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Acute and Chronic Effects of Methanolic Extract of *Teucrium polium* on Blood Parameters and Histopathology of Liver and Kidney in Female Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author IK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NB, SO and NA managed the analyses of the study. Authors AB and LA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Teucrium polium* L. is a medicinal plant largely used traditionally in folk medicine in Algeria, locally named: Khayata. It is effective in the treatment of various diseases.
Methods: The Total Polyphenol and Flavonoid Contents of the methannlic extract of *Teucrium polium* was determined. in order to study any possible toxic effect or changes in normal behavior, Acute Toxicity Assay and Chronic Toxicity Study was evaluated. Experiments were performed on adult female Wistar albino rats. Under ether anesthesy, all the rats were euthanized, blood samples were withdrawn by sinus retro-orbital puncture in tubes containing EDTA and immediately processed for haematological tests using Beckman coulter-automatic For biochemical analyses, a

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volume of blood were collected in a heparinized tube.
Results: The methanolic extraction gave a yield of 8.24% from the aerial parts of *T. polium.* The total polyphenols, flavonoids quantities were 18.63 ± 3.51 mg equivalent gallic acid / g dried material and 9.14 ± 2.39 mg equivalent quercetin / g dried material, respectively. The study of acute toxicity showed a low toxicity with LD_{50} > 2400 mg / Kg of body weights. These data can be used to classify these plant in slightly toxic. However, the sub-acute treatment for six weeks of rats with 75, 150 and 300 mg of methanolic extract / Kg of body weight resulted in significant increases of the most studied haematological parameters. Biochemical analysis revealed significant increase of renal parameters (urea, creatinine, uric acid, Na and K), accompanied by increase of relative weight of kidney, lipidic (cholesterol) and hepatic glutamate oxalo-acetate transaminase (GOT) values in all treated rats. Histopathological examination confirmed the biochemical tests by the observation of perilobular necrosis areas, bile duct and inflammatory infiltration of the liver and presence of marked intracytoplasmic vacuoles in kidney with the dose 300 mg of methanolic extract of *T. polium* / Kg of animal body weight
Conclusion: The use of *Teucrium polium* L. may cause hepatotoxicity and/or nephrotoxicity after prolonged herb administration.

Keywords: Teucrium polium L; methanolic extract; LD_{50}; biochemical parameters; acute and chronic toxicity; liver; kidney.

1. INTRODUCTION

Mountain germander, *Teucrium polium* L. (lamiaceae) is originating from the south-west of Asia, Europa and North Africa [1]. There are various reports about pharmacological properties of *Teucrium polium* L. These include antibacterial [23], anti-inflammatory, analgesic, antispasmodic and hypolipidemic [1,4] anti-ulcer, anti-nociceptive [5], antidiabetic, diuretic [6], antifongic, inotropic and chronotropic [7] effects. Most of these effects seem been attributed to the antioxidant and free radical scavenging properties of *Teucrium polium*.

Phytochemical investigations have shown that *T. polium* contains various compounds such as flavonoids, polyphenols [8], iridoides, tannins, essential oils and alkaloids diterpenoids, principally furano neoclerodanes [9,10]. One of these major components is teucrine A [11,12].

