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Acute and chronic effects of methanolic extract of *Tamus communus* on blood parameters and histopathology of liver and kidney in female Rats

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Abstract

Tamus communis L. is commonly used as medicinal plants in Algeria against a variety of human disease. In this study, the extraction of rhizomesof *T. communis*by methanol gave a dry matter yield of 6.45%. The effects of *T. communis* methanolic extract (TCME) were examined *per os* on female rats *Albino Wistar* for six weeks. Determination of serum parameters biochemical, haematological and evaluation of physical and histopathology of organs of treated rats were studied. The study of acute toxicity showed a low toxicity with LD_{50} > 12000 mg / kg of body weight of rats treated with TCME. These data can be used to classify these two plants in low toxicity and / or slightly toxic. However, the subacute treatment of rats with the doses of 75, 150 and 300 mg of TCME / kg body weight of rats for six weeks resulted in significant increases of haematological parameters: RBC, MCV, RDW, HCT, PLT, MPV, MCH and HGB. Biochemical analysis revealed significant increase of renal. On the other hand, TCME did not cause significant changes biochemical parameters; but a significant decrease ($p \leq 0.05$) of relative mass of ovaries in rats treated. Histopathological examination has not marqued modifications of organs structure.

Key words: *Tamus communis L.*, TCME, $LD_{50,}$ biochemical, hematological-parameters, histopathology.

INTRODUCTION

Common Tamier (F: Dioscoreaceae) Black Bryony, Karm barri, Karma sawda (Al-Khateeb *et al*., 2012; García-Herrera *et al*., 2020) is a perennial, climbing herbaceous plant; its large, blackish rhizome contains an astringent milk (Amraoui *et al*., 2019). *Tamus communis* is native to North Africa, Western Asia, Central and Southern Europe and the Near East, and thrives in woods and undergrowth, hedges, scrub thickets and fences at low altitudes (Slavova *et al*., 2022) .This species, whose active ingredients are mainly phenanthrenes and saponosides derived from diosgenin, are responsible for several biological (Belkhiri *et al*., 2015). Common tamier is often used in pharmaceuticals, this edible plant and often confused with asparagus (Guermah and Medjdoub-Bensaad 2020). Rhizomes and tubers are used in folk medicine in the treatment of rheumatism, osteoarthritis and dermatitis (Zerargui *et al*., 2016), anticancer, antibaterial (Bnouham *et al*., 2006) and anti-inflammatory potential (Roumili *et al*., 2022). .

Phytochemical investigations carried have revealed the presence of numerous components such as: spirostanes, furostanes, glycosides, sterols (the most important of which are: β-sitosterol, stigmasterol, campesterol), carotenoids and hydroxy/alkoxyphenanthrene substituents (Bnouham *et al*., 2006; Slavova *et al*., 2022).This species also contains calcium oxalate crystals and histamines and flavonoids such as diosgenin (Kova´cs *et al*., 2007). Studies have shown that extracts of *T. communis* rhizomes and roots contain saponins (García-Herrera *et al*., 2020) and phenanthrene glycosides normally considered as a dangerous irritant poison and the poisoning symptoms include irritant purgation with burning of the mouth and skin blister (Slavova *et al*., 2022).

The present study aims to investigate *in vivo* toxic effect (acute and chronic evaluation) of TCME. Firstly, work was to determine plant toxic effect after acute administration (Lethal dose 50%). Secondary, to study the target organ for that toxicity and whether there would be any correlation between the toxic effects and of phytochemicals contained in the plant materials after chronic oral administration in female mice.

MATERIALS AND METHODS

Plant material

The medicinal plant used in the present study was *Tamus communis* called ‘karma sawda’, which belongs to the Dioscoreaceae family. *Tamus communis* rhizomes were harvested in November 2007 near Bouandes (north of the wilaya of Sétif, Algeria). Identified by Prof Laouar and a voucher specimen was deposited at the Applied Biochemistry Laboratory, University Ferhat Abbas, Setif, Algeria. The plant was dried and powdered; samples were extracted with absolute methanol. The dry extract was obtained after removing the solvent by evaporation under reduced pressure at 45° C. The extract was stored at – 20° C untill use (Krache *et al*., 2015).

