Evaluation of toxicity, nephroprotective and hepatoprotective activities of Argan oil on CCl_4-induced nephrotoxicity and hepatotoxicity in *Wistar* rats

Nour Elhouda Daoudi[1], Samira Mamri[1], Mohamed Marghich[1], Omar Bouziane[1], Mohamed Bouhrim[1], Ennouamane Saalaoui[1], Mohammed Choukri[2], Hassane mekhfi[1], Abdelkhaleq Legssyer[1], Abderrahim Ziyyat[1], and Mohamed Bnouham[1*].

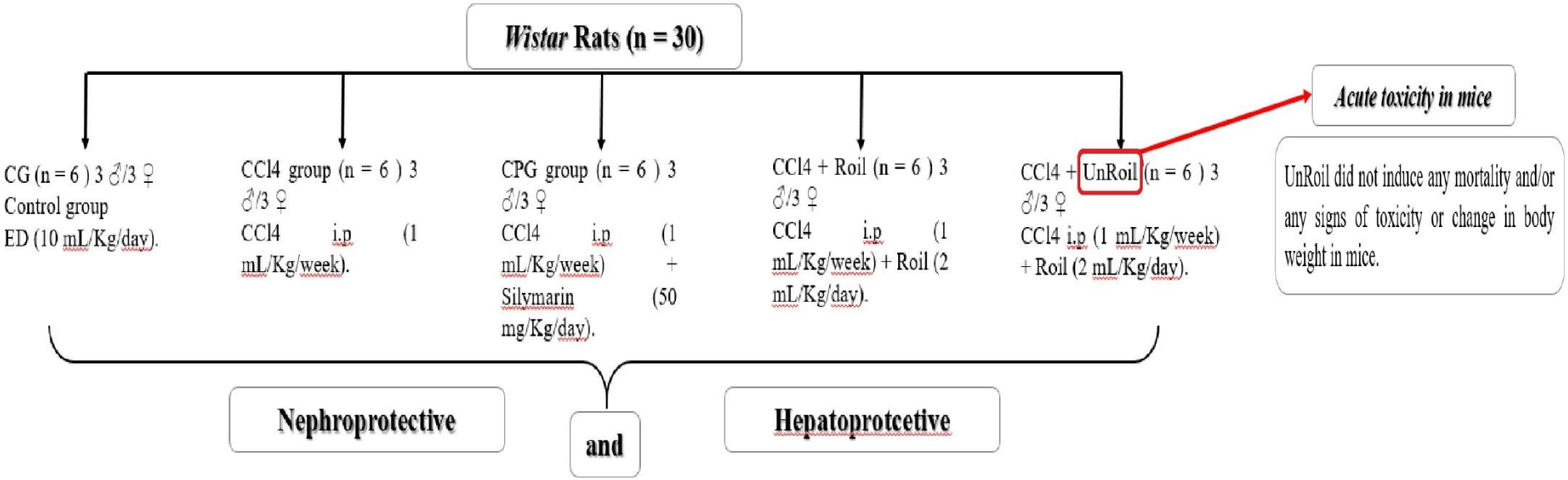
[1]*Laboratory of Bioresources, Biotechnology, Ethnopharmacology and Health, Department of Biology, Faculty of Sciences, University Mohamed First, Boulevard Mohamed VI; BP: 717; 60 000, Oujda, Morocco.*

[2]*Biochemistry Laboratory, Central Laboratory Service - CHU, Mohammed VI, Oujda, Morocco.*

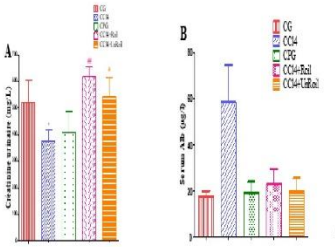
Abstract : In traditional therapy, *Argania spinosa* L. seeds oil used as a nephroprotective and hepatoprotective agent. The present work aims to investigate the acute toxicity of unroasted Argan oil, and studied the nephroprotective and the hepatoprotective activity of both oils Roasted (Roil) and unroasted Argan oil (UnRoil) on CCl_4-induced liver and kidney damages in *Wistar* rats. Animals were divided *into* five equal groups; Control and CCl_4 groups are received only distilled water (10 mL/Kg/day). Control positive group received 50 mg/Kg/day of Silymarin. Roil and UnRoil groups treated with 2 mL/Kg/day of Roil and UnRoil. One week after each pretreatment, the rats are injected intraperitoneally with 1 mL/kg/week of CCl_4. The treatment has lasted for 15 days. The body weight, urinary volume, water, and food intake were measured at the end of the treatment. Then, the animals are sacrificed; the blood and the liver samples were collected for determining the liver weight ratio and biochemical parameters. UnRoil did not show any sign of toxicity up to 5 mL/Kg. In Roil and UnRoil groups the water intake, ALT, AST, total and direct bilirubin, triglycerides, LDL, plasmatic creatinine, urea, uric acid, and MDA levels are reduced significantly as compared with the CCl_4 group. However, body weight,liver weight ratio, food intake, urine urea, urinary creatinine, hepatic glycogen, and GSH levels showed a significant increase compared to the CCl_4 group. Roil and UnRoil showed important nephroprotective and hepatoprotective effects against CCl_4. Although, the roasting process does not influence the ability of Argan seed oils towards these activities.

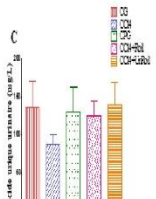
Keywords:*Argania spinosa* seed oil, CCl_4, hepatoprotective, nephroprotective, roasting process.

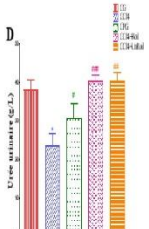
*Corresponding author: Bnouham Mohamed, Tel: +212 6676 27496; E-mail: mbnouham@yahoo.fr



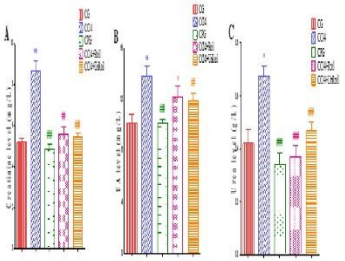
Groups	Water intake (ml/24 h)	Urinary volume (ml/24 h)	Urine pH level
CG	19.16 ± 1.25	6.83 ± 1.56	9.33 ± 0.27
CCl4 group	41.00 ± 4.57 **	10.42 ± 1.1	8.08 ± 0.11 *
CPG	29.16 ± 1.81*	10.83 ± 1.41	9.08 ± 0.39 #
CCl4 + Roil	20.83 ± 2.38 #	8.25 ± 0.44	8.08 ± 0.18 *
CCl4 + UnRoil	20.83 ± 0.56 #	7.5 ± 0.81	8.67 ± 0.23

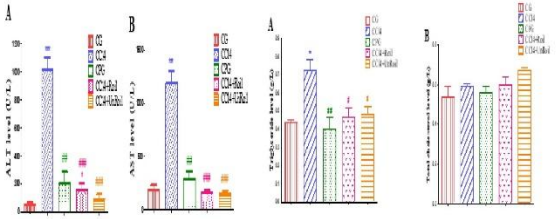


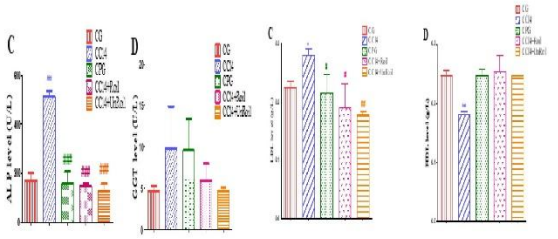


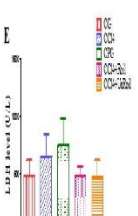


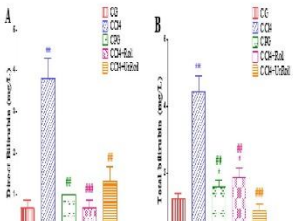
Groups		CG	CCl_4 group	CPG	CCl_4 + Roil	CCl_4 + UnRoil
Na^+(mM)	Plasma	178.83 ± 8.64	175.83 ± 7.01	172.6 ± 6.01	157.17 ± 12.04	157.67 ± 15.96
	Urine	199.17 ± 12.72	126.5 ± 20.75 *	184 ± 24.66	182.83 ± 11.06	203.6 ± 10.83 #
Ca^{2+} (mM)	Plasma	71.58 ± 2.67	89.65 ± 2.64	82.16 ± 3.05	83.5 ± 3.69	82.5 ± 2.76
	Urine	107.83 ± 17.62	138.33 ± 13.11	101.17 ± 14.65	149.2 ± 12.56	181.2 ± 19.20
K^+(mM)	Plasma	4.08 ± 0.23	5.00 ± 0.68	4.17 ± 0.32	3.62 ± 0.39	4.025 ± 0.56
	Urine	177.00 ± 31.81	132.41 ± 21.33	155.96 ± 17.35	187.25 ± 12.96	194.88 ± 14.27
Cl^-(mM)	Plasma	77.67 ± 8.54	81.33 ± 5.44	78.00 ± 4.90	79.5 ± 8.1	66.66 ± 9.07
	Urine	110.83 ± 7	58.83 ± 15.71 *	96.6 ± 8.85 #	101.5 ± 5.31 #	103.2 ± 9.01 #