However, many herbal medicinal plants including *T. polium* were found to induce fatal hepatic effects and severe acute liver failure with marked haematological and biochemical alterations after prolonged administration [13]. Several cases of germander hepatitis were reported in Canada [14] and Spain [15]. Several reports linked the consumption of *T. polium* with hepatitis in man [16,17,18]. *T. polium* is consumed by many people in Mediterranean countries such as Jordanians and Algerians for the treatment of several deaseases, and there is no detailed information on the liver status after the consumption of the plant tea. Cytotoxic effects of *Teucrium polium* on some cell lines (A549, BT20, MCF7, and PC12) has been reported by Nematollahi-Mahani [19]. The IC_{50} values for each cell line were found: A549, 90 µg/mL; BT20, 106 µg/mL; MCF-7, 140 µg/mL; and PC12, 120 µg/mL *T. polium* ethanolic extract. All studies has been focused on aqueous or infusion [20,21], ethanolic [22,19] and ethyl acetate extracts and no toxicological study has reported the effects of methanolic extract which is rich of flavonoids and polyphenols. Finally, it is well known that every drug has been associated with hepatotoxicity almost certainly due to the pivotal role of the liver in drug metabolism. Hepatic metabolism is, first and foremost, a mechanism that converts drugs and other compounds into products that are more easily excreted and that usually have a lower pharmacologic activity than the portent compound [23,24]. A metabolite may have higher activity and/or greater toxicity than the original drug. Metabolites of the drugs that are excreted from kidneys may also cause cellular damage leading to kidney dysfunction [25].

In this report, the focus was on the acute and chronic toxicity tests. The primary concern was to determine how toxic the methanolic extract of *T. polium* may be after acute administration to rats. Secondary, what would be the target organ for that toxicity and whether there would be any correlation between the toxic effects and the flavonoids and polyphénols contained in the plant materials after chronic oral administration to rats.

2. MATERIALS AND METHODS

2.1 Plant Material

The aerial parts of *Teucrium polium* were collected during June 2009 from Bougaa region at the north of Setif province in the north east of Algeria, identified by Prof Laouar and a voucher specimen was deposited at the Applied Biochemistry Laboratory, University Ferhat Abbas, Setif, Algeria. The plant materials were dried at room temperature and powdered. The dry plant samples were extracted with absolute methanol. The dry extract was obtained after removing the solvent by evaporation under reduced pressure at 45℃. The extract was stored at - 20℃ untill use.

2.2 Animals

Experiments were performed on adult female Wistar albino rats, weighting 201.61 ± 7.04 g. The animals obtained from 'Institut Pasteur d'Algérie' were housed in groups of eight to ten in plastic cages at controlled room temperature. Water and food were freely available.

2.3 Determination of Total Polyphenol and Flavonoid Contents

Total polyphenols were measured using Folin-ciocalteu according to Li et al. [26]. the absorbance is measured at 765 nm. The concentration of total polyphenols is calculated starting from the calibration line established with the gallic acid and is expressed in µg of equivalent of acid gallic per milligram of extract.

Flavonoids were quantified using $AlCl_3$ reagent [27]. Flavonoids were measured as quercetin equivalents. One mL of *T. polium* methanolic extract (TPME) samples was dissolved in methanol, 1 mL of $AlCl_3$ (2% in methanol) was added, and after incubation for 10 min, the absorbance was measured at 430 nm [28].

2.4 Acute Toxicity Assay

In order to study any possible toxic effect or changes in normal behavior, 5 groups of 7 rats were used in this experiment. The animals were fasted 24 hours before the treatment [29,30]. The acute toxicity of the plant was studied by preparing four different concentrations of the extract (0.3, 0,6, 1.2 and 2.4 g/kg), and administered orally to four groups of animals. The fifth group was taken as a control and given 1.0 ml NaCl 9‰. The behavioural changes, posture and mortality were checked for 24 hours. The method of Karber [31] was employed for the determination of acute oral lethal dose (LD_{50}).

2.5 Chronic Toxicity Study

Animals were divided into four dose groups of 8 animals /dose. The first group was given 1 ml normal saline and taken as a control. The second, third and forth group were given a single doses of 75, 150 and 300 mg / Kg of TPME extract by gavage daily. Body weight food consumption and clinical observations were monitored daily. Animals were fasted 3 h prior to dosing to facilitate administration of the complete dose. All animals treated for 42 days then, they were fasted for about 3 h and sacrificed by euthanasia. Immediately after decapitation blood samples were obtained directly from the neck for haematological and serum analysis. By thoracic abdominal longitudinal incision the animal abdomen was opened and the liver and left kidney were removed and the wet weights were recorded [32].

