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A study of acute dermal toxicity of *Artemisia herba-alba* Asso essential oils

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Medicinal plants constitute a huge reserve of many therapeutic virtues recognized by their powerful effects since the dawn of time. Our research focused on the study of acute skin toxicity (AST) of *Artemisia herba-alba* Asso (Aha) essential oils, obtained by hydrodistillation. AST has been evaluated, using rats of the species *Rattus norvegicus* with reference to a method proposed by the organisation for economic co-operation and development (OECD) guideline. Biochemical parameters, alanine aminotransferase, aspartate aminotransférase, total bilirubin, direct bilirubin, and creatinine were measured in whole blood after animal sacrifices. In parallel, the histological liver and kidney sections were analyzed. The Lethal dose$_{50}$ (LD_{50}) value was found greater than 5000 mg/kg. In addition, the biochemical markers of the tested rats did not change during the experiment compared to the control group. No tissue damage has been detected. *A. herba alba* Asso essential oil was considered non-dangerous, which can be used to heal against all benign or severe illnesses.

Keywords: Acute skin toxicity, *Artemisia herba-alba* Asso, Essential oils, LD_{50}.
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Introduction

Medicinal plants constitute a main source of many active ingredients that have been known since ancient times to treat severe or benign illnesses. The white wormwood is a perennial plant; the leaves are hairy and silvery. The flowers are all hermaphrodite[1]. Fruits are achenes. Its vegetative growth takes place in autumn; flowering begins in June and develops mainly at the end of summer[1]. This plant is (relatively) abundant in the Iberian Peninsula and reaches the highest population density in the centre of Spain spreading over the eastern, southeastern and southern Spain[2]. Several structural types of sesquiterpenes lactones have been found in the aerial parts of *Artemisia herba-alba* Asso, the eusmanolides followed by the germacranolides. The flavonoids detected are flavones and glycosides of flavonols[3]. Fourteen compounds were identified in *A. herba-alba* essential oil, cis-thujone (25.5%), trans-thujone (17.7%), vanillyl alcohol (11.5%), and nor-davanone (7.8%) were the main components[4]. Essential oils obtained from *A. herba-alba* air-dried leaves and flowers were efficient in inhibiting the oxidation of linoleic acid which is an important issue in food processing and preservation with an IC_{50} value equal to 0.2 mg/mL[5]. At a concentration lower than 242 µg/mL, the antioxidant activity of the *A. herba-alba* oil is lower than the positive control BHT, however, for a concentration higher than 242 µg/mL, the oil exhibited the greatest inhibitory activity reaching as high as 87.82%[(ref 6)]. Evaluation of skin irritancy using animals is one of the most common procedures performed on industrial and pharmaceutical products. Many results were found after exposure to different extracts obtained from the wormwood. The administration of *Artemisia* essential oils in mice by intra-peritoneal route induced a sedative action. The mice remain prostrate and inert until death at a dose of 2 mL/Kg[7]. Rats administered a single 5000 mg/kg dose (regulatory limit dose) of *Artemisia dracunculus* L. survived, gained weight and appeared to be active and healthy with no signs of gross toxicity, adverse pharmacological effect, or abnormal behaviour during the 14-day post-treatment observation period[8]. The purpose of this study is to present new results depending on the toxicity of *A. herba alba-asso* essential oils after skin exposure. However, the chemical compounds of this essential oil have been identified previously[9].

Materials and Methods

A. herba-alba Asso essential oil

The aerial part of *A. herba-alba asso* was harvested in March 2015, in Mascara region (Northwest of Algeria), this plant was previously identified and stored as voucher specimens in the herbarium of the

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institute under the following code (AS00006). The extraction of essential oils was carried out by hydrodistillation[10] and its composition has been revealed previously by the GC/MS, forty chemical compounds were found, whereas, camphor (34.8%) and chrysantenone (19.6%) appeared as majoritar[9].