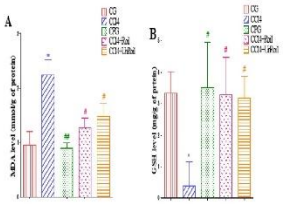


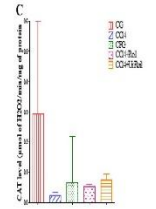


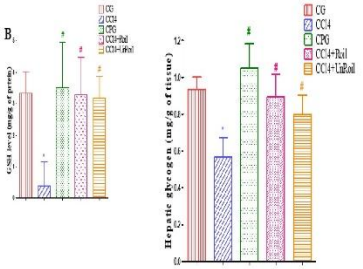












Groups	Liver weight ratio (%)
CG	27.34 ± 0.62
CCl4 group	43.51 ± 1.09 ***
CPG	35.72 ± 1.62 *** #
CCl4 + Roil	38.2 ± 0.9 *** #
CCl4 + UnRoil	37.89 ± 0.92 *** ##

Introduction

In the human body, the liver is considered among the largest organ. It has important physiological functions that are represented by secretory/excretory and metabolic roles (Govind, 2011). Dysfunction of that organ can lead to the appearance of cirrhosis and liver failure. Moreover, it can be damaged by several chemical drugs like acetaminophen, paracetamol, carbon tetrachloride (Kumar et al., 2011; Domenicali et al., 2009) and also by excessive alcohol consumption, autoimmune and infectious problems (Adewusi et Afolayan, 2010). The kidney is a vital organ that plays a key role in the plasma ionic regulation and it is responsible for the removal of nitrogenous metabolic waste products, and detoxification of xenobiotics (Aldahmash et al., 2016). Several factors (chemicals and drugs) and diseases could lead to the appearance of acute kidney tissue injury, which considered a leading cause of death in the world (Safhi, 2018). These nephro-damages could be produced through the generation of free radicals and reactive oxygen species (Ganie et al., 2011).

Aromatic and medicinal herbs play an important role in human health care, since ancient times (Falzon et Balabanova, 2017). Many studies have been demonstrated the effectiveness of some medicinal plants on liver and kidney damages. This effect due to the presence of several compounds that play a key role as antioxidants such as polyphenols, terpenoids, carotenoids, fatty acids, and essential oils (Daoudi et al.,2020b; Daoudi et Bnouham, 2020a). Based on several works, vegetable oils showed effectiveness on kidney, and liver toxicity provoked by nephrotoxic and hepatotoxic agents such as carbon tetrachloride (CCl_4), which defined as the oldest toxins that used widely for experiments induced hepatic and kidney-damages in laboratory animals (Safhi, 2018). It enhanced the production of reactive oxygen species in kidney and liver. In the liver, it causes serious damage in hepatocytes similar to that of the viral infection. When the carbon tetrachloride enters inside the liver, it serves as a xenobiotic. It converts *into* two free radicals: trichloromethyl peroxyl and trichloromethyl causing lipid peroxidation and then serious hepatic diseases.

Argania spinosa L. seeds oil possesses several pharmacological effects such as antidiabetic (Bnouham et al., 2008; Bellahcen et al., 2013; Daoudi et al., 2020c), antioxydant(Drissi et al., 2004), antiobesity(Adlouni et al., 2008), hypocholesterolemia, hypolipemic(H. Berrougui et al., 2003), and antihypertensiveeffects(Berrougui et Alvarez de Sotomayor,2004). It canprevent cancer (Khallouki et al., 2003), and inhibitplateletaggregation(Mekhfi et al.,

2008). Furthermore, it is used in Moroccan traditional therapy as a hepatoprotective agent. It contains a high amount of polyphenols, tocopherol, sterols, and carotenoids, which can play a key role in the treatment of hepatic damages. Generally, there are two types of *Argania spinosa* L. seeds oil: Roasted Argan oil (Roil) or edible oil obtained from the roasting kernels, and cosmetic oil prepared directly from the unroasted kernels (UnRoil). The majority of pharmacological studies that are done on Argan oil studied only the effect of Roil, like that performed by Taghred M. Saber et al, and Er et al.Which demonstrated that Argan oil possesses a nephroprotective effect against sodium fluoride-induced renal damages in male Sprague Dawley rats (Saber et al., 2020) and against acrylamide model that induced liver and kidney toxicity (Er et al., 2020). Moreover, it was shown that Argan oil ameliorated liver mercuric chloride toxicity in *albino Wistar* male rats (Necib et al., 2013). Until now, no available works have studied the protective effect of both Roil and UnRoil against CCl_4-induced both nephro and hepatotoxicities in rats. Therefore, in our investigation, we evaluated the acute toxicity of UnRoil, studied the nephroprotective and the hepatoprotective effects of Roil and UnRoil on CCl_4-induced liver and kidney damages in *Wistar* Rats and verified if the roasting process can affect the hepatoprotective activity of Argan oils.

Materials and Methods

Chemicals and reagents

Carbon tetrachloride (CCl_4), was purchased from Sigma-Aldrich Chemicals (St.Louis, MO, USA). Silymarin from Sigma-Aldrich, Belgium. Standard kits for hepatic, lipidic, urea, creatinine, and electrolyte assay were purchased from Biosystems, Spain. Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Glutathione, 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), and hydrogen peroxide (H2O2) were purchased from Sigma Chemicals, Germany. sulfosalicylic acid purchased from LOBA Chemie, India. All other reagents used in this work were of high quality and analytical grade.

Plant material collection

Argan fruits (*Argania spinosa* L. SKEELS: Sapotaceae) were collected from the Agadir region (southern Morocco) in February 2019. The herb specimen was deposited in the herbarium at the Department of Biology, Mohamed First University (Oujda, Morocco) and identified by a botanist under the reference number HUMPOM431.

Preparation of Argan oil

Argan oil preparation is made in several steps. After the peeling steps of Argan fruits, the seeds are subjected to a roasting process for preparing Roil or they are used directly for preparing UnRoil. Then, the seeds are used for preparing the oils by using a cold-press machine.

The yield of Argan oils was respectively 28.49% for Roil and 12.50% for UnRoil.

Animals

Swiss albino mice and *Wistar* rats of each sex used in this work were taken from the animal's house of the Biology Department of the Faculty of Sciences, Mohammed First University, Oujda (Morocco). The animals were maintained in plastic cages with accessibility *ad libitum* to food and water and in a well-ventilated room. They were kept under standard laboratory conditions (12 h/12h light/dark cycle and with a temperature of 22 to 26 °C). Rats were cared for in compliance with the internationally accepted Guide for the care and use of laboratory animals, published by the US National Institutes of Health (National Institutes of Health 1985).

Acute toxicity study of UnRoil

Acute toxicity study of UnRoil was performed on *Swiss albino* mice, according to the guidelines for testing chemicals, 2008 of the Organization for Economic Cooperation and Development (OECD 2008). This study was performed; using two administration routes: oral (p.o) and intraperitoneal (i.p). For that, two lots of mice were divided *into* 4 groups in each administration route: Control group (CG) received 10 mL/Kg of distilled water p.o or i.p. UnRoil groups received respectively 1, 2, and 5 mL/Kg p.o or i.p. After a single dose administration of UnRoil, the animals were observed for 15 days in the aim to assess any behavioral changes, toxicity signs and/or deaths. Moreover, the body weights of the mice were measured after 0, 1, 7, and 15 days of the administration (Mukinda et Syce, 2007).

Experimental design

The experimental study was conducted in the use of the CCl_4 model as a hepatotoxic and nephrotoxic agent. After 1 week of adaptation, the animals were randomly divided *into* five groups, each containing six rats (3 ♂/3 ♀: The Control group (CG) and CCl_4 group received 10 ml/Kg of distilled water. The control positive group (CPG) received 50 mg/Kg of

silymarin. Roasted Argan oil group (Roil) received 2 ml/Kg of Roil. Unroasted Argan oil group (UnRoil) received 2 ml/Kg of UnRoil. After 1 week of each pretreatment, the animals are injected intraperitoneally (i.p.) with CCl_4 at a dose of 1 mL/kg/day once a week for 15 days (CCl_4 was solubilized in mineral oil v/v). The body weights of the rats were measured after 0, 7, 9, 11and 15 days of the treatment. All animals were treated and observed daily for two weeks. Besides, at the end of the treatment the urinary volume, water intake, and food intake were measured for all animals, by using metabolic cages (Bouhrim et al., 2018).

