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ACUTE AND SUB-CHRONIC TOXICITY STUDY OF CORIARIA MYRTIFOLIA LEAVES EXTRACT IN RODENTS

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ABSTRACT

Coriaria myrtifolia belongs to the Coriariacae's family. It has the largest fruits in the genus Coriaria. It is an Herbal plant commonly used in western Mediterranean area. In the present study, we investigated the acute and sub-chronic toxicity of the crude methanolic extract of leaves of *Coriaria myrtifolia*. For acute toxicity, a single oral administration was performed at a dose of 2000 mg/kg body weight in Swice mice. The study of sub-chronic toxicity was evaluated by daily oral administration with the extract at dose 200 mg/kg to Wistar rats for sixty days. No mortality or signs of toxicity were observed in the acute study. Wistar rats were analyzed for body weight, blood chemical. The results showed no toxicity and mortality in either sex comparing to the control group. After sixty days, the extract of *Coriaria myrtifolia* did produced the increase of neutrophils and decrease of lymphocytes. The extracts of the leaves of *Coriaria myrtifolia* showed a low toxicity. They are good candidates for further pharmacological investigations.

KEYWORDS: *Coriaria myrtifolia*, acute toxicity, Sub-chronic toxicity.

LIST OF ABBREVIATIONS: *C.M*: *Coriaria myrtifolia*, BW: Body weight.

INTRODUCTION

Coriaria myrtifolia L. (*C.M*) called Redoul, is a shrub found in the Mediterranean area that is 2–3 m tall. Allan, 2000a, Although *C.M* is the only indigenous coriariacean species in Europe, potentially toxic exotic species have been introduced including *Coriaria Terminalis* Hemsl and *Coriaria Japonica* Gray (Bruneton, 1999; Merck, 2001). Traditionally, the leaves of redoul were intensively used in tanning and dyeing purposes. Poisoning with this plant is often accidental, by ingestion of the fruit in confusion with blackberries (Flesch, 2005; Nisse and Mathieu, 2002; Skalli et al., 2002; Alonso Castel et al., 2005, 1997; De Haro et al., 2005; Cahen, 1978). It is especially dangerous for children, confusing it with edible berries. *C.M* should be recognized as one of the most neurotoxic plants in western Mediterranean area. The coriamyrtine, a neurotoxin is abundant in other species of the genus including representants in Asia (Kariyone and Sato, 1930; Okuda and Yoshida, 1964; Chang et al., 1996) and in the New World (Reyes et al., 1998). This neurotoxic effect was similar to those of other neurotoxic alkaloids of some plants (Minodier et al., 2003, Girre, 2001). The ingestion of fruits of *C.M* can give rise to different neurological syndromes, some of which are irreversible (Carod Artal , 2003). However one study reported the toxicity of this plant to their leaves (Poyen et al., 1970). This study completed other investigations by the identification of possible target organs involved in the plant toxicity. The present study was carried out to evaluate the acute and sub-chronic toxicity of the leaves of *C.M*.

MATERIALS AND METHODS

Plant Material

C.M was collected from the North of Morocco in April 2010, and was identified by A. Ennabili, botanist in the National Institute of Medicinal and Aromatic Plants. A voucher specimen (N°INP630) has been deposited in the herbarium of Institute.

Extraction Procedure

The dried leaves of *C.M* were extracted in soxhlet apparatus, successively with hexane and methanol 85%. The extracts were concentrated under vacuum (Rotavap: Buchi), at 40°C and dried in a lyophiliser. The final extract was stored at 4°C until further use.

Experimental Animals

The study was related to rodents primarily of the mice IOPS-OFA female adults from approximately 20 to 30 g of body weight, and both of the sexes of Wistar rats weighing 200 - 300 g. They were high under conditions standards of laboratory: the top water, regular food,

maintained at 23- 25°C and cycle of 12h/12h of light/obscurity. The animals were treated according to directives of the Official Journal of the European Community about the care and of the use of the animals in a laboratory.

Acute Toxicity

Acute toxicity study for the extract leaves of the *C.M* was conducted according to the method of Organization of Economic Co-operation and Development, as per 423 guidelines (OECD) (Donald, 1997). Following the fasting period, *C.M* extract was given orally in a single dose; 2000 mg / kg of body (BW), which the single high dose is recommended by OECD guidelines 423 for testing acute toxicity (Kulkarni, 1993). The control group received The normal saline 0.9%. Observations were made and recorded continuously for 4 h and 24h to detect any eventual symptoms of toxicity: changes in physical appearance, skin, pain, stress, abdominal contraction and mortality. They were observed for 14 days after administration of the substances. All the animals were weighed before and during the observation period. Care and treatment of the mice were in compliance with the guidelines of care and use of laboratory animals (commission on life science, national research council 1996).