2.6 Haematological and Biochemical Analysis

Under ether anesthesy all the rats were euthanized, blood samples (2.0-4.0 ml) were withdrawn by sinus retro-orbital puncture in tubes containing EDTA and immediately processed for haematological tests using Beckman coulter-automatic haematology analyzer (USA). The haematological parameters measured were mean cell volume (MCV), red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), platelets (PLT), mean platelet volume (MPV), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). For biochemical analyses, 2 to 3 ml of blood were collected in a heparinized tube and centrifuged at 4000 g/ 5 min. at 4℃. The plasma obtained was stored at –20℃ until use. The biochemical parameters including glucose (Glu), urea (Urea), creatinin (Creat), uric acid (UA), Na, K, cholesterol (Chol), triglycerides (TG), glutamate oxalo-acetate transaminases (GOT), glutamate pyruvate transaminases (GPT), alcaline phosphatase (ALP) were measured at the Central Laboratory of the University Hospital (CHU) of Setif.

2.7 Evaluation of Organs

The animals were weighted and euthanized by ether inhalation, all the organs/tissues were carefully examined macroscopically and the brain, lungs, heart, spleen, liver, kidneys and ovaries were weighed. The specimens were fixed in 10% formalin for 24 h, and standard dehydration and paraffin-wax embedding procedures were used. Sections (5 mm) were cut in a microtome, adhered to glass slides with. Hematoxylin and eosin-stained slides were prepared by using standard methods and evaluated by light microscopy.

2.8 Statistical Analysis

Statistical analysis was performed using Student's *t*-test for significance and analysis of variance (ANOVA) followed by Dunnett's test were done for the multiple comparison of the effect of different extract doses. Values of $p < 0.05$ were considered statistically significant. The comparison of the averages and the variances was done using SigmaStat 3.5, version 3, and SigmaPlot 10.0.

3. RESULTS

The methanolic extraction gave a yield of 8.24% from the aerial parts of *T. polium.* The total polyphenols, flavonoids quantities were 18.63 ± 3.51 mg equivalent gallic acid / g dried material and 9.14 ± 2.39 mg equivalent quercetin / g dried material, respectively.

3.1 Acute Toxicity Studies

Rats were individually observed during the first 30 min and regularly during the first 24 h after TPME administration. Clinical signs observed were summarized in Table 1. Mortality due to different doses administered to female rat was 10% and 20% for 1200 and 2400 mg/Kg, repectively. LD_{50} is higher than 2400 mg/kg body weight.

3.2 Effects of *T. polium on* Body Weight

Percentages of changes in body weights during the administration period are shown in Fig. 1. Values for the group treated with 75 mg of TPME /kg of body weight were slowly decreased compred to those of the controls group but differencies were not significant. Whereas, administration of 150 and 300 mg/kg result in significant decrease except the fifth week with 300 mg/ Kg. It seems that, at the concentration of 300 mg/kg, there is certain adaptation of the anomals wich is not seen the the other concentrations.

The macroscopic analysis of the target organs of the treated animals (liver, lung, heart, brain and spleen) did not show significant changes in color and texture when compared with the control group. Nevertheless, a significant increase in the values of the relative mass of kidneys with the 150 and 300 mg/kg are observed. The results of organs weight are summarized in Fig. 2.

3.3 Effets of TPME on Haematological and Biochemical Paramaters

Haematological parameters of the blood in the four groups of rats are represented in Table 2. A significant increase was observed of the following parameters: RBC, MPV, HCT, PLT and HGB compared to the none treated rats. Similarly, biochemical parameters in the four groups of rats, Glu, Creat, K, Urea, Na, UA and GOT were significantly increased after chronic treatment with 75, 150 and 300 mg / kg and a significant increase in GPT level with the dose of 75 mg/kg and in Chol level with the 300 mg / kg *T. polium* extract compared to controls.