Animal

Experiments were performed on adult female Wistar albino rats, weighting 201.61 ± 7.04 g. The animals obtained from ‘Pasteur Institute of Algeria’ were housed in groups of eight to ten in plastic cages at controlled room temperature. Water and food were freely available and housed for seven days before the experiments in plastic cages under standard laboratory conditions (relative humidity 50-70%, 20-22°C temperature, 12:12 h light: dark cycles). Permission for experimental use was obtained from the Laboratory of Applied Biochemistry, Ferhat Abbas University of Setif 1. All procedures were performed in compliance with

laws and institutional guidelines (Guide for the Care and Use of Laboratory Animals; NIH Publication No. 86-23, 1985).

Oral acute toxicity

Acute toxic evaluation and changes in behaviour experiments were curried in animals already fasted 24 hours before the treatment (Küçükboyaci *et al*., 2015). The acute toxicity of TMCE was studied by preparing four different concentrations (0.3, 0,6, 1.2 and 2.4 g/kg), and orally administered to four groups of seven mice. The fifth group was taken as a control and given 1.0 ml NaCl 9‰. The behavioural changes, posture and mortality were checkedfor 24 hours (Amraei *et al*., 2018).The method of Karber (Krache *et al*., 2015) was employed for the determination of acute oral lethal dose of 50 % (LD_{50}).

Chronic toxicity

Animals were divided into four dose groups of 8 animals /dose. The first group was control (given 1 ml normal saline). The other groups were given daily, *per os*, single doses of 75, 150 and 300 mg/ Kg of TCME respectively. Body weight food consumption and clinical observations have been monitored on a daily basis. To facilitate oral administration of TCME, mices were fasted three hours prior to dosing. All animals treated for 42 days then, they were fasted for about 3 h and sacrificed by euthanasia (Krache *et al*., 2015). After de-capitation, blood samples were obtained directly from the neck for haematological and serum analysis. Ani-mal abdomen was opened after abdominal longitudinal incision, organs (liver and kidney) were removed and the wet weights were recorded (Amraei *et al*., 2018).

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). Blood was collected in a heparinized tube, cen-trifuged at 4000 g/ 5min. at 4°C and plasma obtained was stored at –20° C until use for biochemical analyses. 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.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).

Organs evaluation

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. Organs were fixed in 10% formalin for 24 h, and standard dehydration and paraffin-wax embedding procedures were used. Sections (5 µm) were cut in a microtome, adhered to glass slides with. Hematoxylin and eosin-stained slides were prepared and evaluated by light microscopy (Krache *et al*., 2015).

Statistical analysis

Statistical funding 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. The comparison of the averages and the variances was done using SigmaStat 3.5, version 3, and SigmaPlot 10.0. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The methanolic extraction from the rhizomes of *T. communis* gave a yield of 6.45 % (TCME).

Oral acute toxicity

Female mice and clinical signs were individually observed during the first 30 min and regularly during the first 24 h after TCME administration (Table 1).

Table 1: Signs and symptoms of TCME toxicity on female rat score based on the order of severity.

Dose (mg/kg)	Signs and symptoms	Time
0	Normal	-
1500	Piloerection, stressed rats	30 min
3000	Piloerection, Laboured breathing, immobilization of the rats.	1 h
6000	Sleepiness, labored breathing, convulsion	24 h
12000	Labored breathing, accelerated heart rate, convulsion, severe drowsiness, leg paralysis.	1^{first} min

No sign of mortality was reported. LD_{50} is higher than 12000 mg/kg body weight (Table 2).

Table 2: Determination of the LD_{50} of TCME according to Karbar and Behrens (Krache *et al*., 2015)

Dose (mg/kg)	Nombre de rats (n)	Percentage of death (**%)**
0 (NaCl 9‰)	10	0
1500	10	0
3000	10	0
6000	10	10
12000	10	10

Effects of TCME *on* body weight

The clinical map of rats treated with the doses is free of the severe symptoms of intoxication encountered in humans (vomiting, convulsion, paralysis, accelerated respiratory rate) and of the lethal effect; nevertheless, a decrease in vivacity and diarrhea in treated rats compared to controls were seen.

