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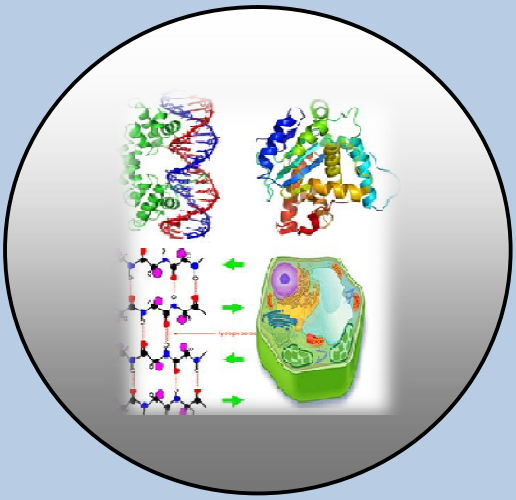

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RESEARCH PAPER

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Phytochemical Screening Chemical Composition and Toxicity of *Euphorbia echinus*

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ABSTRACT

In recent years, interest on plant research has increased all over the world. In this growing interest, many of phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities (Chen et al., 2008; Pesewu et al., 2008; Prachayasittikul et al., 2008; Turker and Usta, 2008). However some searches have shown that many substances, including natural products, are potentially toxic (Veiga-Junior et al., 2005).

The aim of this study was to test the safety of the aqueous extract of Euphorbia echinus a Moroccan plant, widely used in folk medicine; after acute and sub-chronic administration to Wistar rats via oral route. This plant was also subjected to a phytochemical screening and GC-MS analysis of increasing polarity gradient (petroleum ether, iso-hexan, chloroform, ethanol, and distilled water) in order to characterize the chemical composition of the different extracts. The acute toxicity study showed no toxicity when administered up to 5 g/kg body weight orally; however this study revealed some signs of toxicity when the extract was taken for a long period. Urea, acid uric and creatinine demonstrated dose dependent increase; while triglicerides and blood glucose decreased with dose. Therefore histological studies showed some damages in kidney and no alteration in liver at the lowest dose (0.5 g/kg). The phytochemical and GC-MS analysis revealed the presence of several biologically active compounds such us flavonoids, tannins, alkaloids, steroids, phenols, and terpenes; which could serve as a potential source of drugs in herbal medicine; however it also characterize some toxic compounds.

Key words: Euphorbia echinus, Toxicity, Phytochemical Screening, GC-MS and Histology.

INTRODUCTION

Euphorbia echinus belongs to the family of *Euphorbiaceae*, it's a native plant from south Morocco, popularly called "Daghmous". In folk medicine, the plant is widely used to dissolve cysts and also as hypoglycemic, anti-inflammatory, laxative and as a cure for many others remedies. In fact, the genius, *Euphorbia* is stated to possess inflammatory, antiarthritic, antiamoebic, spasmolytic, antiviral, hepatoprotective, and antitumor activity (Bani, 2000; Tona et al, 2000; Semple et al, 1998; Shimura et al, 1990). However up to now, no toxicity study of *euphorbia echinus* has ever been performed. Thus the review of literature didn't reveal any information on chemical analysis.

The aim of this study was to evaluate the acute and subacute toxicity (60 days) of *Euphorbia echinus* aqueous extract. For this purpose, histological sections of liver and kidney were performed, in addition to a biochemical evaluation.

Therefore the chemical composition was also determined in order to characterize the components of the different solvent extracts.

MATERIAL AND METHODS

Plant material

The whole plant of *Euphorbia echinus* was collected from Sidi Ifni, Southern Anti-Atlas, Morocco and was authenticated by Prof. Leila EL GHAZI belonging to Biology Department, Faculty of Sciences, University of Hassan II Casablanca.

To avoid any contamination or dust, the plant's aerial parts were cleaned and spread to dry at room temperature in a clean room.

Extracts preparation

60 g of the powder was extracted in petroleum ether under agitation at room temperature. The solution is filtered on gauze then through Whatman No. 1 filter paper. The filtrates were concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40ºC to achieve etheric filtrate noted PEI. On residual marcs we added Iso-hexan, after filtration and concentration the hexanic filtrate obtained was named HII. The same operation made it possible to obtain chloroformic (CIII) and ethanolic filtrate (EIV).

Boiling water was poured on 10 g of powder, then the mixture stand under agitation at room temperature. The extract was filtered using gauze and Whatman No. 1 filter paper, the filtrate represents the infused solution (IFV).

20 g of the powder were mixed with distilled water in a round-bottom flask, linked to a column connected to a refrigerant. The round-bottom flask was placed at 60°C for 1 hour. The decoction extract was filtered as above and concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40ºC (DVI).