Animals

In the period from 2017 to 2018, twenty-four healthy animals, males and females belonging to the *Rattus norvégicus* strain were supplied by the animal facility of the University of Mascara, weighing 250±10 g, this is approximately between **7**-8 weeks *of* age. Breeding cages were equipped with feeders and bottles to provide them with food and water *ad libitum*. Drinking water was changed every 24 hours. The temperature of the breeding room was kept at 25 °C, with an alternation of 12 hours of light and 12 hours of dark, the humidity was between 50 and 70%[(ref 11)]. The experimental protocols were approved by the Animal Research Ethics Committee of the Mascarian University, Mustapha Stambouli (ARECM) according to the Adelaide University Animal Ethics Committee (Ethics number M/76/98).

Acute skin toxicity

This test was as per the OECD guideline[12]. The animals were divided into two groups, the first received the essential oils and the second representing the control group. Each group had 12 rats, 6 males and 6 females. Exactly 5 g of gelatin (from the brand Vahiné) was placed in 100 mL of sterile distilled water, the whole was placed in a water bath set at 100 °C. Melted gelatin was mixed with *A. herba-alba* essential oils, to obtain a single dose of 5000 mg/kg. Gelatin was used as an emulsifier. Because its hydrophobic aspect gives it wide use in several pharmaceutical applications. This macroprotein was found to be non-toxic, non-irritant, and non-carcinogenic[13]. In parallel, the control group was treated with only the gelatin solution. About 24 hours before the test, the dorsal region of the animals was shaved, with an area of 2 cm^2. The preparations were applied and they were kept in contact with the skin, covered with a bandage to avoid any kind of ingestion by animals. Then the rats were returned to their cages. Observations made during the test were based on changes on the skin in terms of irritation and even oedema, also on behavioural manifestations like ataxia, tremor, loss of balance, and unconsciousness. The animal weight was determined during the experiment. The LD_{50} was calculated according to the Bliss and Quart method[14]. Fourteen days later the animals were sacrificed. Whole blood was collected and organs were removed.

Biochemical analysis of blood parameters

The purpose of the quantification of the biomarkers of blood, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), total bilirubin, direct bilirubin, and creatinine was to deduce the resulting signs of pathology after administration of the extracts by the dermal route. This approach was confirmed by the histological study of the hepatic and renal tissues. The blood was centrifuged at 3000 rpm for 10 minutes. Then the plasma was transferred into new heparin tubes.

Histopathology

In histopathology, the diagnosis of various abnormalities in fresh tissue is difficult. That is why the organs must be fixed in formalin solution (10%). Then, dehydration, clearing, wax infiltration, embedding were carried out to obtain clear and very fine cuts, so that the light passes through them easily[15].

Statical analysis

Statistical analysis was performed using SPSS software. All the experiments were carried out in triplicate. The results were expressed on average ± SEM. However, the P value <0.05 was considered significant. All the graphs were drawn by Graphpad Prism software version 7.

Results

Behavioural manifestations

During the experiment, the animals administering the essential oils by the dermal route survived. This indicated that the LD_{50} was greater than 5000 mg/kg, referring to the scale proposed by Hodge and Sterner[16]. It seemed that this oil was included in the category of non-toxic substances. However, the behaviour of the animals was manifested by weakness, slow breathing, introversion and ataxia compared to the controls. All of these signs disappeared after the first 24 hours.

Weight evaluation

Fig. 1 showed a remarkable weight gain. This parameter was considered important to determine acute skin toxicity because it can reflect directly the physiological state of animals in toxicity cases.

Determination of blood biochemical markers

The biochemical markers of the blood of rats did not change during the experiment (Table 1).