Blood sampling

Twelve hours after the last dose of intraperitoneal injection of CCl_4, all animals were anaesthetized under a light ethyl ether and sacrificed. The blood samples were collected from the carotid arteries and were put *into* dry blood collection tubes. Then, the blood was centrifuged at 4 ℃ for 10 min and at 3000 rpm to separate the plasma. Thereafter, the plasma was kept at -20 ℃ until biochemical analysis.

Liver/Kidney sampling

The livers and kidneys of all the sacrificed groups were weighed and conserved for preparing the liver homogenate (20 % w/v) in phosphate buffer (0.1 mM, pH = 7.4) and kept frozen at −20°C until the evaluation of the level of catalase (CAT), malondialdehyde (MDA) and total glutathione contents.

The effect of Roil and UnRoil intake on the variation of the liver and kidneys weight ratio were estimated according to the following formula:

$$\text{Liver weight ratio (\%)} = \left(\frac{\text{Fresh organ weight}}{\text{Scrifice body weight}}\right) \text{X } 100$$

Biochemical assays

Hepatic, lipidic and renal markers were measured, using ARCHITECT ci4100 Analyzer: ALT, AST, ALP, LDH, GGT, triglyceride, total cholesterol, LDL, HDL, creatinine, urea, uric acid, albumin, total bilirubin, direct bilirubin, electrolyte levels.

Determination of hepatic glycogen

Hepatic glycogen was determined according to the protocol described by (Ong et Khoo, 2000). To prepare the liver homogenate, 2 mL of KOH solution (30%) was added to an amount of 0.3-0.5 g of the liver. The tissue was boiled at 100 °C for 30 min. Then, 4 mL of ethanol (95 %) was added to the mixture, to precipitate the glycogen. Therefore, 1 mL of distilled water was added to solubilize the purified glycogen. The content of hepatic glycogen was determined calorimetrically by adding 5 mL of anthrone reagent. The absorbance was measured at 625 nm and the results are expressed in mg/Kg of tissue.

Determination of malondialdehyde

Lipid peroxidation in the liver was evaluated using the TBA reaction method. After the preparation of the homogenate, 1 ml of the homogenate supernatant was added in 1 ml of TBA reactive constitute with 0.67 % w/v of TBA, and 15% of trichloroacetic acid dissolved in 0.25 N of chloridric acid. Then, the reaction mixture was placed in a boiling water bath for 30 min and centrifuged at 4750 rpm for 5 min. The absorbance was measured at 535 nm and the calculation of MDA amounts was made using the extinction coefficient of 1.56×10^5 M-1.cm-1. The results are expressed in nanomoles of MDA per milligrams of the liver tissue (nmol/mg) (Draper et Hadley, 1990).

Determination of catalase

The determination of the catalase activity was carried out according to the technique described by (Aebi, 1984). In spectrophotometry assay, the reaction mixture contains 20 µL of the homogenate supernatant (1 to 1.5 mg of protein per 1 mL of supernatant), 780 µL of phosphate buffer (100 mM, pH = 7.5), and 200 µL of H_2O_2. The reaction was initiated by the addition of H_2O_2 in the mixture reaction. Then, monitoring the degradation of H_2O_2 to O_2 at 240 nm after 15 s and 60 s of this addition. The catalase activity is determined according to the below formula, and the results are expressed in µmol of H_2O_2 per minute and per mg of protein.

$$\mathbf{IU/g} = \left(\frac{\mathbf{2.3033}}{\mathbf{\Delta T}}\right) \mathbf{x} \left(\frac{\mathbf{logA1}}{\mathbf{A2}}\right)$$

A1: Absorbance after 15 s

A2: Absorbance after 60 s

T: Time intervals per minute.

Determination of hepatic glutathione

The determination of hepatic glutathione was performed by applying the protocol described by (Ellman, 1959) with some modifications. The reagent mixture contains 500 µL of hepatic homogenate, 500 µL of sulfosalicylic acid (4 %) and it was centrifuged at 3500 g for 10 min. Then, 1 mL of 0.1 M phosphate buffer (pH = 7.4), and 400 µL of DTNB (10 mM) were added to 200 µL of the obtained homogenate supernatant. The absorbance was measured at 412 nm and the determination of hepatic glutathione was done by the preparation of different concentrations of GSH (0 - 1000 µg /ml) that used to draw the calibration curve.

Statistical analysis

The results were expressed as the mean ± Standard Error of Measurement (SEM) and were subjected to statistical analyses using ANOVA (GraphPad Prism 5 Software). $P< 0.05$ was considered statistically significant.

Results

Acute toxicity of UnRoil

The results of the acute toxicity of UnRoil in mice showed that doses of 1, 2, 5 mL/Kg administered orally (p.o) or injected (i.p) in mice, did not induce any mortality and did not reveal any behavioral disturbances or signs of toxicity in all treated groups during the 15 days of observation. In addition, UnRoil showed a healthy and normal appearance on the general behavior of the animal and on their body weight (Figure 1).

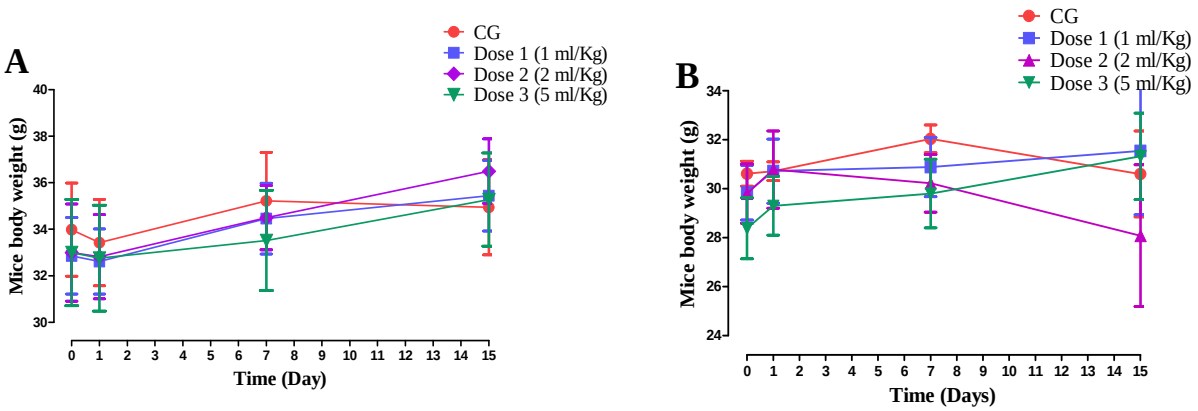


Figure 1: Effect of oral (A) and intraperitoneal (B) administration of 1, 2, and 5 mL/Kg of UnRoil on body weight in Albino mice (n = 5). CG: Control group.

Effect of oral administration of Roil and UnRoil on body weight

Table 1 shows the effect ofthe intraperitoneal injection of CCl_4 at a dose of 1 mL/Kg/week and oral administration of 2 ml /Kg/day of Roil and UnRoil on body weightsvariations. The results showed that after the injection of CCl_4there is no significant changes in body weight gain of all groups, as compared to the control group ($p > 0.05$). After two days of CCl_4 injection, it was marked that the body weight continue to increase in CG. However, it reduced significantly in CCl_4 group ($p < 0.05$). Besides, the pretreatment with Roil and UnRoil improves significantly this decrease ($p < 0.05$ for the both Argan oils as compared with CCl_4group).

Table 1: Effect of oral administration of 2 mL/Kg/day of Roil/UnRoil on body weights in CCl_4-intoxicated *Wistar* rats.

Groups	Average body weight (g)					Body weight gain (g)		% body weight change	
	Day 0	Day 7	Day 9	Day 11	Day 15	Day 7	Day 15	Day 7	Day 15
CG	186.83 ± 8.75	202.33 ± 8.07	204.83 ± 5.52	209.2 ± 5.29	211.16 ± 6.87	15.50 ± 14.01	24.83 ±3.94	10.64 ± 8.12	13.62 ± 2.70
CCl4 group	173.00 ± 7.62	189.17 ± 6.06	180.00 ± 6.12 *	183.33 ± 6.17	185.66 ± 7.85	16.16 ± 9.55	12.66 ± 1.44*	9.82 ± 2.49	7.38 ± 0.36
CPG	185.33 ± 12.79	200.67 ± 9.00	192.83 ± 10.83	199.66 ± 11.22	203.33 ± 13.06	15.33 ± 1.67	18.00 ± 1.22#	9.19 ± 1.71	10.14 ± 0.97
CCl4 + Roil	179.66 ± 7.39	195.66 ± 7.76	190.33 ± 8.98	193.97 ± 8.86	210.83 ± 9.57	16.00 ± 9.79	31.16 ± 6.99#	10.42 ± 5.91	17.78 ± 4.06
CCl4 + UnRoil	170.25 ± 8.67	185.83 ± 9.18	181.66 ± 6.8	182.33 ± 7.07	190.35 ± 8.56	15.58 ± 3.16	20.1 ± 2.82#	9.78 ± 2.35	12.38 ± 2.03

CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl_4: Carbon tetrachloride.