Chronology of body weight evolution

Monitoring of changes in animal body weight during the subacute toxicity experiment showed a moderately significant (7%) increase in weight during the 4th, 5th and 6th weeks with the 75 mg/k dose, and a moderate decrease of 10% during the 1st week with the 300 mg/kg dose in female rats treated with TCME compared with white rats (**Figure 1**).

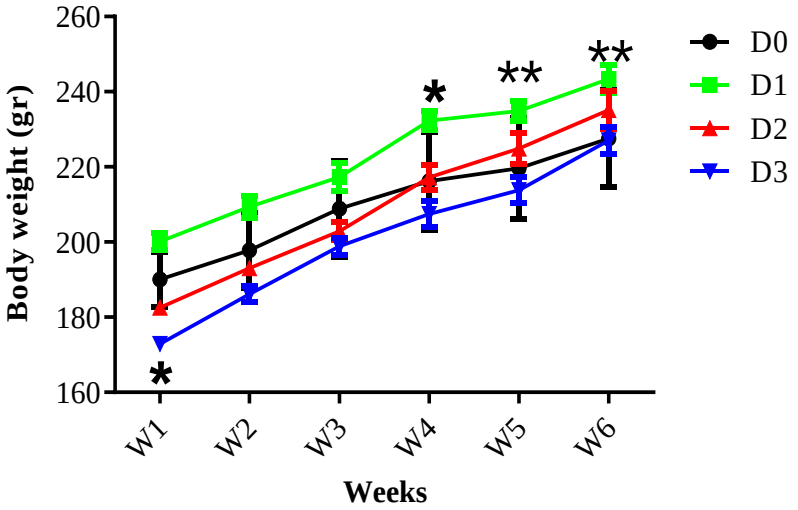


Figure 1: Change in body weight (weight - initial weight) of control and treated female rats under conditions of subacute toxicity with TCME. Values are mean ± SEM. * P≤0.05, ** P≤0.01. Doses: D0, control; D1 (75 mg/kg), D2 (150 mg/kg), D3 (300 mg/kg).

Relative organ weights

Macroscopic examination of the various organs harvested in situ revealed normal size, shape and weight. Relative organ mass values (ovaries, kidneys, spleen, liver, lungs, heart and brain) showed changes in some organs compared with controls: a highly significant decrease in relative ovarian mass was found in rats treated with TCME at all three doses tested (**Figure 2**).