Table 1. Signs and symptoms of *T. polium* toxicity on female rat score based on the order of severity

Extact	Dose (mg/kg)	Signs and symptoms	Time
T. polium	0	Normal	-
	300	piloerection, stressed rats	15 min.
	600	Piloerection, laboured breathing, immobilization of the rats.	15 min.
	1200	Irritability, tremblement, labored breathing, immobilization of the rats	6 days
	2400	Paralysis, tremor, labored breathing, death	30 min.

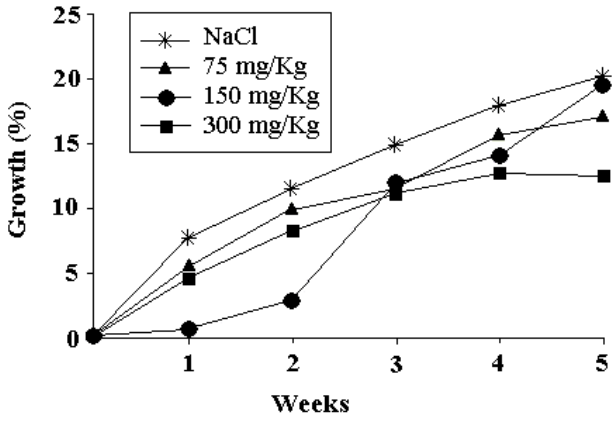

Fig. 1. Changes of body weight growth (%) of treated rats and control

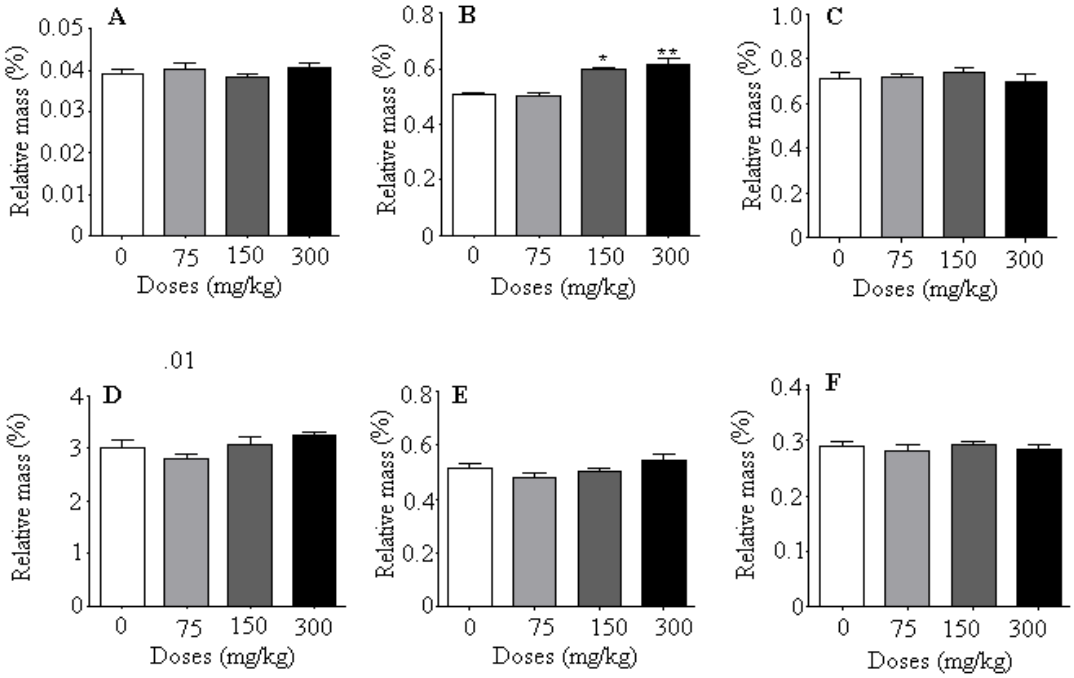

Fig. 2. Effects of methanolic extract of *T. polium* on organ relative weights of female rats in chronic toxicity. A; ovaries, B; kidneys, C; brain, D; liver, E; lungs and F; heart. The values are mean ± SEM (n = 7-8). * p < 0.05, ** p < 0.01