Effect of Roil and UnRoil on the variation of liver and kidneys weight ratio

Table 2 showed the liver and kidneys weights ratio of the entire tested groups. In CCl_4 group, we marked that CCl_4 increased the liver weight ratio ($p < 0.001$). However, the treatment with 2 mL/Kg/day of Roil and UnRoil improve significantly this parameter ($p < 0.05$ for Roil and $p < 0.01$ for UnRoil as compared with CCl_4 group). Besides, it was observed that CCl_4 did not provoke any significant changes in the growth of the left and the right kidneys as compared with CG.

As compared with the CPG, it was found that Roil and UnRoil have the same effect of that of silymarin.

Table 2: Effect of oral administration of 2 mL/Kg/day of Roil/UnRoil on the liver and kidneys weights ratio in CCl_4-intoxicated Wistar rats.

Groups	Liver weight ratio (%)	Kidney weight ratio (%)	
		LeftKidney	Right Kidney
CG	2.734 ± 0.062	0.378 ± 0.026	0.360 ± 0.014
CCl_4 group	4.351 ± 0.109 ***	0.376 ± 0.021	0.362 ± 0.0093
CPG	35.72 ± 1.62 *** #	3.47 ± 0.09	3.59 ± 0.08

CCl_4 + Roil	38.2 ± 0.9 *** #	3.38 ± 0.11	3.49 ± 0.07
CCl_4 + UnRoil	37.89 ± 0.92 *** ##	3.45 ± 0.11	3.49 ± 0.07

CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl_4: Carbon tetrachloride.

Effect of oral administration of Roil and UnRoil on water intake, food intake, and urinary volume in CCl4-intoxicated rats

Table 3 shows that at the end of treatment, water intake increased significantly in the CCl_4 group ($p < 0.01$). While the treatment with Roil and UnRoil at 2 mL/Kg/Day showed a significant decrease in water intake compared to the CCl_4 group ($p < 0.05$). Food intake at the end of treatment was reduced significantly in the CCl_4 group ($p < 0.01$). However, it increased in the groups treated with Roil and UnRoil for 15 days ($p < 0.01$ for Roil and $p < 0.05$ for UnRoil) as compared to the CCl_4 group and the control group. Furthermore, no significant change was observed regarding urine volume in the CCl_4 group and the other groups. Although, the urine pH of the CCl_4 group showed a significant change as compared to the control group ($p < 0.05$). While Roil and UnRoil groups showed no significant change as compared to the CCl_4 group.

Table 3: Effect of oral administration of 2ml/Kg/day of Roil and UnRoil on water intake, food intake, urinary volume, and urine pH level in CCl_4-intoxicated *Wistar* rats.

Groups	Water intake (ml/24 h)	Urinary volume (ml/24 h)	Urine pH level
CG	19.16 ± 1.25	6.83 ± 1.56	9.33 ± 0.27
CCl_4 group	41.00 ± 4.57 **	10.42 ± 1.1	8.08 ± 0.11 *
CPG	29.16 ± 1.81*	10.83 ± 1.41	9.08 ± 0.39 #
CCl_4 + Roil	20.83 ± 2.38 #	8.25 ± 0.44	8.08 ± 0.18 *
CCl_4 + UnRoil	20.83 ± 0.56 #	7.5 ± 0.81	8.67 ± 0.23

CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl_4: Carbon tetrachloride.

Effect of Roil and UnRoil oral administration on hepatic markers

The measurement of the plasma levels of ALT, AST, ALP, GGT and LDH are presented in figure 2. At the end of treatment, it is noted that the group injected with 1 mL/Kg/Week of CCl_4 induced a very significant increase of ALT ($p < 0.001$), AST ($p < 0.001$) and ALP ($P < 0.001$) as compared to CG. However, treatment with Roil and UnRoil at a dose of 2 mL/Kg/day attenuated significantly these parameters compared with the CCl_4 group. In addition, the results showed that the ALT, AST, and ALP levels in the groups

treated with Roil and UnRoil were statistically similar to the values in the CPG. For the levels of GGT and LDH, we notice that there is no significant change in the CCl_4 group compared to the other groups.

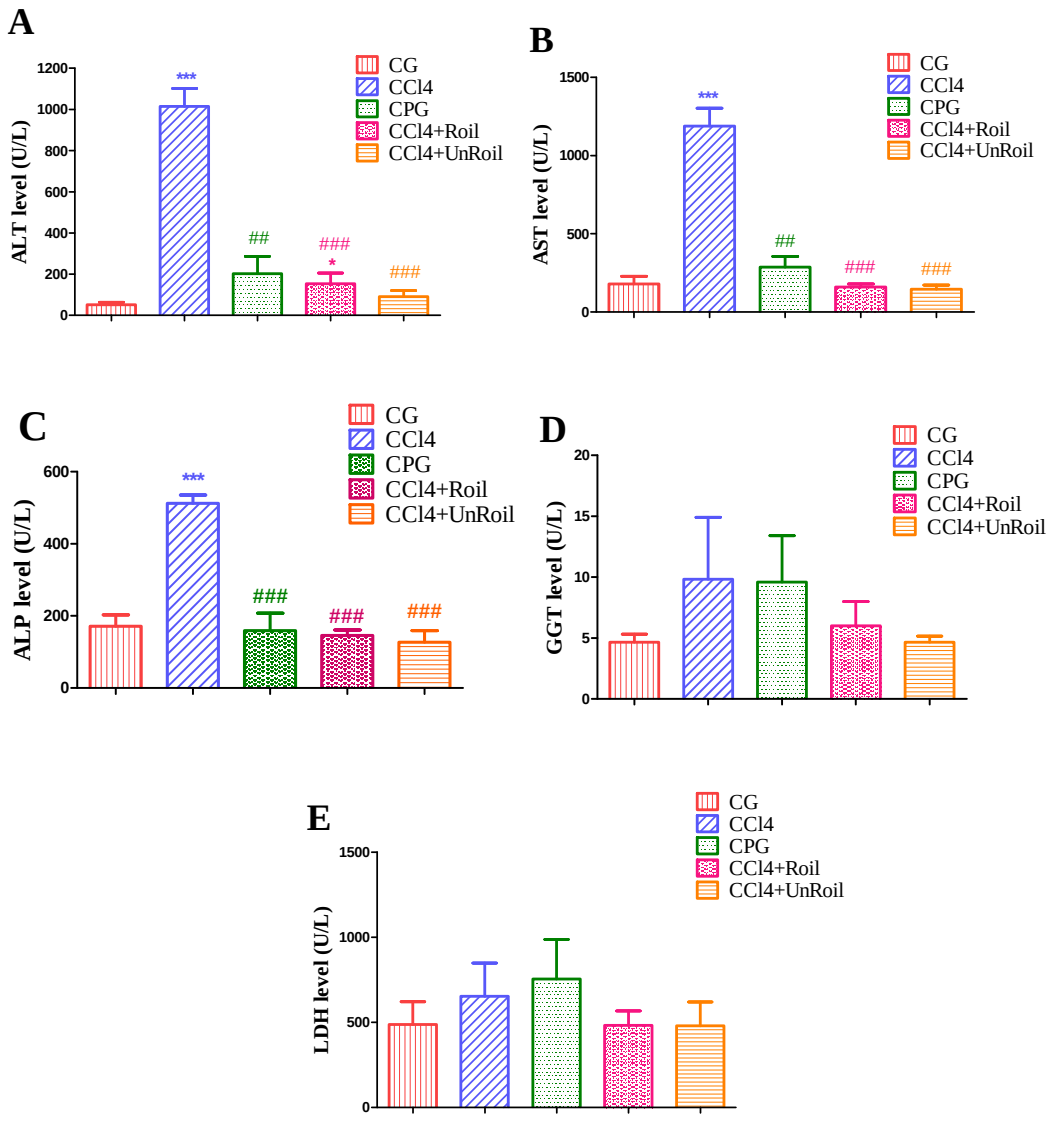


Figure 2: Effect of Roil and UnRoil on hepatic markers, ALT (A), AST (B), ALP (C), GGT (D), and LDH (E) in CCl_4-intoxicated rats (n = 6). Values are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.01 compared with CG. #P < 0.05, ##P < 0.01, ###P< 0.01 compared with the CCl_4 group. CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl_4: Carbon tetrachloride.