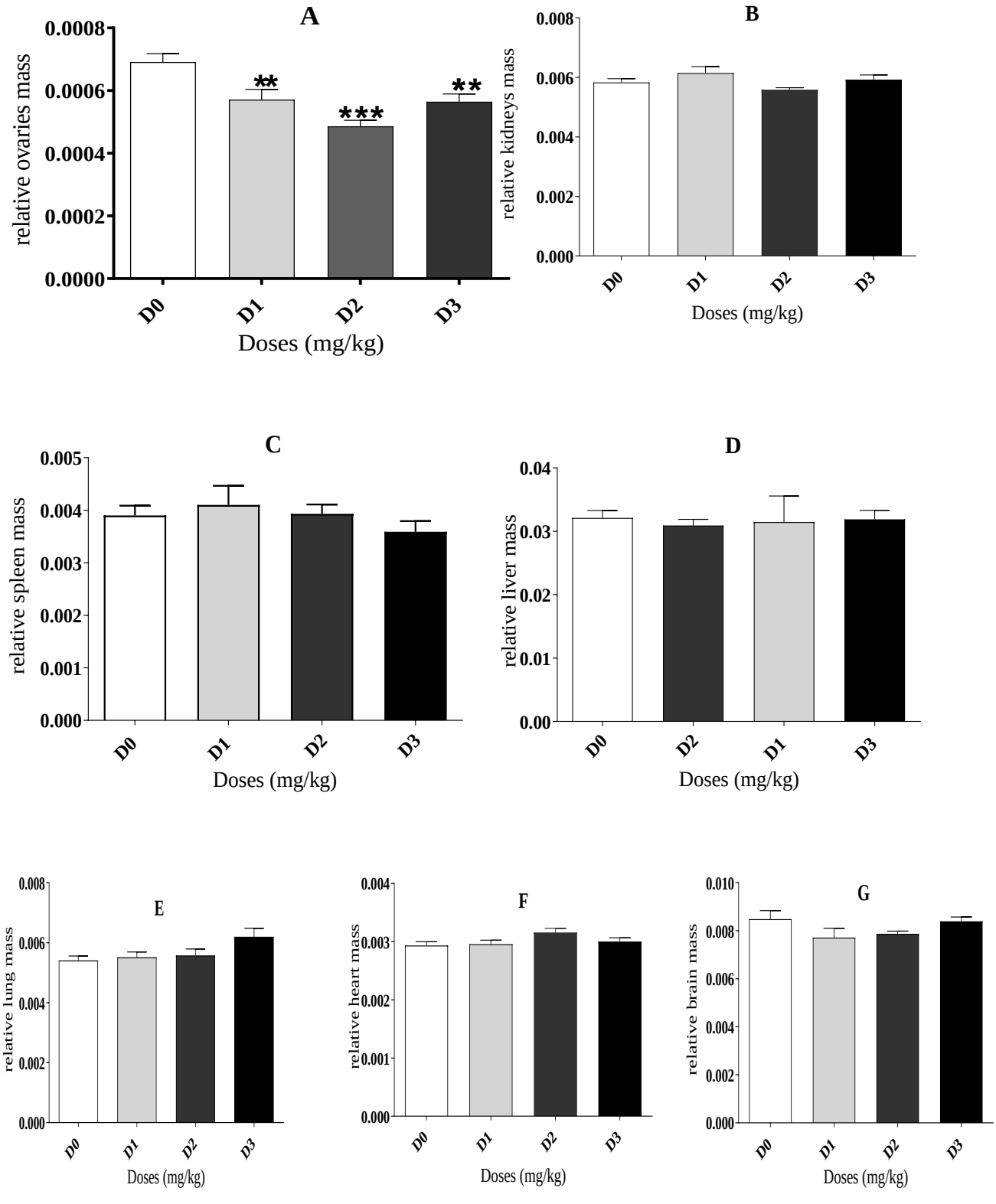


Figure 2: Variations in relative organ mass values for different doses during subacute treatment with TCME. Values are means ± SEM (n = 7-8) .Stars represent significance according to ANOVA test; * (P≤0.05), ** (P≤0.01), *** (P≤0.001). Groups: A (relative ovary mass), B (relative kidney mass), C (relative spleen mass) and D (relative liver mass), E (relative lung mass), F :(relative heart mass) and G (relative brain mass). D0 doses: control; D1, D2, D3 groups treated with 75mg/kg, 150 mg/kg and 300 mg/kg respectively.

Effects of TCME on biochemical and haematological parameters

Biochemical studies on TCME-treated rats show a significant decrease in the levels of the following parameters: Urea with doses D1,D2 and D3, Na with doses D2,D3 and UA with doses D1 andD2 and GPT with the 2nd dose compared with control groups D0 (table 3).

Table 3: Analyses biochimiques de traitement subaigu des rats femelles par TCME avec les doses D_0 (0 mg/kg), D_1 (75 mg/kg), D_2 (150 mg/kg), D_3 (300 mg/kg). (n = 8). Les données sont exprimées par moyenne ± S.E.M. * (P≤0.05), ** (P≤0.01).

Biochemical tests	**D_0**	**D_1**	**D_2**	**D_3**
Glu (gr/ L)	1.40 ± 0.08	1.34 ± 0.07	1.23 ± 0.07	1.37 ± 0.13
Urea (gr/ L)	0.70 ± 0.01	0.57 ± 0.01*	0.51 ± 0.01**	0.63 ± 0.02*
Creat (mg/ L)	8 ± 0.62	8.93 ± 0.30	6.98 ± 0.25	8.87 ± 0.14
Na (mEq/L)	171.14 ± 3.67	167.83 ± 4.42	145 ± 1.24**	155.33 ± 5.04*
K (mEq/L)	3.40 ± 0.24	4.09 ± 0.26	2.88 ± 0.07	3.78 ± 0.31
UA (mg/ L)	24 ± 2.35	15 ± 1.10**	18.20 ± 1.32*	21.38 ± 1.93
Chol (gr/ L)	0.26 ± 0.02	0.31 ± 0.03	0.26 ± 0.01	0.30 ± 0.02
TG (gr/ L)	0.79 ± 0.06	0.92 ± 0.08	0.62 ± 0.08	0.95 ± 0.10
GOT (UI/L)	105.50 ± 7.30	105.29 ± 12.53	97.60 ± 6.84	108.17 ± 12.13
GPT (UI/L)	54.25 ± 2.59	48.57 ± 2.45	28.20 ± 1.27**	58.50 ± 3.28
PAL (UI/L)	94.17 ± 5.64	107.17 ± 12.43	120.43 ± 10.44	116.89 ± 10.94