3.4 Histopathological Examination

The observation of the histological slices of the liver and kidneys of treated rats compared to controls are presented in Figs. 3 and 4, respectively. The examination of kidneys revealed presence of intracytoplasmic vacuoles, precisely on cortical area when the dose of 300 mg/kg of *T. polium extract* was used. Histological examination of the liver showed moderate portal inflammatory infiltrates, a vascular congestion around vessels. Mild lobular necrosis, a vascular congestion and steatosis was also seen of the cuts of the rats treated with group treated by 75 mg/kg *T. polium* extract. A perilobular necrosis and an inflammatory infiltrate around the portal vein of the cuts of the rats treated with the second group. A moderate inflammatory infiltrates in portal tracts were seen, proliferation of bile ducts and a portal fibrosis was noted on 300 mg/kg of *T. polium extract*.

4. DISCUSSION

4.1 Acute Toxicity

No sign of acute toxicity for female rat treated by *T. polium* extract was seen; this suggests that LD_{50} is higher than 2400 mg/Kg of body weight. The results is in agreement with the study of Autore et al. [2] and Rasekh et al. [21]. Hasani et al. [33] dismount that the administration of this plant is influenced by the nature of the sex. However, Khleifat et al. [13] determined LD_{50} of 262.0 mg/kg in the rats treated by intraperitoneal way by ethanolic extract of *T. polium*. This data is correlated with the route of adopted administration which targets directly the liver [24,34].

Table 2. Haematological data for female rats orally treated by *T. polium* extract for 6 weeks

Haematologic tests	Non-treated	75 mg/kg	150 mg/kg	300 mg/kg
RBC (10^6/mm^3)	5.41 ± 0.29	7.65 ± 1.03**	6.36 ± 0.16*	7.41 ± 0.32**
HCT (%)	29 ± 1.83	39.06 ± 0.32**	38.96 ± 0.71**	36.52 ± 2.17*
PLT (10^3/mm)	303 ± 27.76	548 ± 19.78**	532.29 ± 20.93**	638.60± 34.42**
MPV (µm^3)	6.46 ± 0.07	7.04 ± 0.05**	6.71 ± 0.09*	7.12 ± 0.11**
HGB (gr/dl)	9.90 ± 0.59	14.23 ± 0.08**	13.93 ± 0.25**	13.62 ± 0.61**
WBC (10^3/mm^3)	7.46 ± 0.82	5.80 ± 0.53	6.76 ± 0.55	6.48 ± 0.43
MCV (µm^3)	50.84 ± 0.67	50.40 ± 0.50	49.29 ± 0.47	49.08 ± 1.11
MCH (pg)	17.81 ± 0.23	18.35 ± 0.13	17.42 ± 0.26	17.63 ± 0.37
MCHC (g/dl)	35.35 ± 0.41	36.32 ± 0.19	35.53 ± 0.23	35.93 ± 0.41

** P<0.05, **P<0.01*

Table 3. Serum biochemical data for female rats orally treated by *T. polium* extract for 6 weeks