Table 1 — Effects of *Artemisia herba-alba* Asso essential oils administered via a dermal route on biochemical parameters in rats

Biochemical parameters	Male	Male control	Female	Female control
Total bilirubin (mg/L)	0.5±0.14	0.3±0.02	0.4±0.2	0.3±0.1
Direct bilirubin (mg/L)	0.59±0.03	0.6±0.01	0.2±0,0	0.3±0.03
Aspartate aminotransferase (µ/L)	49.7±0.5	54.7±0.7	38.2±1.2	38± 2
Alanine aminotransferase (µ/L)	24.7±0.9	35.1±0.6	24±0,0	34±0,9
Creatinine (mg/L)	4.8±0.19	4.7±0.12	3±1.3	2.9±1.0

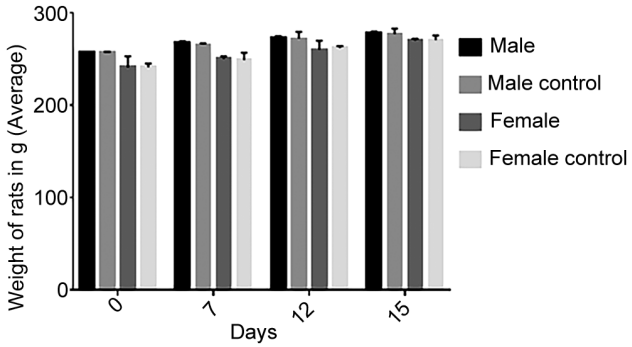

Fig. 1 — Effects of *Artemisia herba-alba* Asso essential oil administered via the dermal route on the weight of rats.

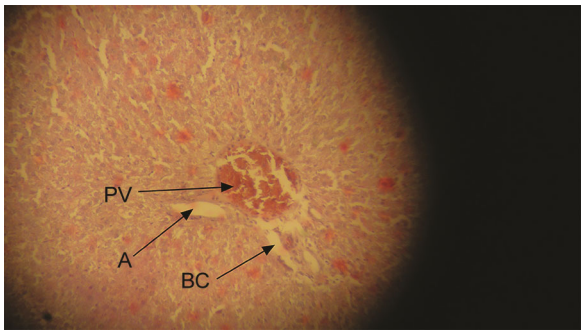

Fig. 2 — Microscopic observation of a normal hepatic lobule without inflammation, which shows an artery (A), portal vein (PV) and biliary canaliculus (BC) with magnification (X100).

Histopathology

Macroscopically, the livers of the rats tested showed liver parenchyma of a brownish appearance. Whereas, microscopic observation of the liver fragments (Fig. 2), presented a parenchymal structure of lobulated form, composed of the hepatic lobule of hexagonal shape, centred by a centro-lobular vein and delimited by portal spaces, which showed an artery, a vein and a canaliculus biliary. With the absence of necrosis, periportal interstitial hepatitis, steatosis, infiltration of mononuclear cells and cholestasis. In parallel, macroscopic observation of the kidneys (Fig. 3) showed renal parenchyma with a brownish appearance. The microscopic observation of the renal fragments demonstrated the presence of the glomérulo with the total absence of any thyroid or lateral aspect

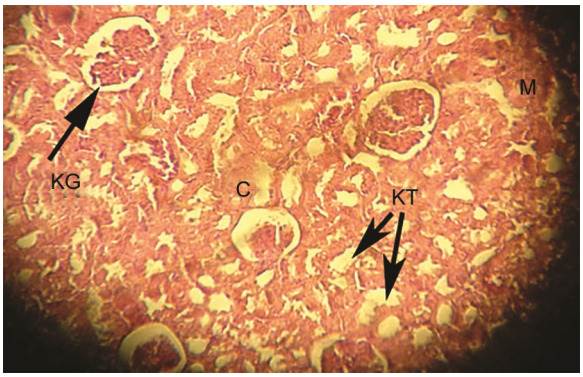

Fig. 3 — Microscopic observation at the cortical level in kidney cuts (C) with the presence of Kidney glomerulus (KG), medullary (M) and kidney tubules (KT) with magnification (X100).

of hyaline. The blood capillaries were preserved, while the renal tubules showed regular cuboids cells with complete absence of any signs of inflammation.

