CCl_4$, followed by pretreatment with OEME (Table 3).

In our findings, necrosis, portal inflammation and vacuolization were observed in hepatocytes and were significantly lower in groups that were treated with OEME. It is assumed that this effect is related to the high content of total phenols and flavonoids as was demonstrated in the study of Antioxidant properties of methanolic extract of *Origanum vulgare* effect by Cervato [40] and Canberk [41]. Flavonoids are considered as the most active antioxidant phenolic compounds due to their chemical structure [42]. It was reported also that they have a vascular protection effect. Thus, the hemorrhage caused by $CCl_4$ in the liver was minimized by use of plant extract as described by Stoclet [43]. Our results are also supported by Corchete [39] who observed that silymarin, a flavonoid complex, co-treatment with $CCl_4$, totally prevented the changes seen in the positive control as the hepatic necrosis, ballooning degeneration, lymphocytes infiltration and reduced the elevated serum enzymes [44]. Pradhan and Girish [45] reported also the hepatoprotective effect of the silymarin which is used in treatment of hepatic toxic injury and many liver diseases [46]. Many mechanisms are employed in the protective action of silymarin as antioxidant in vivo and in vitro were demonstrated by Singh K [47] and Féher J [48].These results reconfirmed the protective functions of silymarin against the $CCl_4$ induced liver damage [49].

## 5. CONCLUSION

In conclusion, the results of the present study indicate that, under the experimental conditions, the pre-treatment with OEME has a hepatoprotective activity against CCl4 induced hepatic injury in rats, by preventing the changes seen in the histological sections from liver of the tested rats and by reducing the elevated serum enzyme levels diagnosed by biochemical assays. Furthermore, it has proven to be effective as Silymarin, so it can be used as hepatoprotective or therapeutic drug. But other effects as reduced body weight and appetite, need to be clarified in future studies.

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## CONSENT

Not applicable.

## ETHICAL APPROVAL

All authors hereby declare that All manipulations of animals were in agreement with local legislation. Moreover, all procedures used in animal experimentation complied with the French National law, implementing the European Communities Council Directive 86/609/EEC. All efforts were made to minimize the number of animals used and their suffering, and the study met the ethical standards of Chronobiology International.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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