Biochemical data	Non-treated	75 mg/kg	150 mg/kg	300 mg/kg
Na (mEq/L)	134.57 ± 2.10	169.07± 4.74**	170.13 ± 1.32**	165.55 ± 1.81**
UA (mg/ L)	8 ± 1.21	27.14 ± 1.97**	17.13 ± 1.29*	16.53 ± 2.90*
Urea (gr/ L)	0.51 ± 0.03	0.64 ± 0.05	0.65 ± 0.03	0.61 ± 0.06
Chol (gr/ L)	0.34 ± 0.08	0.33 ± 0.026	0.35 ± 0.03	0.57 ± 0.041**
TG (gr/ L)	0.47 ± 0.084	0.39 ± 0.02	0.49 ± 0.03	0.39 ± 0.04
ALP (UI/L)	93.80 ± 7.07	74.33 ± 2.67	97.83 ± 3.31	86.30 ± 41.98
GOT (UI/L)	43.86 ± 3.77	96.67± 7.84**	85.17 ± 4.39**	85.00 ± 16.16**
GPT (UI/L)	96.67 ± 8.66	35.57 ± 3.09**	59.43 ± 5.22**	56.00 ± 16.76**
Creat (mg/ L)	6.58 ± 0.37	6.15 ± 0.27	6.79 ± 0.36	6.0 ± 0.45
Glu (gr/ L)	1.47 ± 0.11	1.27 ± 0.11	1.30 ± 0.06	1.24 ± 0.05

*Values are expressed as mean (n = 8) ± SED, * p ≤ 0.05, ** p ≤ 0.01.*

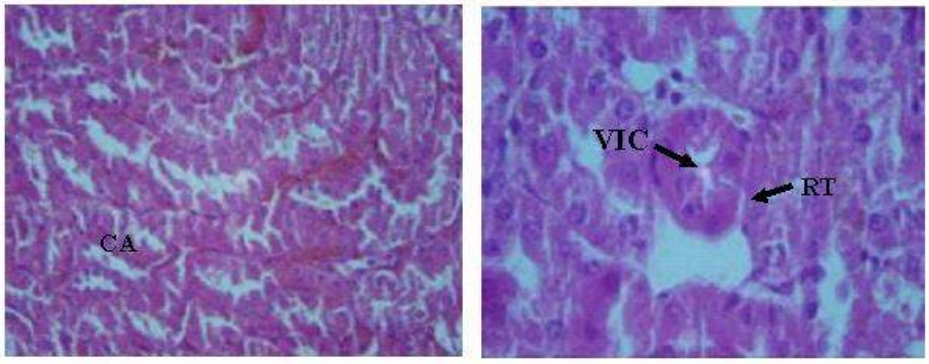

Fig. 3. Renal histological cuts of control group and treated rats with 300 mg/kg *T. polium* extract. (hematoxyline, eosin; Ã—600). CA: cortical area, RT: renal tube, VIC: vacuoles intracytoplasmic