Phytochemical screening

The plant extracts were screened for saponosides, alkaloids, flavonoids, tannins, terpenes, sterols, phenols, saponins and reducing compounds as described by (Bekro et al., 2007; Karumi et al., 2004; Wagner, 1983; Ronchetti, 1971; Hegnauer, 1973). Tests were based on the visual observation of color changes or formation of a precipitate after the addition of specific reagents as it's resumed in table 1.

GC-MS analysis

The GC/MS analysis of the samples of *Euphorbia echinus* was performed using Agilent GC-Mass 6890 model gas chromatograph-5973N model mass spectrometer equipped with a 7683 series auto-injector (Agilent, USA). The system is equipped with a DB-5MS column (30 m x 0.25 mm x 0.25 µm film thickness, Agilent, USA). The temperature was programmed from 50°C to 260°C at 15°C min^{-1} and then held at 260°C for 20 min. Helium gas at a constant flow rate of 1 ml min^{-1} was used as carrier gas. The samples of 1.0 µl were manually injected in the split less mode. MS interface temperature was 230°C. For CG mass detection, an electron ionization system with ionization energy of 70 eV was used and the scan range was 30 -700 amu.

Identification and percentage composition of the compounds was performed using MS library and the NIST 98 spectrometer data bank.

Experimental animals

Tests concerned 30 adult male Wistar rats weighting between 160 to 200 g. They were housed under standard environmental conditions of temperature at 24 ± 1°C under a 12 h dark-light cycle, and allowed free access to drinking water and standard pellet diet. Rats were deprived of food but with access to water *ad libitum*16-18 hour prior the experiments.

Acute toxicity test

Acute toxicity test was performed according to the World Health Organization (WHO) guideline (WHO 2000) and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals 420 (OECD 2001). The aqueous extract (decoction) was prepared at the concentration of 1g/ml in distilled water. Five rats were administrated a single oral dose of 5g/kg body weight while the control group received a saline solution (NaCl 0.9%). Signs of toxicity and mortality have been followed at the first, second, fourth and sixth hours after administration; then once daily for 15 days.

Sub-chronic toxicity test

The method was performed according to WHO guideline (WHO 2000) and the OECD guideline for testing of chemicals 452 (OECD 1981) Healthy male were randomly divided into six groups (n = 5). Animals received daily saline water-vehicle orally (control group) or *Euphorbia echinus* aqueous extract with different doses of 0.5 g/kg, 1.5 g/kg, 2.5 g/kg, 3.5 g/kg and 5 g/kg. The behavior of rats was noted daily, and their weights were recorded once per week. After 30 days all rats were anesthetized by urethan (0.5-1.5 g/kg), and their blood samples were collected into heparinezed centrifuge tubes for biochemical and haematological analyses, respectively. The liver and kidney were removed for macroscopic features and histology tests.

Blood analysis

Biochemical parameters

Following the sacrifice the fasting blood glucose was determined in blood plasma by glucose oxidase method (Trinder, 1969). Total cholesterol, high density lipoprotein-cholesterol (HDL-cholesterol) and triglyceride using blood serum, were estimated by modified enzymatic method from Sigma Diagnostics (Wassan et al., 2001).

The effect of the aqueous extract on certain biochemical parameters were also examined and compared with those of the control group. The blood samples collected with heparinized tubes were centrifuged at 6000 rpm for 10 minutes to obtain clear plasma for

the following tests: urea was determined according to Urease-Berthelot method (Weatherburn, 1967) and plasma creatinine was estimated using Jaffe reaction (Perone et al., 1992) Alanine Amino Transferase (ALT) and Aspatate Amino Transferase (AST) also known as GOT and GPT respectively were measured by using enzymatic method (Horder and Sampson, 1991).

The samples were analyzed for haemoglobin (Hb) content by Cyanmethaemoglobin (Drabkin) method (Dacie and Lewis, 1984).

Haematological and biochemical analyses were performed at LABOMAC, serum biochemistry tests were performed using an automated analyser (Cobas 6000) according to the manufacturer's instruction using reagents purchased from Fortress Diagnostics (France).

Histological analysis

Freshly dissected liver and kidney from group 1 and 2 were cut rapidly and fixed in Bouin for 48 hours, then stored in ethanol 60% for histological assessment. The tissues were dehydrated in ascending grades of ethanol (70%, 80%, 90%, 95% and 100%), cleared in 2 changes of toluene, impregnated with 2 changes of molten paraffin wax, and finally embedded in wax using an automate (LEICA TP1020). After that wax blocs were cooled to facilitate sectioning. Three replicates sections of 3-4 μm were taken using LEICA microtome. Then blocs were monted on slides, dried for 30 minutes in 60°C for dehydration and stained with haematoxylin and eiosine. Coverslips were mounted with eukitt. Preparations were examined using microscope (CETI) to evaluate histological alterations (Pearse, 1985).