Sub-Chronic Toxicity Test

We used Wistar rats divided into two groups (ten rats per group) at single doses of (control; normal saline 0.9%), and 200 mg/kg body weight by oral gavage for 60 consecutive days.

The animals were weighed and observed daily for clinical symptoms including hemorrhage, diarrhea, convulsions, sedation, stimulation, colic and death.

Hematological and Biochemical Analysis

After 60 days of the treatment, blood was withdrawn through retro-orbital sinus of all rats under thiopental anesthesia. The blood was placed into EDTA vials for hematological assay and in plain vials for clinical biochemistry determination. The blood for hematological assay was immediately analyzed using a hematological analyzer "SYSMEX SERIE XE-2100". The parameters measured were white blood cell (WBC), red blood cell (RBC) and platelets (PLT). All biochemical parameters were studied by an auto-analyzer "Olympus AU 640". Clinical Biochemistry values determination were for liver profile (total protein, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST). Renal profile parameters measured were urea and creatinine. Other biochemistries were glucose, cholesterol, and triglycerides for lipid profile.

Statistical Analysis

Results were expressed as the mean ± standard error of mean (SEM). Statistical analysis of data was carried out using student's t-test. Differences in mean were considered to be significant when $P < 0.05$.

RESULTS

Acute Toxicity: The result showed no mortality of mice in dose of 2000mg/kg of *C.M*. And no toxic effect was observed during the period test. Physical observation indicated that any mouse showed signs of toxic effect such as changes on skin and fur, eyes, behavior pattern, tremors, salivation, diarrhea, sleep, and coma. Mean body for mice treated with 2000 mg/kg of *C.M* extract is illustrated in table 1. The results indicated that no change of body was remarked.

Table1. Body weight (g) of control and mice treated with *C.M* leaves extract recorded during acute toxicity study.

	T0	Week 1	Week 2
Control	27,5±1,7	28±1,6	26,17±1,20
2000 mg/Kg	26,78±1,65	24,02±3,15	25,31±2,31

Values are expressed as mean ± SEM, n = 6. No significant difference was observed

Sub chronic Toxicity

Bodyweight, Mortality, and Clinical Signs: In the subchronic toxicity, all the rats used survived until the end of the observation period, but visible signs of less mobility, sedation, inductor of spasm were noted after administration orally. The effect of oral gavage was also evaluated on body weight. The results of in vivo toxicity show that treated groups at the administered doses of 200 mg/kg/day appear normal, presented a significant weight gain in both sexes of the rats. There was no mortality recorded in the group treated by the extract at dose 200 mg/kg. The result of the effect of the extract on the bodyweight is presented in Table2.

Table 2. Body weight (g) of control and rats treated with *C.M* leaves extract recorded during subchronic toxicity study.

T0	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Control 288,92±25,48	299,69± 25,51	308,01± 24,72	318± 25,35	325,86± 25,15	306,17± 18,44	315,91± 29,78	310,29± 32,19	308,91± 25,18
200mg/kg 258,42±14,23	258,42± 14,23	253,73± 14,6	249,85± 15,72	258,09± 14,72	271,63± 15,05	270,9± 16,13	283,2± 14,55	282,01± 13,93

Values are expressed as mean ± SEM, n = 10. No significant difference was observed.

Heamatological Analysis: In general the results showed no change in RBC and WBC values. Furthermore, the values for the neutrophiles and platelets were slightly increased in treated group compared to the control after oral administration for 30 and 60 days (p<0, 05). However, lymphocytes were decreased 62, 27±2,82 vs 70,33±0,68 after 30days, and 56,23 vs 70,33 after 60 days (table 3).

Table 3. Heamatological parameters of control and rats treated with *C.M* leaves extract after 30, 60 days.

	Control	200mg/kg BW	
		30 days	60 days
RBC (10^6/µL)	8, 39±0,48	8,98±0,18 ^{NS}	9,94±0,14 ^{NS}
WBC (10^3/µL)	9,57±1,94	10,88±1,86 ^{NS}	10,98±1,86 ^{NS}
Neutrophiles (%)	20,45± 0,72	29,17±3,27 *	32,03±4,88 *
Lymphocytes (%)	70,33±0,68	62,27±2,82 *	56,23±5,88 *
Monocytes (%)	4,76±0,67	4,52±0,41 ^{NS}	5,62±0,14 ^{NS}
PLT (10^3/µL)	530±24,63	707±41,67 *	722±128**

Data are expressed as mean ±S.E.M from 10 independent experiments. NS: no significant, *p value less than 0.05, (p < 0.05): significant value, **: P< 0.01: Statistically very significant from control by Student's test.