Effect of Roil and UnRoil oral administration on direct and total bilirubin

Figure 3 shows the effect of Roil and UnRoil administration on direct and total bilirubin levels in all the tested groups. At the end of treatment, it is noted that the level of direct and total bilirubin in the CCl_4 group increased significantly ($p < 0.001$) compared to the normal group. Therefore, a dysfunction in the excretory function of the hepatocytes is possible in this case. Treatment of the rats with Roil and UnRoil at 2 mL/Kg for 2 weeks significantly attenuated the level of direct and total bilirubin in comparison with the CCl_4 group.

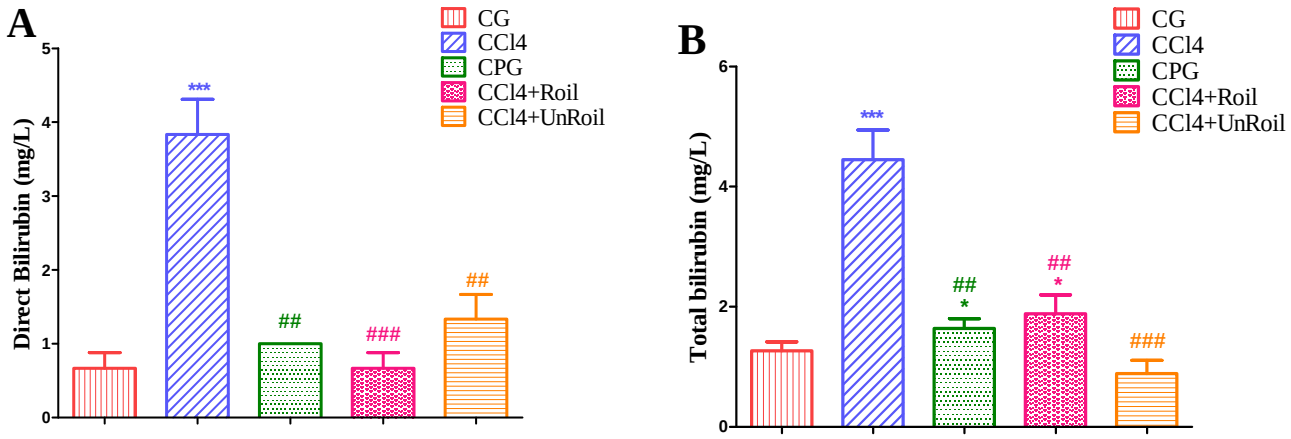


Figure 3: Effect of Roil and UnRoil on plasma direct bilirubin (A) and total bilirubin (B) in CCl_4-intoxicated rats (n = 6). Values are presented as mean ± SEM. ***P < 0.01 compared with CG. ##P < 0.01, ###P< 0.01 compared with the CCl_4 group. CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl_4: Carbon tetrachloride.

Effect of Roil and UnRoil oral administration on lipidic profile

In this test, we evaluated the effect of Roil and UnRoil on the metabolic function of the liver. For this, we determined the levels of triglycerides, total cholesterol, LDL and HDL for each group (Figure 4). At the end of 2 weeks of treatment, we notice that in the CCl_4 group, there is a significant increase in the triglycerides ($p < 0.001$) and LDL ($p < 0.05$) levels, and a very significant decrease in the level of HDL ($p < 0.001$) compared to CG. While no change was observed in total cholesterol levels in the tested groups. Treatment with 2 mL/Kg/Day of Roil and UnRoil in CCl_4-intoxicated rats improved lipid parameters compared to the CCl_4 group and these results appear statistically similar to that of silymarin at a dose of 50 mg/Kg/Day.

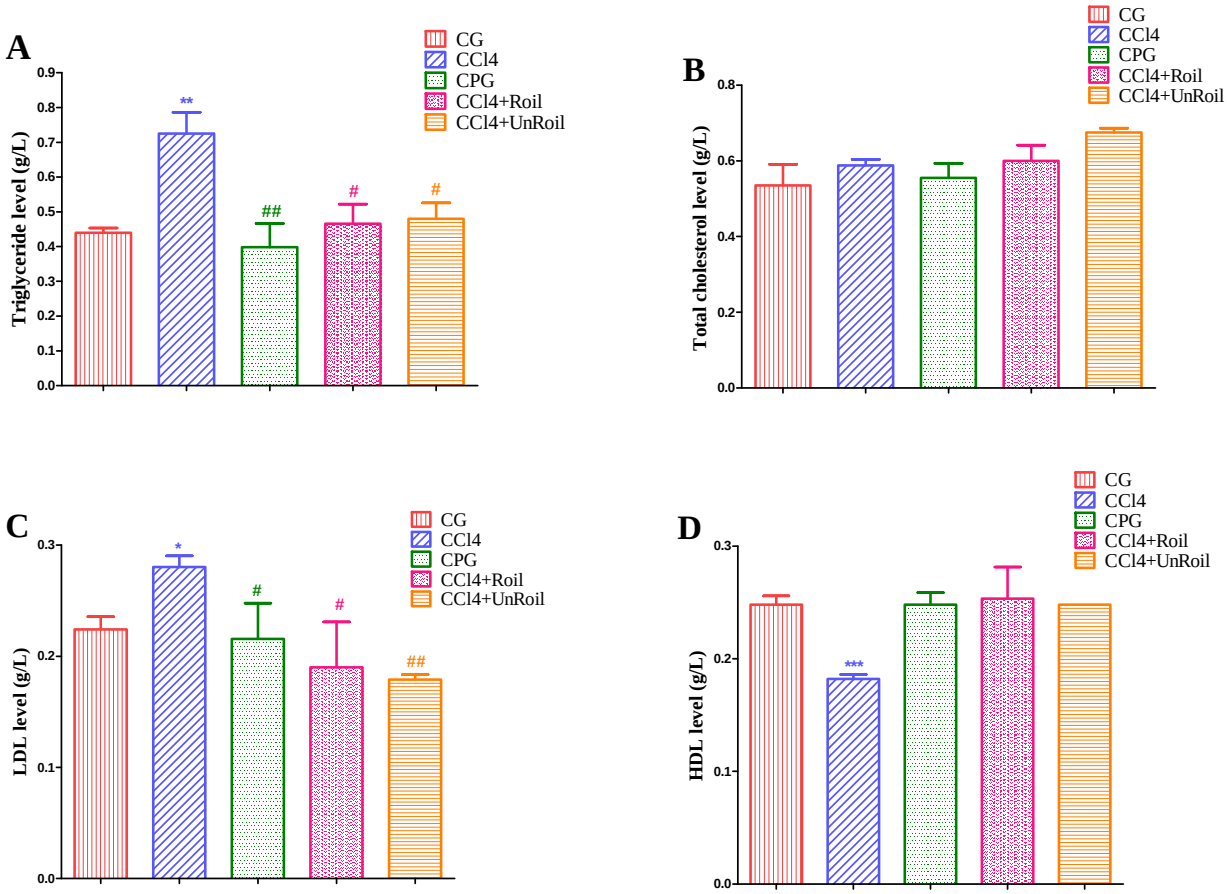


Figure 4: Effect of Roil and UnRoil on lipid profile: Triglycerides (A), Total cholesterol (B), LDL (C), HDL (D) in CCl_4-intoxicated rats (n = 6). Values are presented as mean ± SEM. *P < 0.05, ***P < 0.01 compared with CG. #P < 0.05, ##P < 0.01compared with the CCl_4 group. CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl_4: Carbon tetrachloride.

Effect of Roil and UnRoil oral administration on urinary parameters

The determination of the urine parameters in the CG group showed the presence of a creatinine level of 522.59 ± 54.82 mg / L, 17.66 ± 1.63 µg / L of albuminuria, 135, 58 ± 24.56 mg / L of uric acid and 37.97 ± 2.08 g / L of urea. The intraperitoneal injection of 1 mL/Kg/week of CCl_4 showed a significant decrease in urinary creatinine ($p < 0.05$) and urea ($p < 0.05$) and a significant increase in the level of albuminuria ($p < 0.05$) compared to CG. Treatment with 2 mL/Kg/ Day of Roil or UnRoil for 15 days improved significantly the level of these parameters in comparison with the CCl_4 group (Figure 5) and these values are statistically similar to those of the group treated with silymarin. Moreover, it was found that

there is no significant difference of the tested urinary parameters between Roil and UnRoil ($p > 0.05$).