Statistical analysis

Data are expressed as the mean ± S.E.M and statistics were performed using one-way analysis of variance (ANOVA). Significant differences between the control and treatments groups were determined using t-test and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening results are listed in table 2; all solvents revealed the presence of phenolic compounds, tannins, alkaloids, reducing compounds and also indicate the presence of flavonoids except in petroleum ether and iso-hexan for the latter. However both infusion and decoction were negative for heterosides, moreover chloroform for polyterpenes. The analysis also showed the absence of Heterosidic triterpenes in infusion extract. The aqueous extract was positive for saponins (persistant foam = 4 cm).

Phytochemicals found in *Euprhorbia echinus* are known to be biologically active. For example, polyphenolic compounds exhibit antioxidant activity by inactivating lipid free radicals oxidation or prevent decomposition of hydroperoxides into free radicals (Fecka et al., 2007; Pokorny et al., 2001). It was also found that flavonoids functioned to reduce blood-lipid and glucose and enhance human immunity (Atoui et al., 2005). It has reported that monoterpene and sesquiterpene compounds such as zingiberene and α-and β-turmerone are used as carminative, antifungal and as antiplatelet agent (Lee, 2006). Saponins are a special category of isoprenoid glycosides that form colloidal solutions with water and foam when shaken. They posses a broad range of biological and pharmacological properties including molluscicidal, anti-viral, hypoglycemic, and immunomodulating activities

(Harmatha et al., 2000; Lacaille-Dubois and Wagner., 2000; Morrissey and Osbourn., 1999; Lacaille-Dubois., 1999; Lacaille-Dubois and Wagner., 1996).

Acute toxicity

Results indicated that *Euphorbia echinus* acute treatment via a single oral administration at 5 g/kg did not produce any sign of toxicity or death in rats during 15 days of observation. Therefore, the LD50 could not be estimated, and it is possibly higher than 5 g/kg.

Sub-chronic toxicity test

During the 60 days following the repeated administration of *Euphorbia echinus* aqueous extract, 5 rats died: 2 in group 6 and 1 in group 3, 4 and 5 (table 3). All animals gained weight, however groups 1 and 2 showed an elevated spleen weight compared to the other groups which demonstrated a very low weight gain compared to control group.

Chemical parameters are presented in table 4; generally all significant changes are within normal ranges. No statistically significant differences exist in hemoglobin, cholesterol and LDL. However there was a significant increase in urea (group 2, 4 and 6), uric acid (group 2, 4, 5 and 6), creatinine (group 2, 3, 4, 5 and 6) and HDL (group 6) compared to the control. The treatment with *Euphorbia echinus* extract showed a significant decrease in glucose (group 2, 3, 5 and 6), triglycerides in all treated groups, and also in ALAT (group 3 and 6) and ASAT (group 4 and 5).

Urea and creatinine are sensible markers to kidney alterations, mainly when these markers increased concomitantly (Satyanarayana et al., 2001). Therefore we cannot exclude that the elevated urea, creatinine and uric acid levels could be an initial, indication of the toxic effects in kidney by *Euphorbia echinus* at high doses 3.5 g /kg and 5 g/kg. ALAT and ASAT are reliable indices of liver toxicity (Hayes, 1989); so the decrease of these hepatic enzymes showed that the extract did not cause any damage to the liver. The slight decrease of glucose, Triglycerides and HDL may suggest the presence of hypoglycemic and hypolipidemic agents in the extract.

The results of the GC-MS analysis identified the various compounds present in different extracts. Chemical compounds possessing anti-inflammatory activity were identified in all solvent extracts for instance: taraxasterol a monohydroxy triterpene (table 5 and 6) (Akihisa et al., 1996), 2 4 6-dimethoxyacetophenone (table 5) (Favier et al., 1998; Sala et al., 2001), 18-Acetoxy-ent-kaur-16-ene (table 7) (Chavan et al., 2011), Phenylethanol (table 8) (Jassim and Naji, 2003; Pan et al., 2003; Korkina, 2007), menthol (table 9) (Juergens et al., 1998) and sclareol (table 10) (Huang et al., 2012). Plants of *Euphorbia's* genus are known to possess medicinal compounds that can cure inflammatory disorders (Satti *et al*., 1988).