Blood results showed a significant increase in the following parameters: RBC with all three doses, MCV and MCH with the 2nd dose, RDW with the 1st and 3rd doses and HCT, PLT, HGB with the 2^{nd} and 3^{rd} doses compared with controls(table 4).

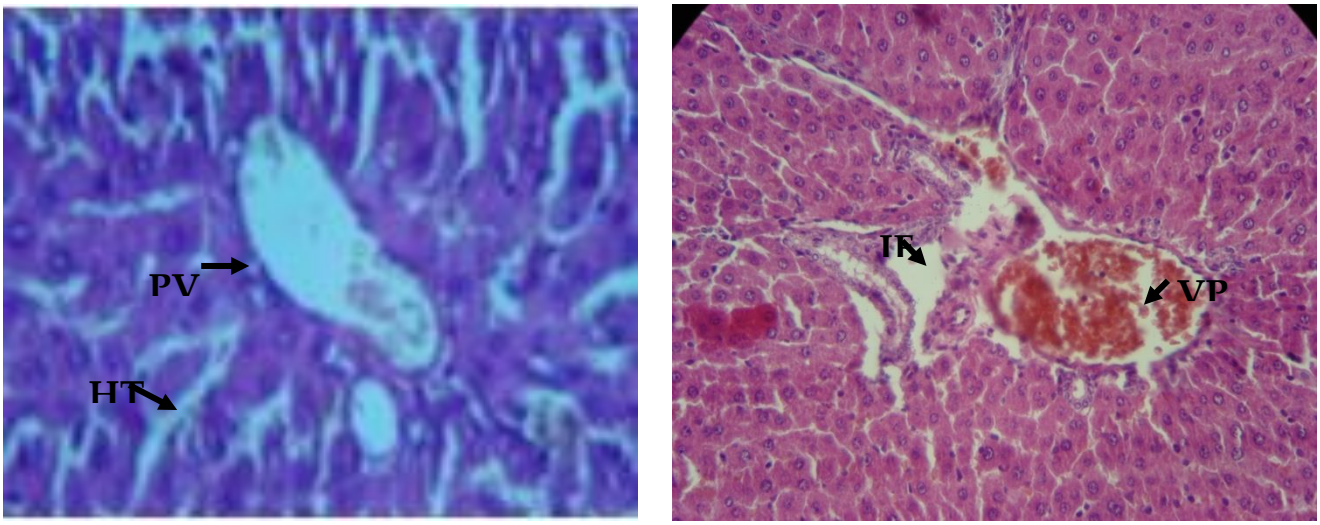
Table 4: Hematological analyses of female rats after subacute treatment with TCME doses D0 (0 mg/kg), D1 (75 mg/kg), D2 (150 mg/kg), D3 (300 mg/kg) (n = 8). Data are expressed as mean ± S.E.M. * (P≤0.05), ** (P≤0.01)

Hematological tests	**D_0**	**D_1**	**D_2**	**D_3**
RBC (10^6/mm^3)	6.34 ± 0.25	7 ± 0.14*	7.54 ± 0.12**	7.40 ± 0.17**
MCV (^{3}µm)	49.72 ± 0.52	49.89 ± 0.81	53.49 ± 0.50**	49.63 ± 0.61
RDW (%)	10.36 ± 0.25	11.76 ± 0.62*	10.27 ± 0.15	11.07 ± 0.19*
HCT (%)	32.79 ± 0.99	35.11 ± 0.75	40.80 ± 0.35**	38.31 ± 0.80**
PLT (10^3/mm)	346.50 ± 27.35	428.88 ± 42.32	550.33 ± 21.08**	482.57±19.36**
MPV (^{3}µm)	6.71 ± 0.095	6.69 ± 0.07	6.91 ± 0.05	6.68 ± 0.08

