Biomedicine & Pharmacotherapy 108 (2018) 144–152

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Effect of *Hypericum humifusum* aqueous and methanolic leaf extracts on biochemical and histological parameters in adult rats

Afef Nahdi[a,*], Imen Hammami[a], Ridha Ben Ali[a], Olfa Kallech-Ziri[b], Ahmed El May[c], Michèle Véronique El May[a]

[a] Research Unit n° 17/ES/13, Tunis El Manar University, Faculty of Medicine, 15 of Djebel Lakhdar Street, 1007, Tunis, Tunisia
[b] National Research Institute of Physico-chemical Analyses, INRAP, Sidi Thabet, 2020, Ariana, Tunisia
[c] Salah Azaiez Cancer Institute, Place Bab Saadoun, 1006, Tunis, Tunisia

ARTICLE INFO

Keywords:
Hypericum humifusum
Oxidative stress
Liver
Histology
Rats

ABSTRACT

Hypericum genus is traditionally known for its medicinal use and its therapeutic and antioxidant effects. However, the toxic effect of this plant has not been much explored. Our study aimed at investigating the effect of *Hypericum humifusum* (*Hh*) leaf extracts on oxidative stress parameters in male rats. For it, we first focused on the phytochemical analysis of the aqueous and methanolic extracts of *Hh* leaves. Hence, Wistar rats were treated per gavage for 30 days and divided into Control (1 mL/rat, distilled water), A200 group (200 mg/kg body weight (bw) aqueous extract), A400 group (400 mg/kg bw aqueous extract), M10 group (10 mg/kg bw methanolic extract), M20 group (20 mg/kg bw methanolic extract). The phytochemical analysis revealed the presence of tannins, flavonoids, steroids, carbohydrates, and phenolic compounds. Biochemical and histological investigations were performed in plasma and liver tissue. Liver tissue homogenates were used for the measurement of malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) levels. At the same time, alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH) were assayed in plasma samples. Histological study was also conducted in liver. We showed that *Hh* extracts reduced relative liver weight and increased ALT, AST, LDH activities in treated groups compared to control group. These results were associated with an increase of MDA levels and a decrease of antioxidant enzyme activities (CAT and SOD) in liver tissues of treated rats. Histology of liver demonstrated several alterations showing necrosis, altered hepatocytes and lymphocyte migration mainly in A200 group and dilated sinusoids, foamy appearance of hepatocytes and lymphocyte accumulation in the other treated groups. This original work indicated that chronic consumption of *Hh* leaf extracts has no antioxidant effect but instead it induces oxidative stress and enhances markers of cell damage which was confirmed by histological study of liver rats.

1. Introduction

Medicinal plants have therapeutic properties due to the presence of hundreds or thousands of bioactive natural compounds called secondary metabolites accumulated in various plant organs and sometimes in specialized cells. Many of these secondary metabolites have biological activities present in plant extracts and their components like polyphenols, flavonoids and essential oils that have a beneficial effect on health and a role of natural antioxidants [1]. Among these medicinal plants *Hypericum* genus is cited that comprises 484 species worldwide [2] and can be found spontaneous in Minor Asia, Northern Africa and Mediterranean region. In Tunisia, the *Hypericum* genus is widely distributed especially in the humid and semi-arid regions [3]. It includes eight species: *H. perforatum L.*, *H. humifusum L.*, *H. tomentosum L.*, *H. perfoliatum L.*, *H. triquetrifolium L.*, *H. Richeri L.*, *H. androsaemum L.* et *H. ericoides L.* [4]. Traditionally, *Hypericum* species and particularly *H. perforatum* (*Hp*) extract, well known worldwide and most studied, have been used for the treatment of trauma, burns, hemorrhoids, rheumatism, neuralgia, gastroenteritis, ulcers, hysteria and depression [5,6]. Furthermore, they also showed anti-inflammatory and antibacterial action, and antioxidant, antiviral, antidepressant and analgesic effects [7,8]. *Hp* extract is best known for its use in the treatment of mild to moderately severe depressive disorders [9]. It demonstrated antioxidant properties and increased gene expression of antioxidant enzymes [10]. Aydin showed that *Hp*, as antioxidant agent, can protect liver against ischemia-reperfusion injury [11]. Pharmacological

* Corresponding author.
E-mail address: afef.nahdi@gmail.com (A. Nahdi).

https://doi.org/10.1016/j.biopha.2018.09.034
Received 27 March 2018; Received in revised form 21 August 2018; Accepted 5 September 2018

activities of *Hypericum* extracts are attributed to hypericin and hyperforin [12,13], and also to flavonoids, the main component present in plant, which might play a role in preventing several kinds of cancer [14]. The effect of these compounds may be different depending on the method of preparation of the *Hypericum* extracts and the process of harvesting, storage, drying and extraction [15]. Other authors suggested that *Hp* has a potential to prevent stress memory disorders [16]. However, it has been shown that *Hp* extracts administered to rats during pregnancy and breastfeeding are toxic [17]. Most scientific works were reported on *Hypericum perforatum* extract and its antioxidant effect, while the effect of other species of *Hypericum* such as *Hypericum humifusum* (*Hh*) was poorly explored. The latter is known for its use in Spanish folk medicine as an agent to treat digestive disorders and skin diseases [18], and for its antiseptic, astringent and antispasmodic properties [19]. This species is also used against viruses thanks to its high content of naphtodianthrones, and have antibacterial and cytotoxic activities thanks to the considerable amounts of phloroglucinol [20]. But, few studies have determined the exact effective dose of this plant for human usage and its toxicity level.