Discussion

The present study was conducted to determine the acute skin toxicity of Aha essential oils, mainly used in the traditional pharmacopoeia of the Mascara region. The essential oil of this herb has anthelmintic, disinfectant, and antispasmodic effects[17]. The oil marked by its variable composition according to geographic factors and the most abundant compounds appears to be α-thujone, β-thujone, chrysanthenone and trans-sabinyl acetate[18]. Cases with severe intoxications in humans have been reported after consumption of essential oil rich in thujone[19]. The acute skin toxicity of *Artemisia* essential oil has not been previously reported. But, these plants of the same genus have been subjected to oral toxicity or other toxicities. The present study confirmed that camphor and chrysantenone, previously found in these essential oils had no toxic effect on organs after dermal exposure. In an acute toxicity test, animals treated with *Artemisia* did not show any visible toxicity at the concentration of 3 g/kg, with a decrease in locomotor activity[20]. The oral LD_{50} of *Absinthe* essential oils was found at around 0.37 g/kg[21]. Also, no significant change was observed in body weight

followed for two weeks after exposure to a dose of 2 g/kg of *Artemisia campestris* administered by gavage[22]. Numerous doses of essential oils of Aha administered at a rate of 0.01 mL/g by intra-peritoneal injection to rats gave an LD_{50} of 615 mg/kg[23]. It should be mentioned, that the incorporation of the dry powder of the aerial part of *A. herba-alba* in the food of the flesh cushions had no effect on the blood parameters, cholesterol, globulin, albumin, and total proteins[24]. No deaths were recorded within 72 h in LD_{50} assay in animal groups after administration of *A. herba-alba* extracts at 1 and 2 g/kg[25]. No significant histopathological changes were noted in the studied organs (liver, heart, kidney, brain, and medulla spinalis) in *A. herba alba* group receiving aqueous extracts after chronic and acute treatment[26]. On the contrary, *A. herba-alba*, can cause seizures in children and pregnant women[27]. The aqueous extract obtained from the flowers of white wormwood did not significantly alter the activity of alkaline phosphatase or the concentration of urea and creatinine in the plasma of rabbits, however histopathological examination in mice and rabbits revealed mild hydropic degeneration in hepatocytes and proximal convoluted tubules. Glomeruli appeared normal. In the duodenum, there was mild oedema of the substantia propria of the mucosa[28]. In addition, a dose of 2000 mg/kg *A. campertis* essential oil taken orally, showed intense ulceration of the gastric mucosa[22]. At the concentration of 2 µg/mL of the essential oil, leishmanias do not grow beyond the 3rd day. There are immediate and total leishmanicidal effects after application of 4 and 10 µg/mL[29]. The ingestion of *A. herba-alba asso* by adult female rats did not have a negative effect on fertility without the increase in ovarian weights and a decrease in viable fetus's number[30]. Acute renal failure was caused by *A. herba-alba*[31]. On the other hand, the toxic effect of the essential oils of *A. herba-alba* was used also in oncology to reduce uncontrolled cell division. The cytotoxic activity of aerial part essential oil of *A. herba-alba* on CEM cancer cell lines with IC_{50} = 6 µg/mL[32]. Results indicated that the methanolic extracts obtained from *A. herba-alba* leaves specifically target carcinoma cells lines inducing their killing[33]. It seemed also that the essential oil of this plant was used as an insecticidal agent against the pest. *A. herba-alba* and two other species of *Artemisia* genus have insecticidal effects against *Acanthoscelides obtectus* (Say)[34]. *A. herba-alba* essential oil was more toxic against insects (*O. surinamensis* and *T. castaneum*) at all tested concentrations with lethal concentration LC_{50} equal to 3.05 µL/L[35].

Conclusion

The acute skin toxicity results of the present research may give important proofs regarding the therapeutic safety of this essential oil on the proper functioning and survival of the organism. In perspective, It is better to have wide use of the essential oil of *A. herba-alba* in the pharmaceutical field and to consider it as an alternative source of synthetic drugs.

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Conflict of interest

None

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