Petroleum ether extract showed the presence of 2 classes of terpenes: diterpenes alcohol (phytol and sclareol) and triterpenes (Taraxasterol, Friedeline and Beta Amyrin). It also identified an alkaloid: Theophylline and two phytosterols: Stigmasterol and beta Sitosterol. Phytosterols contribute to lowering serum cholesterol levels (Cherif et al., 2010).

It is reported that prolonged administration of Pyritinol found in hexanic, aqueous and infusion extracts significantly reduced streptozotocin-induced changes in free carbonyls, dityrosine, malondialdehyde and advanced oxidative protein products (Jiménez-Andrade et al., 2008).

Ferruginol is a diterpene phenol found in chlroroformic extract, possesses a strong antibacterial activity (Li et al., 2008). Ethanol extract revealed the presence of Bornyl salicylate that has an anti-inflammatory effect, which is related, at least in part, with decrease of mediators as PGE2, NO and pro-inflammatory cytokines (Vasconcelos et al., 2012). A natural fungicide was identified in Infusion extract, it was reported that tau-muurolol exhibited excellent activity against F. oxysporum, R. solani, C. gloeosporioides, and F. solani, with IC50 < 50 µg/mL (Ho et al., 2012). Decoction revealed a large variety of important biological compounds such as antioxidants: alpha tocopherol. It also revealed many sesquiterpenes as ledol, globulol and cedrol, alcohol sesquiterpenes, trans-z-alpha-bisabolene epoxide an oxygenated sesquiterpenes, beta-elemene, and Beta-ionone a cyclic sesquiterpene. Some kinds of sequiterpene compounds induced apoptosis in cancer cells (Furuya et al, 1994; Woynarowski et al., 1997), costunolide exhibited preventive effects on intestinal carcinogenesis (Mori et al., 1994). It is also indicated that HOBS (highly oxygenated bisabolane-type sesquiterpenes) might be able to normalize malignant SMMC-7721 cells by inhibiting cell proliferation and inducing redifferentiation (Miao et al., 2008). In addition to this, it is reported that alcohol sesquiterpenes as cedrol and globulol are fungistatic (Aleu et al., 2001). Beta-Amyrin was also found in aqueous extract, a pentacyclic triterpenoid which according to a study has similar properties in some respects to mianserin and might possess a sedative action (Subarnas et al., 1993). Aloe-emodin normally present in *Aloe vera* leaves was detected in *euphorbia echinus*, which has a specific *in vitro* and *in vivo* antineuroectodermal tumor activity (Pecere et al., 2000). Beta sitosterol was not only found in decotion, but also in ethanol and ether extracts, a phytosterol that inhibits cholesterol absorption in the intestine (Matsuoka et al., 2008). Because the structure of β-sitosterol is similar to that of cholesterol, β-sitosterol takes the place of dietary and biliary cholesterol in micelles produced in the intestinal lumen; this reduces cholesterol absorption in the body (Moreau et al., 2002).

Table 1. Reagents and tests of chemical group's characterization.

Chemical groups	Reagents	Reaction indicating that the test is positive
Flavonoids	Cyanidine	Heat then pink-orange or purplished colouring
Polyphenols	Ferric chloride	Blackish-blue or green ± dark colouring
Alkaloids	Dragendorff Buchard	Precipitate or orange colouring Reddish-brown precipitate
Catechic tannins	Stiasny	Precipitate in large flakes
Gallic tannins	Stiasny	blue-black deep colouring
Polyterpenes and sterols	Liebermann	Crimson or purple ring, changing blue then green
Heterosides		Fugacious purple colouring
Reducing compounds	Keller-Kiliani	2 phases : reddish-brown colouring (acetic acid) and blue or green colouring (sulfuric acid)
Heterosidic triterpene	Salkowski	Reddish-brown colouring of the interface

Histological tests

Histological sections of kidney derived from group 1 (control NaCl 0.1 %), showed normal appearance of histological features; the section indicated a detailed cortical parenchyma and the renal corpuscles appeared as dense rounded structures with the glomerulus surrounded by a narrow Bowman's spaces and cubic distal renal tubules with macula densa (figure 1), however some damages were observed in treated group with 0.5 g/kg. The kidney section showed a vascular congestion of glomerulus showing a glomerular obsolescence. It also identified apoptosis nucleus illustrated by a condensation of the chromatin (figure 2). Probably toxic agents can cause all these changes observed in the kidneys which means that *euphorbia echinus* total aqueous extract contains compounds that could cause damage to kidney. This is supported by the increase of creatinine, urea and uric acid levels with doses. The CG mass also revealed the presence of some toxic compounds such as Phenanthrene (Roe and Grant, 1964), dibutyl phthalate and diethyl phtalate. An in vitro study reported that diethyl phthalate inhibited uridine diphosphate glucuronyl transferase (UDPGT) activity of rat liver microsomal preparations (Gollamundi et al., 1985). UDPGT is an important enzyme involved in the Phase II conjugation and detoxication of many endogenous and xenobiotic substances. It also revealed the presence of some herbcid and pesticide (Arsenous acid) even if the material was washed properly before use.