Biochemical Analysis: The data in table 4 showed no difference for the various biochemical parameters for most of the rats groups between 30 days and 60 days. No Significant reductions were recorded in the plasma concerning ALT, AST and ALP.

Table 4. Biochemical parameters of control and rats treated with C.M leaves extract.

	Control	200mg/kg BW	
		30 days	60 days
Liver profile			
AST (U/L)	154,4±19,2	132±3,33 ^{NS}	115,5±29,4 ^{NS}
ALT(U/L)	75,2±7,05	60,6±7,12 ^{NS}	57 ±12,42 ^{NS}
ALP(U/L)	436,33±87,18	359,8±65,38 ^{NS}	419,33±227 ^{NS}
Renal profile			
Urea (g/l)	0,28±0,02	0,35±0,03 ^{NS}	0,35±0,03 ^{NS}
Creatinine (mg/l)	4,6±0,24	4,98±0,31 ^{NS}	5,2±0,37 ^{NS}
Blood chemistry			
Glucose (g/l)	1,12±0,12	1,32±0,10 ^{NS}	1,14±0,05 ^{NS}
Cholesterol (g/l)	0,72±0,05	0,61±0,02 ^{NS}	0,63±0,02 ^{NS}
Triglycerides (g/l)	0,99±0,25	0 ,84±0,17 ^{NS}	0 ,57±0,11 ^{NS}

Values are expressed as mean ± standard deviation, n = 10. NS: no significant.

DISCUSSION

According to the system of global harmonization of Chemicals (GHS), this product is classified Category 5, which the higher lethal dose 50 (LD50) is 2000 mg/kg. Toxicological research and testing helps to live safely and to derive benefit from natural and synthetic substances. In this study no changes attributable to treatment were found in body weight and any macroscopic changes that could point to the cause of the death observed up to the maximum dose of 2000 mg/kg (BW) of the extract. This result suggested that extracts of *C.M* don't cause any acute toxicity. The LD50 is more than 2000 mg/kg after oral administration comparing to intraperitoneal administration LD50 200mg/kg. Plant may contain compounds that will not be absorbed when it taken orally which would lead to decrease toxicity (Brander et al., 1991). Several authors prove that the phytochemicals' compositions toxicity of some vegetables are responsible to their toxicity (Orech et al., 2005). Our results suggest that the toxicity of *C.M* by i.p administration was related probably to their composition of tanins found in the extract (Boudkhili et al, 2011). The interest of the plant requires an approach to its toxicity.Furthemore, our results could be explained by the absence of the coriamyrtin, which is may be eliminated by the protocol of extraction.

Body weight is known to be one of the most sensitive indicators of adverse effects may be due to the plant toxicity (Raza et al., 2002; Teo et al., 2002). The slight increase in body weight gain is a simple and sensitive index of no toxicity to this plant. The liver and heart release ALP and an elevation in the plasma concentrations are indicators of hepatic and cardiac damage and toxic activity in the tissues (Mythilpriya et al., 2007). The aminotransferases are the most frequently utilized and specific indicators of hepatocellular necrosis. The ALT is primarily localized to the liver and the AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain and liver (Friedman et al 2003). Our results indicate that the methanolic extract of *C.M* when taken for long periods of time might not cause a liver damage after oral administration. All other biochemical parameters were remained normal without any significant difference.

Results showed that during the treatment with the extract at dose 200 mg/kg/day, some clinical significant changes are observed like abdominal contraction. The behavior of treated rats of illness effects and pains abdominal could be a symptom of the depression in this extract **(Boudkhili et al., 2014)**. In this context, author proved that the depression often coexist with chronic pain (Dworkin et al., 1991).The serum hematological parameters were

studied to evaluate the possible inflammations or depressions. Some modifications in hematological parameters after oral administration of *C.M* were noted like the increase of lymphocytes and decrease of neutrophils. There are no significant changes noted on RBC and WBC. Our result is in concordance with literature (Dhabar and McEwen , 2001, Ceddia MA et al, Maes et al., 1992). In fact, Thurin and Baumawn, 2003 proved that depression cause the decrease of lymphocytes and increase of neutrophils. Stress leads to a redistribution of leukocytes, neutrophiles accumulate in the blood, whereas cells migrate from the blood to the mucous membranes.

CONCLUSION

There have been few previous studies on *C.M* toxicity. These studies report variable values for the clinic cases poising in human cases, most of them related to the fruits, while no toxicological parameters are reported. Furthermore, no toxicological data exist on the extract of leaves of C.M until now. The recent study was undertaken to give further contribution to the knowledge on the oral acute toxic effects in mice of leaves of C.M and the oral subchronic toxicity in rat plan a new pharmacological investigation under traditional use.

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