In this work, we focused on the phytochemical analysis of the aqueous and methanolic extracts of *Hypericum humifusum* leaves. Our study aimed too at investigating the effect of 30 days-chronic administration of the two different leaf extracts of *Hh* on oxidative stress parameters in male rats. Biochemical assessments of liver MDA level, CAT and SOD activities, as oxidative stress indices, plasma ALT, AST and LDH levels, as liver function test, and complete blood counts were conducted as well as a liver histological study.

2. Material and methods

2.1. Sample collection

Hypericum humifusum (*Hh*) was harvested in the forest region of northwestern Tunisia (Beja-Gbolat) in March 2015. The fresh leaves were identified by Pr. Olfa Kallech-Ziri (National Research Institute of Physico-Chemical Analysis, INRAP, Sidi Thabet, Tunisia). The Voucher specimen was deposited at the herbarium of the Higher Institute of Biotechnology of Beja (Tunisia).

2.2. Plant extraction

Two different extraction methods from leaves were used in this study to obtain Aqueous (A) or Methanolic (M) extracts.

2.2.1. Aqueous extract

Fresh leaves of *Hh* were weighed (52 g), washed then carefully crushed in distilled water. The mixture was stirred for 48 h at room temperature, and then filtered three times using Whatman paper. The filtrate was diluted in distilled water and used to prepare two doses (200 and 400 mg fresh leaves/kg body weight) of aqueous extract. Finally, solutions were stored at 4 °C until use.

2.2.2. Methanolic extract

The dried (40 °C for 24 h) and powdered plant leaves underwent lipid degradation by Soxhlet method with pure hexane. The latter was eliminated by evaporation and the residue was dried and weighed. 20.23 g of plant powder were extracted at room temperature for 48 h with methanol (200 ml) by maceration. Then, solvent was evaporated under reduced pressure at 40 °C using a rotavapor followed by lyophilization with nitrogen to completely remove the solvents used. The powder was diluted in distilled water to obtain two concentrations of methanolic extract (10 and 20 mg/kg body weight). Solutions were stored at 4 °C until use.

2.3. Phytochemical screening of Hypericum humifusum extract

2.3.1. Phytochemical qualitative analysis

Chemical tests were carried out using standard procedures to identify secondary metabolites in the *Hypericum humifusum* leaf extracts. These latter were screened for the presence of flavonoids, anthocyanins, tannins, phenolic compounds, saponins, anthraquinone, reducing compounds, starch, organic acids, terpenoids and sterols by using the standard tests as described in literature [21].

2.3.2. Phytochemicals quantitative analysis

Total phenol and flavonoid contents in the *Hypericum humifusum* extracts were assayed using the Folin-Ciocalteu reagent following Rigane et al. procedure [22]. The flavonol content in the extract was estimated according to the reported method in Almaraz-Abarca et al. [23]. The anthocyanin quantification was performed using the pH differential method described by Zhe et al. [24] based on the color change of anthocyanins depending on pH.

Reducing carbohydrates were assayed using Miller's method, and the optical density was measured at 540 nm [25]. The protein content was performed according to Bradford's method and the absorbance was read at 595 nm [26]. All determinations were performed in triplicates.

2.4. Animals and treatment

Thirty adult male *Wistar* rats weighing between 200 and 250 g (15 week-old) were purchased from Pasteur Institute of Tunis, Tunisia, housed in stainless steel cages and maintained in a controlled environment under standard conditions of temperature (23 ± 2 °C), humidity (55 ± 5%) and light (12 h light:dark cycle, lights on 07:00–19:00 h). The rats were allowed to acclimatize in the laboratory for a period of 1 week before the beginning of the study. During the study, they received commercial pellet diet (Industrial society of food, Sfax, Tunisia) and water *ad libitum*. All the studies on animals were conducted in accordance with the European Convention (2010) for the protection and use of vertebrate animals and approved by the animal care committee of the Faculty of Medicine of Tunis.

The filtrate of aqueous extract and the lyophilisate of the methanolic extract are both diluted in distilled water before being administered to rats. The latter extract contains alcohol which is toxic chemical element *in vivo*, for that we perform a lyophilization of the methanolic extract of *Hh* with a yield of 5% (Y%). We choose the aqueous extract doses (200 and 400 mg/kg bw) according to Husain et al. study [27], while we choose the lyophilisate methanolic extract doses by analogy with those of the aqueous extract based on the yield of an alcoholic extraction after lyophilization by applying the following formula:

Dose of lyophilisate methanolic extract = Dose of aqueous extract × Yield lyophilization extraction

The *Hypericum humifusum* extracts were administered to rats *per os* by gavage in a volume of 1 mL/200 g bw. The rats were weighed and randomly assigned into five groups of six animals each and treated for 30 days, as follows:

- C: the Control group received 1 mL/200 g bw of distilled water
- A200: rats received 200 mg/kg bw of *Hh* aqueous extract
- A400: rats received 400 mg/kg bw of *Hh* aqueous extract
- M10: rats received 10 mg/kg bw of *Hh* methanolic extract
- M20: rats received 20 mg/kg bw of *Hh* methanolic extract

The rats were weighed daily and the doses of *Hh* extracts were adjusted according to the body weight change during the treatment period. At the end of the experimental period, the animals were anaesthetized by peritoneal injection, using chlorhydrate of Ketamine (50 mg/kg, IP) then sacrificed by decapitation. Blood was immediately