WBC (10^3/mm^3)	6.87± 0.46	7.41 ± 1.16	7.58 ± 0.44	6.57 ± 0.68
HGB (gr/dl)	10.53 ± 0.57	11.52 ± 0.63	14.27 ± 0.17**	13.26 ± 0.32**
MCH (pg)	17.15 ± 0.29	17.38 ± 0.23	18.72 ± 0.17**	17.56 ± 0.28
MCHC (g/dl)	34.49 ± 0.36	34.86 ± 0.37	34.90 ± 0.17	35.43 ± 0.45

Histopathology examination

Histological sections of the liver and kidney of treated rats compared with controls showed that the cellular architecture (lobular and tubular) of these two organs was preserved, although a few peculiarities were considered. Centro-lobular necrosis and an inflammatory infiltrate around the portal vein and space were recorded in some liver tissues of rats treated with TCME at a dose of 300 mg/kg (Figure 3). Histological sections of the kidney showed no structural changes compared with control sections.



Control D$_0$ **Treated D$_3$**

Figure 3: Histological sections of liver tissue from rats treated with D0 (0 mg/kg) and D3 (300 mg/kg) doses of TCME. Eosin/hematoxylin staining/600X magnification. HT: hepatocyte trabeculae, IF: inflammatory infiltrate, PV: portal vein**.**

Discussion

Acute toxicity

The clinical map of female rats treated under conditions of acute toxicity was characterized by an accelerated heart rate, probably due to blockage of M2 muscarinic receptors; these symptoms are similar to those observed during intoxication by *Datura stramonium* (Kenneth *et al*., 2001) respiratory distress, leg paralysis and convulsions, probably due to damage to the central nervous system by blockage of acetylcholine production in the synapses of the central nervous system (Goulle *et al*., 2015). Acute toxicity tests carried out on rats treated with the methanolic extract of the plant according to Karbar and Behrens showed that the LD_{50} is greater than 1200 mg/kg. However, according to the Hodge and Sterner toxicity classification scale (Krache *et al*., 2015), *T. communis* is almost non-toxic and its extract has a good safety margin. Capasso *et*

al., (1983) determined LD_{50} in treated rats using doses of 100, 200, 400, 600, 800 and 1200 mg/kg per os; this was estimated at 800 mg/kg based on mortalities after one week's treatment. Other publication reported all doses used (150, 300 and 600 mg/kg of *T. communis* methanolic extract studied do not show any toxic symptoms or mortality (Amraoui *et al*., 2019).This value is lower than that found in the present study, although it should be noted that for these authors, the LD_{50} determination technique was not mentioned and the sex of the rats chosen, in addition to genetic variability and rhizome chemotype.

Subacute toxicity

Female rats treated under conditions of subacute toxicity by TCME showed no serious symptoms of immediate intoxication or lethality (Capasso *et al*.,1983; Amraoui *et al*., 2019). Nevertheless, a decrease in vivacity and diarrhoea in some treated rats compared with controls was observed from the first days of treatment, possibly due to the handling conditions which put the rats under stress. Nevertheless, after a period of stabulation, they resume their usual life cycles (Muibat *et al*., 2007). Monitoring of the variation in total animal weight during subacute treatment showed for rats treated TCME (100 gr total weight/ ml NaCl 9‰): a moderately significant (7%) increase in weight during weeks 4,5 and 6 with the 75mg/kg dose; a significant (10%) decrease in weight recorded during week 1 with the 300 mg/kg dose compared with controls. This may be due to the tolerance of oral treatment with this extract (adaptation of the animals) during the six weeks with the 75mg/kg dose; whereas the slight decrease in weight of rats in the 3rd group probably occurred as a result of possible dose/absorption interactions (Manahan, 2003). From the second week onwards, the rats returned to their normal body development (adaptation to working conditions) .From these arguments; we conclude that this extract presents a good margin of safety after sub-acute administration up to a dose of 300 mg/kg.