Figure 3 and 4 showed respectively histological structure of liver in both group 1 and 2. The liver is divided into hepatic lobules formed of radially arranged strands of hepatocytes that extend from the central vein to periphery of the lobule. Hepatocytes strands are separated from each other by blood sinusoids that are lined with the endothelial cells and Kupffer cells. No difference was identified when the control and the treated tissues were compared.

Table 2. Results of phytochemical analysis of Euphorbia echinus.

Extracts/ Compounds	petroleum ether	Iso-hexan	chloroform	Ethanol	Aqueous (infused)	Aqueous (decoction)
Flavonoids	-	-	+	+	+	+
Polyphenols	+	+	+	+	+	+
Alkaloids	+ (B/D)	+ (B/D)	+ (B/D)	+ (B/D)	+ (B/D)	+ (B/D)
Catechic tannins	+	+	+	+	+	+
Gallic tannins	+	+	+	+	+	+
Polyterpenes and sterols	+	+	-	+	+	+
Heterosides	+	+	+	+	-	-
Reducing compounds	+	+	+	+	+	+
Heterosidic triterpenes	+	+	+	+	-	+
Saponins	-	-	-	-	-	+ (Foam = 4 cm)

Table 3. summary table of the sub-chronic toxicity results* significantly different from control at P< 0.05. The number of rats used in each group was 3.
D:Death, T: total.

Group number	Dose (g/kg)	Number of mice	Weight gain (g)	D/T
1	0	5	92.83±6.33	0/5
2	0.5	5	101.07±27.06	0/5
3	1	5	51.93±23.51*	1/5
4	2	5	66.18±41.65	1/5
5	3	5	42.91±11.46*	1/5
6	5	5	77.35±44.19	2/5

CONCLUSION

The present study revealed the presence of several biologically active phytochemicals such as: polyphenols, flavonoids, tannins, alkaloids, and terpenes. These compounds are known to show medicinal activity as well as exhibiting physiological activity.

Table 4. Clinical blood chemistry of male rats in the subchronic toxicity test of Euphorbia echinus aqueous extract. * Significantly different from control at P< 0.05. The number of rats used in each group was 3.

Groups/tests	T	0.5g/kg	1g/kg	2 g/kg	3 g/kg	5 g/kg
Glucose (g/l)	1.1±0.01	0.92±0.05*	0.86±0.09*	0.94±0.14	0.87±0.06*	0.84±0.25*
Hemoglobin (%)	6.7±0.1	6.7	6.8	6.83±0.05	6.73±0.05	6.73±0.05
Urea (g/l)	0.33±0.06	0.45±0.005*	0.35±0.07	0.51±0.02*	0.42±0.04	0.49±0.005*
Uric acid mg/l	4.28±1.9	4.68±0.27*	4.23±0.52	5.08±0.72*	5.48±0,89*	10.24±2.3*
Creatinine (mg/l)	2.11±0.34	4.85±0.23*	2.71±0.32*	5.13±0.64*	6.2±0.54*	5.91±0.61*
Cholesterol g/l	0.42±0.12	0.48±0.02	0.42±0.11	0.45±0.03	0.46±0.03	0.61±0.01
Triglicerides (g/l)	0.34±0.03	0.23±0.04*	0.27±0.01*	0.22±0.06*	0.13±0.02*	0.17±0.01*
HDL (g/l)	0.10±0.01	0.13±0.001	0.13±0.02	0.12±0.03	0.11±0.01	0.21±0.01*
LDL (g/l)	0,33±0.08	0.32±0.03	0.35±0.08	0.3±0.08	0.35±0.04	0.36±0.02
LDL/HDL (g/l)	3.19±0.09	2.51±0.03*	2.56±0.09*	2.4±0.10*	3.18±0.04	1.73±0.03*
SGOT UI/l	141±20.51	104±22.3	88±7.5*	130±30.9	121±14.15	94±5.29*
SGPT UI/l	43±7.23	38±3.78	46±14.7	30±6.08*	28±3.05*	34±3.53

Table 5. Chemical compositions of petroleum ether extract of *Euphorbia echinus.*