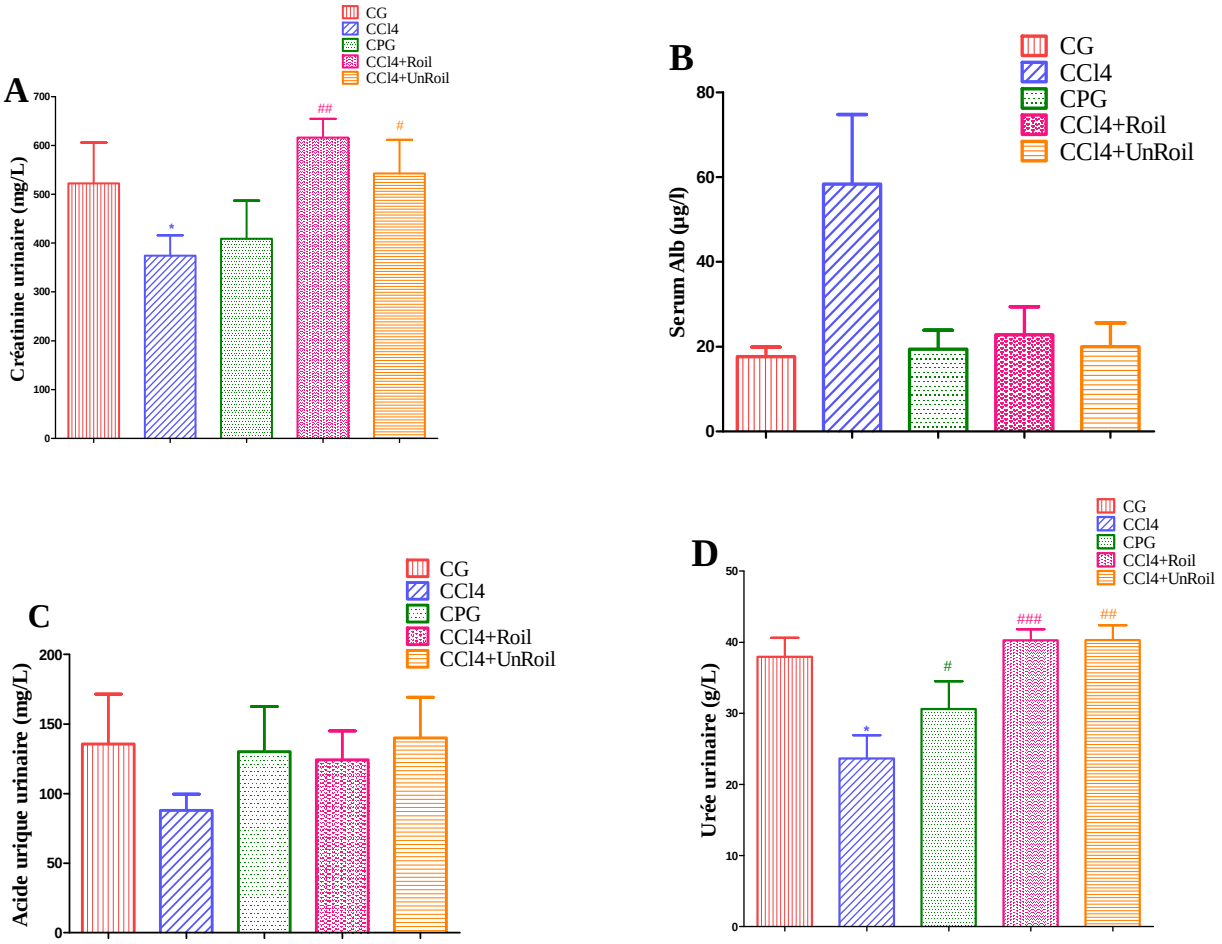


Figure 5: Effect of Roil and UnRoil on creatinine (A), Albumine, uric acid, and urea (D) in urine of CCl4-intoxicated rats (n = 6). Data are presented as mean ± SEM. *P < 0.05 compared with CG. #P < 0.05, ##P < 0.01, and ###P < 0.001 compared with the CCl4 group. CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl4: Carbon tetrachloride.

Effect of Roil and UnRoil oral administration on plasmatic renal markers

The dosage of creatinine, uric acid and urea levels are shown in figure 6. In the CCl_4 group, it is noted that there is a significant increase in creatinine levels ($p < 0.001$), uric acid ($p < 0.001$) and urea ($p < 0.05$) compared to CG. While, the treatment with 2 mL/Kg/Day of Roil or UnRoil for 15 days in CCl_4-intoxicated rats improved significantly the level of these parameters in comparison with the CCl_4 group and these values are statistically similar to

these from the group treated with silymarin. Besides, the comparison between the both Argan oils, showed that Roil decreased significantly the level of urea ($p < 0.01$) than UnRoil.

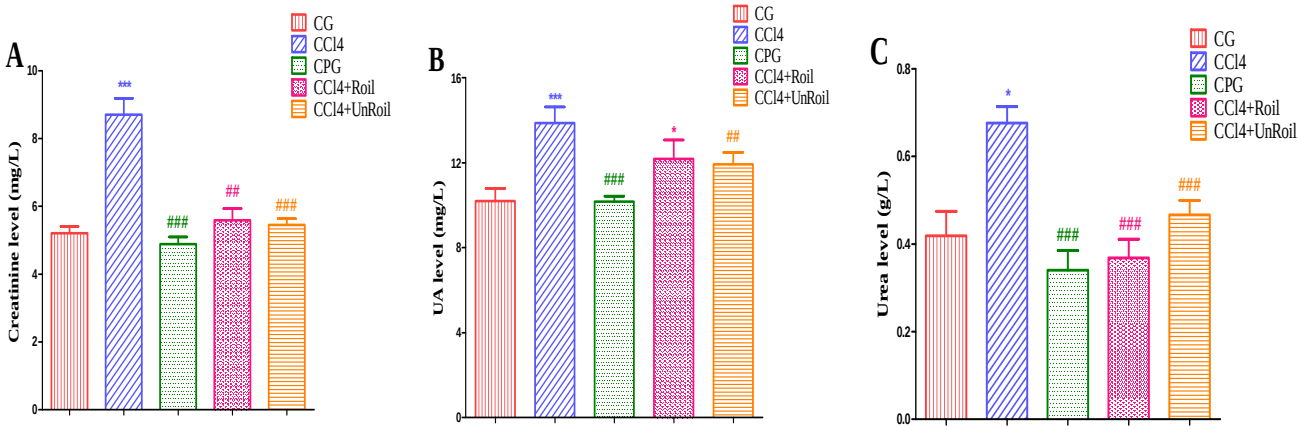


Figure 6 : Effect of Roil and UnRoil oral administration on plasma creatinine (A), Uric acid (B), and Urea (C) in CCl4-intoxicated rats (n = 6). Data are presented as mean ± SEM. *P < 0.05, ***P < 0.001 compared with CG. ##P < 0.01, and ###P < 0.001 compared with the CCl4 group. CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl4: Carbon tetrachloride.

Effect of Roil and UnRoil administration on the level of electrolytes.

The effect of CCl_4 on *Wistar* rats treated with 2 mL/Kg/Day of Argan oils and 50 mg/Kg/Day of Silymarin showed no significant change in serum Na^+, Ca^{2+}, K^+, and Cl^- levels in comparison with CG and the CCl_4 group. While the effect of Roil and UnRoil on the level of electrolytes in the urine is present also in table 4. The results show that the injection of CCl_4 induced a significant decrease in the level of Na ($P < 0.05$), and Cl ($p < 0.05$) compared to the control group. Treatment with Roil and UnRoil prevented the decrease in urinary Cl levels. However, the Na level was improved by the presence of UnRoil.

Table 4: Effect of Roil and UnRoil on electrolytes levels in rats with or without CCl4 administration.

Groups		CG	CCl_4 group	CPG	CCl_4 + Roil	CCl_4 + UnRoil
Na^+ (mM)	Plasma	178.83 ± 8.64	175.83 ± 7.01	172.6 ± 6.01	157.17 ± 12.04	137.67 ± 16.96
	Urine	199.17 ± 12.72	126.5 ± 20.75 *	184 ± 24.66	182.83 ± 11.09	203.6 ± 10.81 #
Ca^{2+} (mM)	Plasma	78.58 ± 2.67	89.65 ± 2.64	82.16 ± 3.09	85.5 ± 3.69	82.5 ± 2.76
	Urine	107.83± 17.62	138.33 ± 13.11	101.17 ± 14.65	149.2 ± 12.36	181.2 ± 19.20
K^+ (mM)	Plasma	4.08 ± 0.23	5.00 ± 0.68	4.17 ± 0.32	3.62 ± 0.39	4.025 ± 0.36
	Urine	177.00 ± 10.81	132.41 ± 21.33	155.96 ± 17.39	187.28 ± 12.98	194.88 ± 14.27

Cl^- (mM)	Plasma	77.67 ± 8.84	83.33 ± 5.44	78.00 ± 4.90	79.5 ± 8.1	66.66 ± 9.07
	Urine	110.83 ± 7	58.83 ± 15.71 *	96.6 ± 8.85 #	101.5 ± 5.31 #	103.2 ± 9.01 #

CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl4: Carbon tetrachloride.