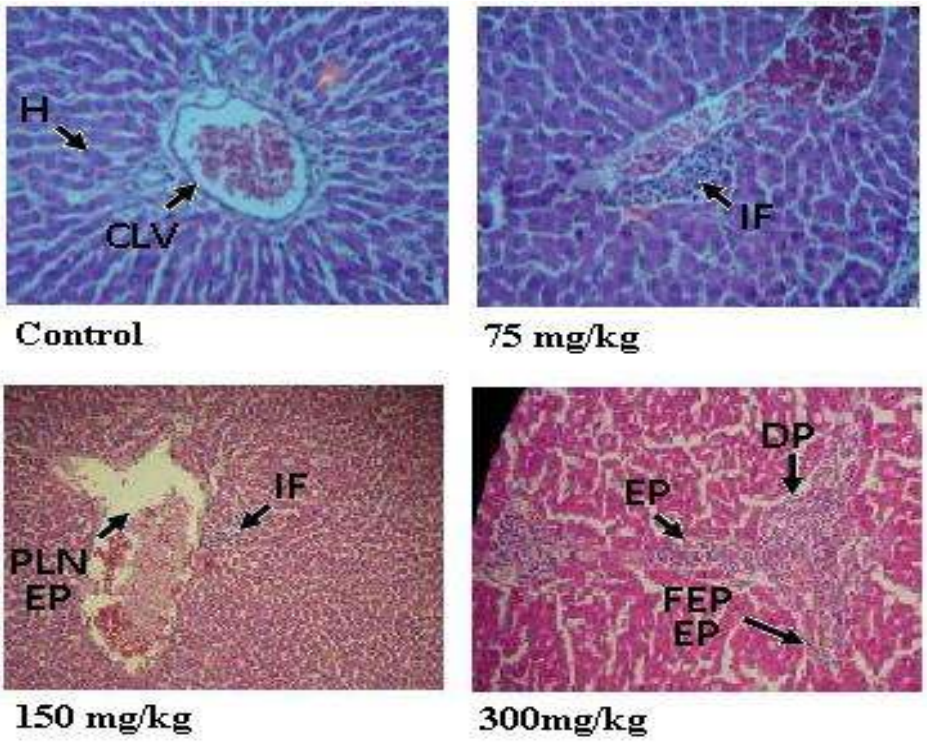

Fig. 4. Hepatic histological cuts of control group and rats treated with 75 mg/kg, 150 mg/kg and 300 mg/kg *T. polium* extract. (hematoxyline / eosin). H : hepatic cell, CLV : centrolobular vein, IF : Inflammatory infiltrate, PLN : perilobular necrosis, EP : espace porte, DP : duct proliferation, FEP : Fibrosis in bridge

4.2 Chronic Toxicity

4.2.1 Effect on body weight

The rats present a moderately significant reduction (3%) of their weights during the sixth week with the 75 mg/kg of methanolic extract. Indeed, the loss of rat's weight is correlated in a physiological state of the animal and can be explained by a reduction of consumption of food. This result agrees with those published by Rasekh et al. [21] where female rats show a significant reduction of body weight ($p<0.05$) with dose of 100 mg/kg of aqueous extract of *T. polium*. The other groups show a normal evolution of their body weight as shows compared to the control during all the experimentation duration as Shahraki shows it and others [35].

4.3 Haematological Biochemical and Parameters of Rats

According to the bibliography [13,21], none disturbance of hematologic values was reported in rats treated by the aqueous and/or ethanolic extract of *T. polium*. Conversely the methanolic extract induced an increase in the number of red globules cells observed with the three groups treated (75, 150, 300 mg/kg), implying a potentiality of erythropoiesis. However, these data are associated with hematocrit and haemoglobin increases with doses of 75 mg/kg and 300 mg/kg. The significant increase in hemoglobin is associated with a significant increase of MCV and MCH witch indicate a tendency to macrocytose and hypochromy [36] latelets level increase on 75 and 300 mg/kg doses can be commented on by secondary hyperplaquettose associated an attack of spleen, a martial deficiency and with the myéloprolifératifs syndromes [37].

The methanolic extract of *T. polium* shows a significant reduction in glucose with the dose of 75 mg/kg, this agrees with works of Gharaibeh et al. [6]; Esmaeili and Yazdanparast, [38]; Ghoraishian, [39]; Bhonde et al. [40]. Several flavonoids such as quercetin and different terpenoids discovered in *T. polium* decrease the

serum glucose level only in the diabetic rats. It is, therefore, possible that these effects of the areal parts of *T. polium can* be due to the flavonoids and/or terpenoids. Zal and their collaborators [20] accorded the hypoglycemic effects of aqueous *T. polium* extract (area of Kerman, Iran) to their composition in ions: potassium, Zinc, Cadmium and Chromium .This suggests that the hypoglycemic property of this plant depends on the type of ground and the geographical area of harvest. Richness of *T. polium* in flavonoidss and polyphenolic compounds such as cirsimaritin, apigenin-7-glucoside, vicenin and luteolin-7-glucoside gifted of antioxydant activities in particular with the dose of 50 and 100 mg/kg [41,42], thus explain the use of *T. polium* in folk medicine in the treatment of the diabetes [33]. The Chol level was appreciably increased after chronic treatment with the dose of 300 mg/kg *T. polium* extract. This is in agreement with the observations at the human ones [13,17].