Components	RT, mn	Area %
2 4 6- dimethoxyacetophenone	8.65	0.16
n-hexadecanoic acid	9.63	0.74
Phytol (diterpene alchool)	10.27	0.18
Longifolenaldehyde	10,52	0,1
Sclareol (diterpene alchool)	11.09	0.07
3 beta etiocholanolone	11.14	0.48
Theophylline (alcaloide)	13.22	0.06
1,3-Benzodioxole	13.3	0.22
Beta tocopherol	15.42	0,01
D alpha tochopherol	16.59	0.02
Stigmasterol (phytosterols)	18.19	0.06
Lanost-8-en-3-one	18.33	0.22
beta Sitosterol (phytosterols)	19.16	0.16
Beta Amyrin (triterpenes)	19.56	0.06
Taraxasterol (Monohydroxy triterpene)	20.5	0.31
Friedeline (triterpene)	21.21	0.03

Table 6. Chemical compositions of Hexanic extract of *Euphorbia echinus.*

Components	RT, mn	Area %
Nonane	2.35	0.33
Stearyl alcohol	2.82	0.11
Octoacosane	3.44	0.06
Undecane	3.79	0.18
Hexatriacontane	7.1	0.94
n-hexadecanoic acid	7.23	0.85
Phytol	10.29	0.04
Dibutanoylmorphine	10.39	0.07
4,14 retro-retinol	11.58	0.06
Taraxasterol	13.93	0.02
D alpha tochopherol	16.61	0.01
Pyritinol	18.25	0.01
lanost-8-en-3-one	18.36	0.05
gamma sitosterol	19.16	0.16

Table 7. Chemical compositions of Chloroformic extract of *Euphorbia echinus.*

Components	RT, mn	Area %
n-hexadecanoic acid	7.5	0.19
Ledol	8.35	0.02
Globulol	8.53	0.07
Benzofuranone	8.77	0.28
18-Acetoxy-ent-kaur-16-ene	10.15	0.05
Thumbergol	10.6	0.33
Retinoic acid	12.41	0.16
Ferruginol	12.52	0.37

Table 8. Chemical compositions of ethanolic extract of *Euphorbia echinus.*

Components	RT, mn	Area %
Propanoic acid	2.83	0.12
2,3-butanediol	2.95	0.57
Phenylethanol	4.25	0.04
Ionol (antioxidant)	7.04	0.16
E-15 Heptadecenal	7.54	1.39
Benzophenone	7.8	0.29
Tetradecanoic acid	8.57	0.26
1-octadecene	8..68	1.01
3,4 dimethoxybenzylidene	8.84	0.56
n-hexadecanoic acid	9.63	1.42
1-Eicosene	9.69	0.77
heptadecanoic acid	10.05	0.18
Kaur-16-ene	10.14	0.26
gamma Sitosterol	10.24	0.05
Phytol	10.27	0.09
Bornyl salicylate anti-inflammatoire	10.42	2.05
Octadecanoic acid	10.51	0.25
Phenanthrenol	10.89	0.33
Thumbergol	11.06	0.17
Sclareoloxide	11.37	0.2
1,4 Naphthoquinone	11.45	0.43
Campesterol (phytostérol)	11.66	0.22
Danthron	11.81	0.9
D alpha Tocopherol	16.55	0.17
Lanost-8-en-3-one	18.31	0.05
Beta sitosterol	11.66	0.22
Beta Humulene	20.47	0.07

They may possess anti-oxidant, anti-inflammatory, anti-tumor and hypolipidemic propertie and so on; which could serve as valuable ingredients for pharmaceutical cosmetic and food industries. Therefore chronic toxicity revealed some signs of toxicity; illustrated by histological kidney alteration and biochemical tests perturbation. That's why additional studies must be conducted to isolate and purify plant constituents and to test their pharmacological potential in order to ensure their safe use in vivo.

Table 9. Chemical compositions of aqueous extract (decoction) of *Euphorbia echinus*.

Components	RT, mn	Area %
Methoxy-phenyl-oxime	3.35	1.27
Tioconazole	3.71	0.6
Thiocyclam	4.04	0.51
Pyritinol	6.38	0.58
Cannabidivarol	6.49	0.9
Linoleic acid	6.8	2.27
hexahydroxy 2 (3h)-Benzofuranone	7.15	0.19
Myristic acid	7.45	0.31
Glibornuride	7.59	0.19
Diethylphthalate	7.63	0.26
trans-z-alpha- bisabolene epoxide	7.95	0.21
Hymexazol	8.37	0.61
Beta-ionone	8.42	0.28
3-hydroxy-beta-ionone	8.45	0.28
Globulol	8.55	0.25
Ledol	8.61	0.3
tretradecanoic acid	8.68	1.21
2-Oxazolidinone, 5-[(2-methoxyphenoxy)methyl]-	8.95	1.24
4-Aminosalicylic acid	8.85	0.18
9,10-anthracenedione; 1-amino	9,42	0.74
n-hexadecanoic acid	9.69	3.64
Menthol	10.27	0.14
beta-elemene	10.45	0.6
Octadenoic acid	10.51	1.64
4-camphenyl butan-2-one	10.6	0.1
Androstérone	11.15	0.14
-Nimbiol	11.23	0.42
Thumbergol	11.54	0.14
Cedrol	11.63	0.11
4,14 retro-retinol	11.64	0.24
Beta sitosterol	11.74	0.13
Aloe-emodin	11.97	1.19
alpha tocopherol	16.59	0.2
beta-Amyrin	19.59	0.05