Effect of Roil and UnRoil oral administration on hepatic glycogen

Figure 7 shows the effect of Roil and UnRoil on hepatic glycogen in CCl_4-intoxicated rats. In CG, the hepatic glycogen level was 0.93 ± 0.04 mg/g of tissue. While in the CCl_4 group the quantity of hepatic glycogen decreased significantly ($p < 0.05$) compared to CG with a value of 0.57 ± 0.06 mg/g of tissue. Daily treatment with Roil and UnRoil in rats injected with CCl_4 for 2 weeks, attenuated significantly the decrease of this parameter ($p < 0.05$ for Roil and $p < 0.05$ for UnRoil) as compared to the CCl_4 group. Besides, no significant difference of hepatic glycogen level was observed between Roil and UnRoil.

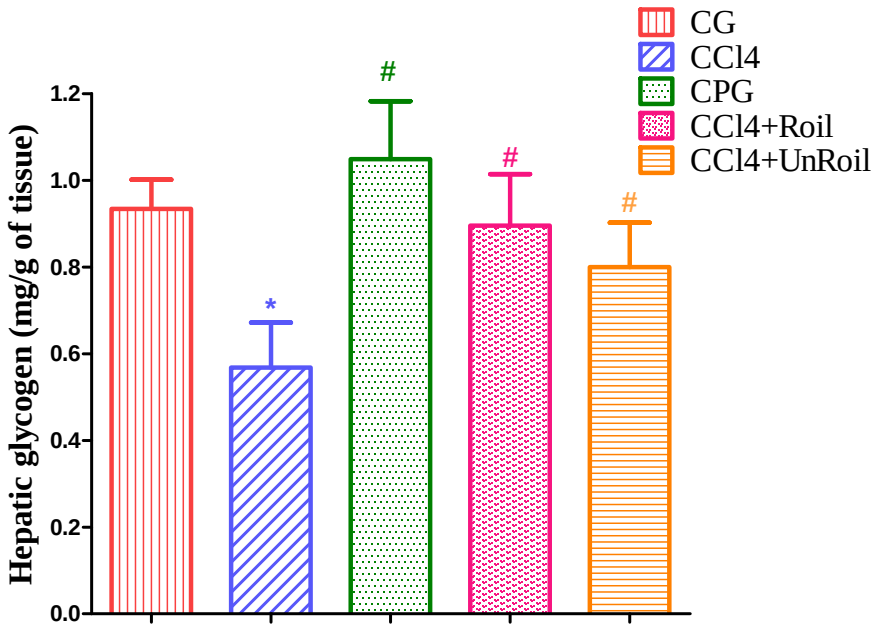


Figure 7: Effect of Roil and UnRoil oral administration on hepatic glycogen in CCl4-intoxicated rats (n = 6). Data are presented as mean ± SEM. *P < 0.05 compared with CG. #P < 0.05 compared with the CCl4 group. CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl4: Carbon tetrachloride.

Effect of Roil and UnRoil oral administration on MDA, GSH and CAT levels

Figure 8 shows the effect of Argan oils pretreatment (Roil and UnRoil) on lipid peroxidation (A), GSH (B), and CAT (C) in rats injected with CCl_4. The results show that the

intraperitoneal injection of 1 mL/Kg/week of CCl_4 caused a significant increase in the level of MDA ($P < 0.01$) and a significant decrease in the level of GSH ($P < 0.05$) in comparison with CG. While pre-treatment with Roil and UnRoil in rats injected with CCl_4 significantly improved the levels of MDA and GSH. Although, no significant change was observed in CAT levels in all treated groups in comparison with CG ($p > 0.05$). Furthermore, the comparison between Roil and UnRoil showed that there is no significant changes of MDA and GSH levels of the both oils ($p>0.05$).

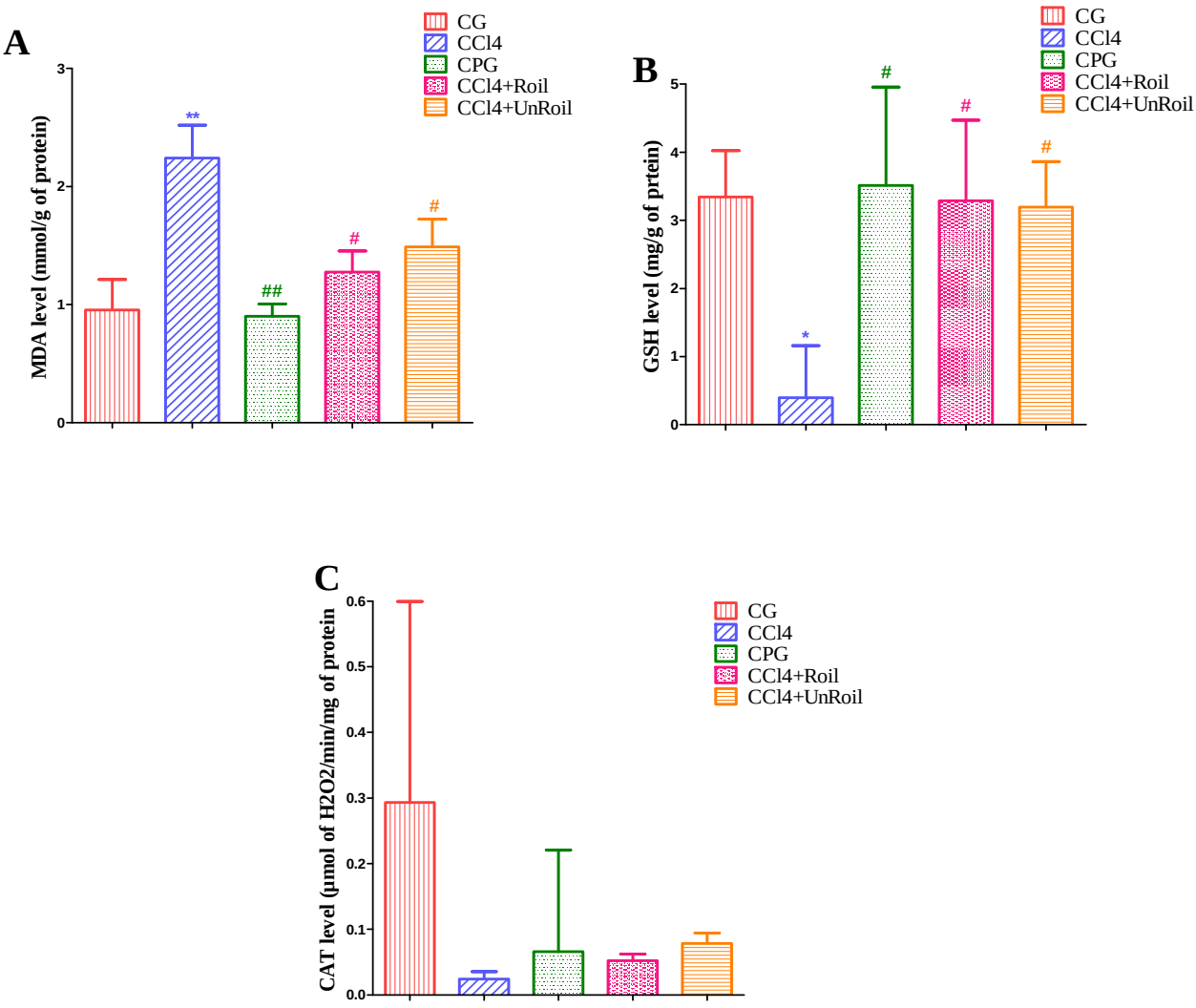


Figure 8: Effect of Roil and UnRoil oral administration on MDA (A), GSH (B), and CAT (C) levels in CCl4-intoxicated rats (n = 6). Data are presented as mean ± SEM. *P < 0.05, **P < 0.01 compared with CG. #P < 0.05, ## < 0.01compared with the CCl4 group. CG: Control group, CPG: Control positive group, Roil: Roasted Argan Oil, UnRoil: Unroasted Argan Oil, CCl4: Carbon tetrachloride.

Discussion

Liver and kidney diseases could be caused by a viral infection and also by hepatotoxic agents as CCl_4-related to serious damages. CCl_4 is the most agent that is used commonly as a model of liver and kidney injury in animals associated with oxidative stress and free radicals, and to study the hepatoprotective and nephroprotective effect of products (Janbaz el al., 2002). In the liver, this agent causes direct tissue alteration and initiate hepatic inflammation, which considered as an early step of fibrosis (Hafez et al., 2014). In the kidney, CCl_4 possesses a higher affinity towards this organ. Therefore, it distributed with a higher concentration in the kidney than in the liver, because it contains predominantly cytochrome P450 in its cortex (Sanzgiri et Bruckner, 1997). The action mechanism of CCl_4 is progressed by the cytochrome P450 oxygenase system. Besides, cytochrome P450 enhances the bioactivation of CCl_4 and the production of free radicals (trichloromethyl peroxyl and trichloromethyl) that play a key role in the increase of protein denaturation, and lipid peroxidation by binding *into* the intracellular proteins and lipids. Then, the development of hepatic and kidney diseases (Boll et al., 2001).