The renal parameters showed a very significant in level of urea, creat Na and K. Urea increases could be explained by an increase in degradation of protein compounds, but also by an injure of renal function [24]. Really, kidneys were clearly damaged and its histological aspect indicated a remarkable cytoplasmic vacuolization of tubular cells of cortical area and which explains the increased in relative weight of the kidney (mainly with the doses of 150 and 300 mg/kg), while the other values of the various studied organs are normal. These results agree with works of Rasekh et al. [21], [Khleifat et al. [13] and Iriadam et al. [43] who showed a significant increase in urea and creatinin level in diabetic rabbits after treatment by aqueous extract of *T. polium* (82 mg/kg *per os*), confirmed by an apoptose in some renal sections.

The values of hepatic analysis presented a significant higher in TGO with the three groups and a reduction in TGP in the first group. This reduction does not agree with work of Mattéi et al. [16] and of Rasekh et al. [21]. However, results of Rasekh and collaborators (2005) showed that its high level only with the dose of 300 mg/kg after 44 days of treatment.

Mazokopakis and collaborators [16] reported that a dysfunction of the liver appears after one month of treatment by an infusion of *T. polium*. In parallel, Mattéi et al. [16] defer two cases of lethal hepatitis due to the treatment containing germander. Histological examinations of liver are the same ones observed in this our study in particular in treated rats with 150 and 300 mg/kg doses. These results are also supported by preceding work of Rasekh et al. [21] an acute and serious failure liver at a man after prolonged administration of *T. polium, T. chamaedrys and T. capitatum*.

Although the mechanism of hepatotoxicity of *T. polium* is not well elucidated, teucrine A and several diterpenoids neoclerodans, present in aeral parts, were to suspect like hepatotoxic precursors of this plant [21]. Experiments in mice showed a formation of toxic metabolites starting from these diterpenoids which interact with

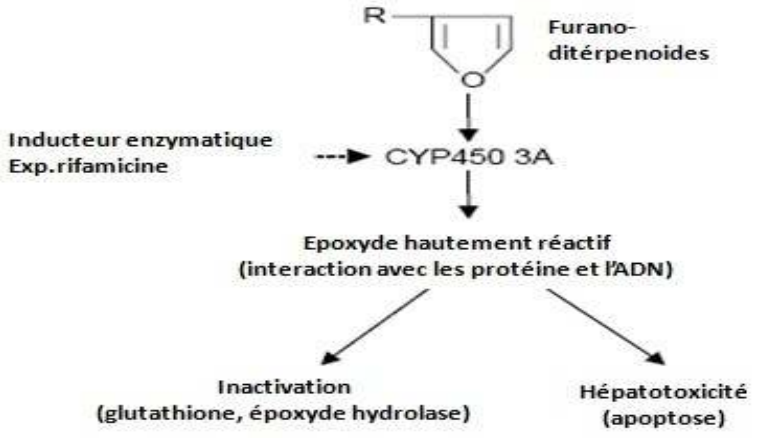

Fig. 5. Toxic mechanism of furano-diterpenoids extracts from *Teucrium polium* [44]

cytochrome P450 3A and the inactivation of glutathione. In fact, the detoxified diterpenoids are effective inductors of hepatocytes apoptose [44,45]. That suggests liver necrosis it is attenuated by a duel mechanism, a direct toxicity and a series of secondary immune reactions [46]. The direct cytotoxicity is known for being the fundamental cause of damage of liver in certain cases, while in others the immunological mechanisms or even a mixture of cytotoxicity and immunogenicity can be implied [47]. A covalent bond of the epoxy hydrolase will take place on the external surface of human hepatocytes and in the presence of teucrine A, could start immune reactions and induces a formation of autoantibody leading the cells to the apoptose (Fig. 5).

5. CONCLUSION

Methanolic *Teucrium polium* extract perturb biochemical serum and hematological parameters related to hepatic and renal function after prolonged administration. The liver histology can indicate image of an acute or chronic hepatitis, cases of cholestasis, cytoplasmic vacuoles characteristic of renal tubes. People should consider about the use *T. polium*, particularly if they are not officially informed of their possible unfavorable reactions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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