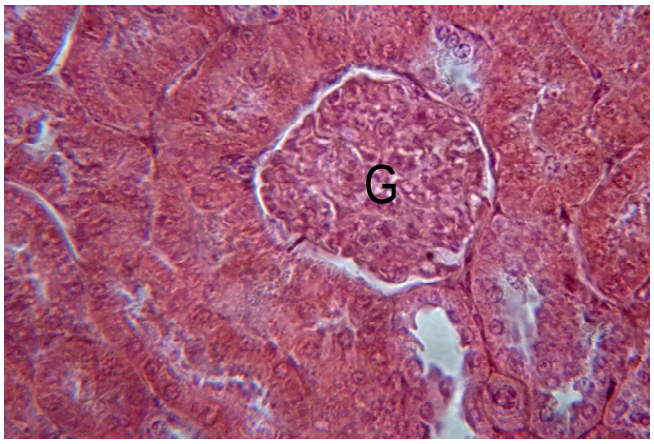

Figure 1. Section in kidney of a control rat (x400) G: glomerulus, Rt: renal tube.

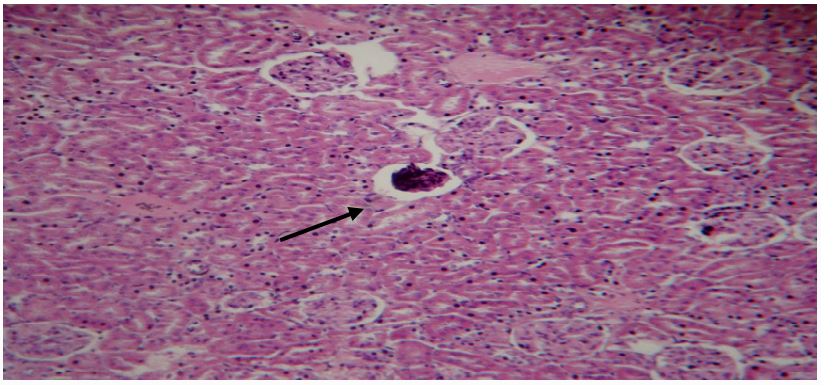

Figure 2a. Section in kidney of treated rat (x 100).

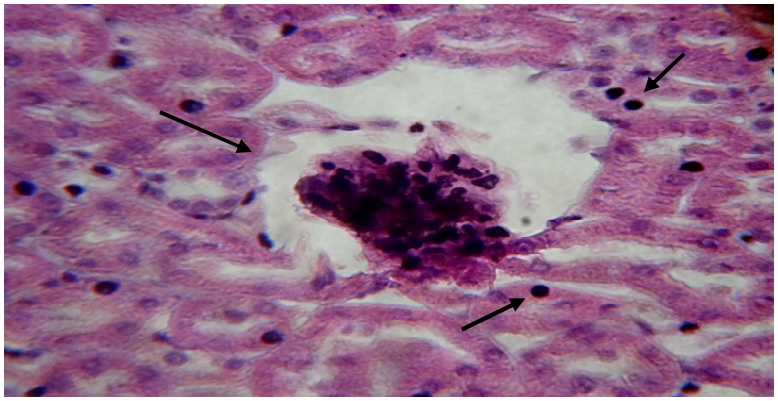

Figure 2b. Section in kidney of treated rat (x 400).

Figure 2. Histological sections of treated kidney with 0.5 g/kg of *euphorbia echinus* aqueous extract.