The antioxidants present in many medicinal plants possess important nephroprotective and hepatoprotective activities that could scavenge the free radicals resulting from the different toxins. *Argania spinosa* L. seed oil is one of the important natural products in Morocco that used in traditional therapy as a hepatoprotective and nephroprotective agent. It contains a high amount of antioxidants such as catechin, caffeic acid, ferulic acid, vanillic acid, and resorcinol that may play a role in the attenuation of chronic diseases (Bakour et al., 2018). Although, to confirm this hypothesis it is necessary to carried out experimental tests using nephrotoxic and hepatotoxic agents such as CCl_4. For that, we have tested in this work, andfor the first time, the nephroand the hepatoprotective effect of cosmetic Argan oil (UnRoil) on CCl_4 induced kidney and liver damages in *Wistar* rats. Furthermore, comparing its effect with that obtained of edible oil (Roil) in the aim to determine if the roasting process influences the activity of the molecules responsible for these effects.

In our work, we found that the administration of Roil and UnRoil possess hepatoprotective and nephroprotective activities on CCl_4-induced kidney and liver toxicity in the *Wistar* rat model. Both oils can protect the organs towards the damages produced by CCl_4, by decreasing the values of ALT, AST, ALP, total/direct bilirubin, plasmatic creatinine, urea and uric acid levels, and increasing urinary creatinine, and urine urea levels. Although, the administration

of CCl_4 to the animals, caused the increase of serum creatinine, urea, and uric acid levels and decrease urinary creatinine, urine urea and urine sodium levels. Besides, it has been declared that the plasma levels of ALT, AST, and ALP are the most important markers of liver injury(Anand et al., 1992). The increase level of AST, ALT, and ALP serum enzymes, have been attributed to the liver damage because they are cytoplasmic origin and are released *into* the blood after hepatic damage(Sharma et Shukla, 2011).However, creatinine and urea plasmatic levels are reliable indicators of kidney function. An increase in creatinine levels means the presence of a kidney disease, which explains the nephrotoxicity effect of CCl_4. Creatinine concentration is a more powerful indicator than the urea concentration in the early stages of kidney disease (Feyzi et al., 2020,Hasanvand et al., 2019,Gilbert et al., 1989). Moreover, in the current study, we investigated the effect of Roil and UnRoil on body weight gain, liver weight ratio, metabolic function (triglycerides, total cholesterol, HDL-c, LDL-c and hepatic glycogen) and excretory function (total and direct bilirubin) of the liver. In fact, the intraperitoneal administration of CCl_4 provoked a significant rise of direct bilirubin, total bilirubin, triglycerides, LDL-c, and liver weight ratio. In contrast, CCl_4 produced a significant reduction in hepatic glycogen, HDL-c and body weight gain as compared to the control group. In addition, CCl_4 did not affect the total cholesterol, urinary uric acid, Ca^{2+} and K^{+} levels.The elevation of total and direct bilirubin levels are associated with the appearance of hepatocytes problems, obstruction to biliary excretion *into*duodenum, hemolysis, in defects of hepatic uptake and conjugation of bilirubin pigment such as in Gilbert's disease(Bigoniya et Rana 2010).Likewise, the cytoplasmic accumulation of triglycerides and LDL-c in the hepatocytes leads to hepatic steatosis(Al-Mehdar et al., 2015).While, the liver weight ratio rise, possibly due to the accumulation of lipids and collagen in the liver cells(Villanueva-Toledo et al., 2020). Hepatic glycogenis a form of glucose storage in the liver. It reflects the stimulation of glycogen synthase and the inhibition of glycogen phosphorylase resulting from insulin action(Vats et al., 2004).The reduction of hepatic glycogen in CCl_4 group could be due to the insulin-deficiency of the animals and the influence of CCl_4,the effect of insulin in the liver.Therefore, Roil and UnRoil are able to improve the excretory function of the liver, by decreasing the total and direct bilirubin plasma levels. And it could also ameliorate the metabolic function of the liver by the restoration of the triglycerides, LDL-c, HDL-c, and hepatic glycogen to normal values as compared with the CCl_4 group. In addition, it was shown that Roil and UnRoil ameliorated the growth performance by increasing the body weight gain and decreasing liver weight ratio.

Lipid peroxidation is one of the main indicators of oxidative stress and an important marker of CCl_4 damaged liver(Cheng et al., 2013).Lipid peroxidation as well as the augmentation of MDA causes membrane cells damages inducing hypoperfusion of the membrane and then the appearance of cytosolic enzymes in the blood(Bigoniya et Rana, 2010). In our work, we found that the level of MDA increase significantly in liver tissue after the administration of CCl_4. The treatment with Roil and UnRoil has shown a significant attenuation in the MDA level. This action may be due to the antioxidant properties of Argan oil, which scavenges free radicals. Then, inhibiting the lipid peroxidation. Besides, glutathione is considered as one of the most abundant tripeptides that acts as antioxidants, and CAT is a haemoprotein that plays a key role in the reduction of H_2O_2 and protects then the tissues from the reactive oxygen species and free radicals (Naik et Panda, 2007). In the present work, we remarked that the lethal dose of CCl_4 reduced significantly the activity of GSH.This reduction indicates an increase in the production of free radicals and depletion of antioxidant enzymes.Whereas the treatment with Roil and UnRoil reversed this effect. Although, CCl_4 did not affects the CAT levels.

Our results are in agreement with previous studies of Necib et al., Er et al., and Saber T.M et al., which demonstrated that Argan oil possesses both, hepatoprotective and renoprotective effect against mercuric chloride, acrylamide, and sodium fluoride models that induced kidney and liver toxicities in animals.It has been showed that the treatment with Argan oil could normalize the cytosolic activity of GSH dependent antioxidant enzymes GST.In fact, the toxic effects of hepatotoxic and nephrotoxic agents leads to the increase of lipid peroxidation accompanied with the reduction in GSH level and the increase in MDA levels in liver tissue. Thereby, the production of oxidative stress that considered as the main cause of tissue injury (Liver and Kidney)(Saber et al., 2020; Er et al., 2020; Necib et al., 2013). Thus, the beneficial effect of Roil and UnRoil is due to their ability to suppress oxidative stress and improve the mitochondrial function of the kidney and the liver of the treated animals.

Argan oil is rich in vitamin E that exhibit a potential anti-inflammatory effect and able to reduce oxidative DNA damage(Usoro et Mousa, 2010). Moreover, it has been stated that it contains mono-unsaturated, saturated fatty acids, and phenolic compounds such as vanilic acid, syringic acid, tyrosoland ferulic acid that may protect the liver and kidney against oxidative stress induced by toxic agents(Khallouki et al., 2003). In addition, Argan oil is a source of coenzyme Q10 (CoQ10) which is mitochondrial antioxidants and play a key role in

the mitochondrial bioenergetics by participating as cofactor of dehydrogenases in the transports of protons and electrons and in ATP production(Er et al., 2020).Based on our finding,the roasting process of Argan oil did not show any significant changes in all tested parameters. Although, it influences the total bilirubin and urea levels, which mean that the bioactive molecules responsible for the improvement of these parameters are affected by this process.

The use of silymarin as a positive control, at a dose of 50 mg/Kg, produced a significant decrease in hepatic markers (AST, ALT, ALP), liver excretory function (total/direct bilirubin), improve the renal excretory and metabolic functions. The effect of this drug appears statistically similar to that produced by Roil and UnRoil in the majority of tested parameters. Silymarin acts as an antioxidant, it scavenges hydroxyl radicals, by decreasing MDA level and increasing the intracellular concentration of GSH. Furthermore, it plays a role in the protection of the tissue towards injuries, caused by xenobiotic, by enhancing the permeability of the cellular membrane. It possesses also a role in the regeneration of hepatocytes (Abdel-Kader et al., 2017).

In the current study, we investigated also the acute toxicity of UnRoil. The obtained results confirm that the administration of this oil did not induce any mortality and/or any signs of toxicity or change in body weightin mice.

Conclusions

Based on our findings, we conclude that the UnRoil is healthy and it did not possess any adverse effects. Moreover, Roil and UnRoil protect significantly the kidney and liver toxicity caused by CCl_4. They may intercept the free radicals released by CCl_4, by acting as a free radical scavenging agent. Besides, the roasting process of Argan seed oils does not influence the hepatoprotective and nephropretective effect. However, further experimental works are required to examine exactly the action mechanism of Argan seed oils against CCl_4-induced liver disturbances.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Funding Statement

This work was supported by grants from the CNRST of Morocco (Project PPR2).

Acknowledgments

We would like to thank BADRAOUI Mustapha and Ramdaoui Karim for their technical assistance

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Arabian Journal of Medicinal and Aromatic Plants

www.ajmap.info ISSN 2458-5920