After macroscopic examination of the various organs sampled, the relative mass values of certain organs (ovaries, kidneys and liver) showed changes compared with the controls: a highly significant reduction in the relative mass of the ovaries of rats treated with TCME at all three doses. In fact, the oestrous cycle of the rat is sensitive, taking 4 to 5 days and accompanied by the following cytological characteristics: Pro-oestrus and oestrus take 12 hours, Met-oestrus takes 21 hours and Di-oestrus takes 57 hours (Sharp Parrick and La Regina, 1998). Estrogenic chemicals are known to cause infertility by reducing the period of ovulation, disrupting the oestrous cycle and reducing the levels of sex hormones such as progesterone and endometrial development hormones. Treatment of rats with TCME reduced ovarian weight, which could be attributed to the presence of phyto-oestrogenic principles, which bind to the same intracellular receptors as oestradiol (but with a much lower affinity) and generate physiological responses similar to oestradiol, including hypertrophy of the accessory glands in males, anti-gonadotropic effects in the hypothalamic-pituitary glands and disruption of gonadal levels in both sexes in mammals (Maruo *et al*., 2003).On the other hand, phytoestrogens can reduce pituitary hormone levels by inhibiting the negative feed-back mechanism of pituitary gonadotropins (Shibeshi *et al*., 2006). Another possibility is that sex hormones (which are in turn influenced by total cholesterol levels) can affect the weight of the reproductive organs (ovaries), which are extremely sensitive to variations in these hormones (Nakamura *et al*., 2001).

Most studies relating to toxicity at the cellular level (in human or animal models) are only relevant for chronic toxicity; they are setup to ‘cure’ the conditions and prevent further damage. Although we are focusing on prevention, we include them here for completion (Martinez-Hurtado *et al*., 2018). *Tamus communis* L. young shoots, which could have an important on their nutritional composition. These processes are frequently necessary to improve their edibility and/or to remove undesirable compounds, such as oxalic acid; while in other

cases, the process is part of the traditional behavior of its consumption. In black bryony (*T. communis*) and white bryony (*B. dioica*), some toxic compounds (saponins and triterpene glycosides, respectively), or some ribosome inactivating proteins, can be found in different parts of the plant, such as fruits and subterranean tubers, and the cooking process traditionally applied to the edible parts of these species may reduce those toxic substances, by either solubilization into cooking liquid or heat degradation which destroys the toxic principles (Dogan, 2012 ;García-Herrera et al., 2020).

The significant decrease in the levels of renal balance values: Urea, Na and AU and TGP reported with doses 75, 150 and 300 mg/kg, of rats treated with TCME, respectively, may be interpreted by the administration of rhizome extractto female rats not causing changes in these parameters up to the 300 mg/kg dose.

The serum results observed in rats treated with MCT showed a significant increase (generally observed with the three doses used) in RBC, MCV, RDW, HCT, PLT, MPV, MCH and HGB levels compared with the control groups. TCME induced an increase in the number of red blood cells observed with the three doses (75, 150, 300 mg/kg), implying a potential for erythropoiesis. However, possible increases in erythropoiesis were associated with increases in haematocrit and haemoglobin with the 150 and 300 mg/kg doses. The significant increase in haemoglobinemia associated with a significant increase in MCV and MCH levels would indicate a tendency towards macrocytosis and hypochromia. In addition, these results show once again that haemoglobinemia can only be validly assessed in the context of erythrocyte cytological parameters (Ndoutamia and Ganda, 2005).

The erythrocyte distribution RDW corresponds to the coefficient of variation of an erythrocyte population is slightly increased with the 75 mg/kg dose for TCME and can be explained by an increase in the variability of erythrocyte size. The hyperplaquettosis observed with doses of 150 and 300 mg/kg may be explained by secondary hyperplaquettosis associated with damage to the spleen, martial deficiency and myeloproliferative syndromes (Bain and Gupta, 2003).

CONCLUSIONS

TCME disturbs some serum parameters biochemical parameters related to liver and kidney function and hematological parameters mainly the red blood cell count during sub-acute treatment pertreatment. We recommend that chronic toxicity studies be carried out on be carried out on the plants studied to determine whether they can be used in the manufacture of medicines. In addition, toxicity study would be required to determine the LD_{50}. Plant extracts are generally used in their raw state, which is why we have used high doses for thefor the present tests compared with reference products. To compensate this, it is preferable to isolate the active ingredients of the various plant extracts plant extracts and present them in acceptable, non-toxic galenic form toxicity. There is a need to raise awareness of the use of traditional medicine, as the risk involved is closely correlated with the notion of dose.

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