Table 10. Chemical compositions of infused extract of *Euphorbia echinus.*

Components	RT, mn	Area %
Pyritinol	4.23	2.37
Carbendazim	4.36	1.03
Arsenous acid	5.64	0.3
Myristic acid	7.48	0.16
Glibornuride	7.69	0.65
Globulol	8.55	0.25
Ledol	8.61	0.18
9,10 Anthracenedione	8.85	0.8
Tau-Muurolol	9.07	0.14
Dibutyl phtalate	9.12	0.34
4-methoxybenzyl phenyl	9.44	0.28
Kaur-16-ene	10.46	0.35
Pregnane-3,20-dione	10.56	0.13
Sclareol oxide	11.11	0.07
Androstérone	11.15	0.14
7-methoxy-1-methyl-anthraquinone	11.77	0.08

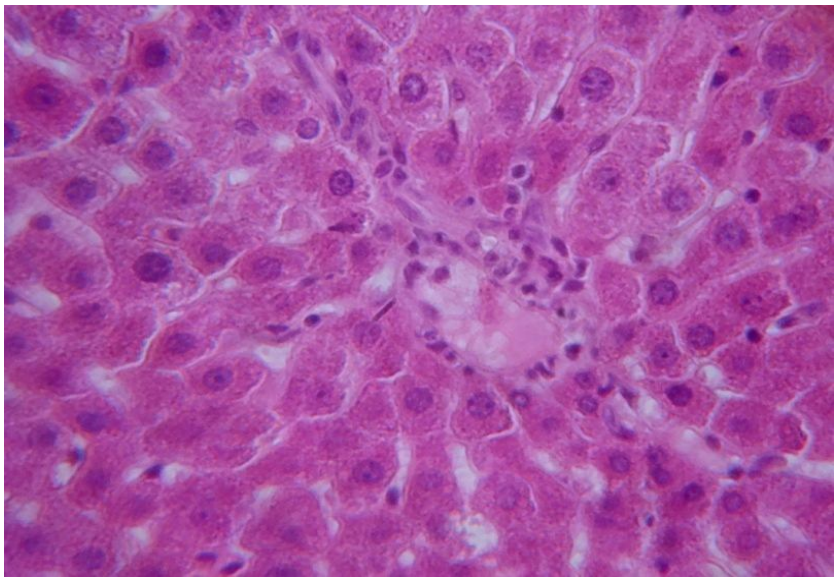

Figure 3. Section in liver of a control rat (x400).

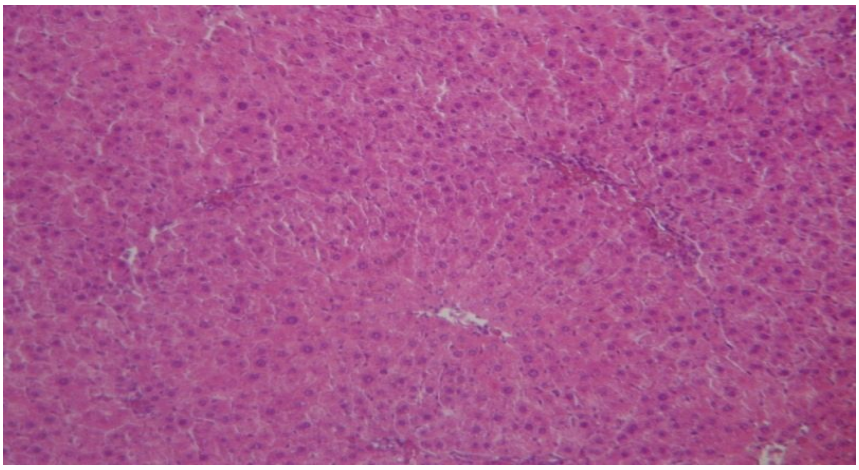

Figure 4a. Section in liver of a treated rat (x100).

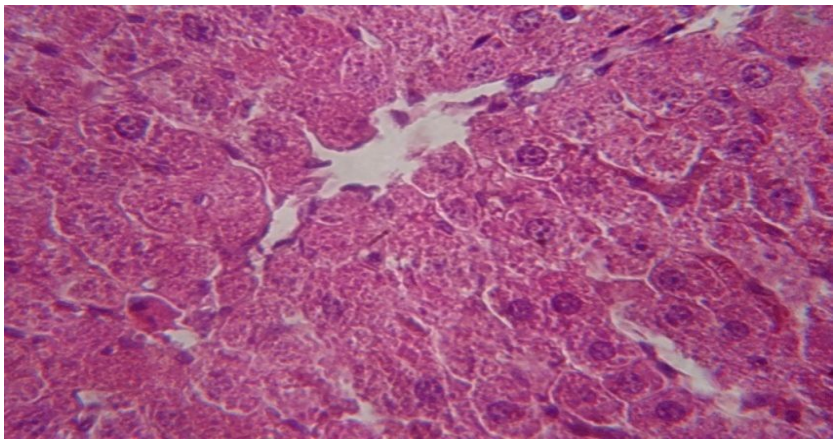

Figure 4b. Section in liver of a treated rat (x400).

Figure 4. Histological sections of control and treated liver with 0.5 g/kg of *Euphorbia echinus* aqueous extract.

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