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EVALUATION OF ERYTHROCYTES TOXICITY AND ANTIOXIDANT ACTIVITY OF ALKALOIDS OF *FUMARIA CAPREOLATA*

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ABSTRACT

Objective of the present work was to evaluate alkaloids extract of *Fumaria capreolata* for possible toxicity and antioxidant potential. Antioxidant activity was measured using the 2, 2 0-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging. The significant correlations exist between extract concentrations and percentage scavenging activity of radicals in all models. These results clearly indicate that the alkaloid extract from *Fumaria capreolata* is effective free radical scavenger and chain breaking antioxidant. In order to assess the toxicity of the alkaloids extract, acute and cell toxicological studies were carried out in mice and erythrocytes. After acute administration of doses 200, 1000 and 2000 mg/kg of total alkaloids, there were no remarkable changes in general appearance and no deaths occurred in any experimental group in both sexes.

KEYWORDS: **Acute toxicity, Antioxidant, Alkaloids, Erythrocytes, *Fumaria capreolata*.**

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INTRODUCTION

The genera *Fumaria* (*Fumariaceae*), is widely distributed in Europe and Northern Africa. Fumitory or earth smoke has been used in many countries for the treatment of skin diseases, rheumatism, hypertension or infections. Nowadays, this herb is a component in several phyto-pharmaceuticals, mainly used to treat functional diseases of the hepatobiliary system discernible as colicky pains affecting the gallbladder, biliary system and the gastrointestinal tract. These biological activities of *Fumariae herba* are mainly associated with the presence of isoquinoline alkaloids. Various types of isoquinolines alkaloids have been isolated from this family [1]. Oxidative stress resulting from the toxic effects of free radicals on the tissue plays an important role in the pathogenesis of various pathological conditions such as ageing process, anemia, arthritis, asthma, inflammation, ischemia, neurodegeneration, Parkinson's disease, and perhaps dementia. Antioxidants are radical scavengers, which protect the human body against free radicals.Free radical also induces liver damage. Likewise, metabolism of certain drugs like paracetamol, produce free radicals, which cause liver damage [2]. Antioxidants may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by other mechanisms and thereby help in preventing the free radical induced diseases. Toxicology is the aspect of pharmacology that deals with the adverse effects of bioactive substances on living creatures along with their diagnosis and clinical use. In order to develop and establish the safety and efficacy level of a new drug, toxicological studies alkaloids extract from *Fumaria capreolata* are very essential.

MATERIALS AND METHODS

(i) Plant material and chemicals

Aerial parts of herbarium was collected from Bejaia area, in the North east of Algeria in May 2012 when they were at the flowering and fruit setting stage, and verified from Flora of Algeria [3]. Plants were authenticated by a Taxonomist of the Laboratory of Botany of Bejaia University. 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2 0- azino- bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Potassium ferricyanide, Trolox, cafeic acid, and ethylene diamine tetra acetic acid (EDTA) were obtained from Sigma (Sigma–Aldrich, Germany).

(ii) Extraction of alkaloids [4]

Aerial parts of the plants were dried in oven at 40°C for overnight and ground into fine powder using a grinder. The powder samples (20 g) from several individuals of each population, were extracted with methanol (300 ml) in a Soxhlet apparatus for 8 hours, and then evaporated to 2 mL in vacuo. The methanolic residue was taken up in 20 ml of 2.5% hydrochloric acid and filtered. The aqueous acid solution was adjusted to pH= 9.50 with concentrated ammonium hydroxide and extracted with dichloromethane (3 x 20 ml). The extracts were dried over magnesium sulphate and the solvent evaporated to afford a crude extract of alkaloids. After evaporation the yield of each fraction was calculated. The alkaloid extract (AFC) thus obtained is stored at 4°C until further use.

(iii)Acute toxicity study

Healthy adult male NMRI mice were allowed to fast by withdrawing the food and water for 18h and divided into four groups (n=6). Group I-IV animals were orally fed with alkaloid extract of *Fumaria capreolata* in increasing dose levels of 200, 1000 and 2000 mg/kg respectively, while group IV (untreated) served as control. The animals were observed continuously for first 4 h for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality if any, and next 14 days for any lethality or death. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals of SAIDAL, Algeria.

(iv) Determination of erythrocytes toxicity [5]

Veinous blood was collected from a healthy volunteer after obtaining informed consent and delivered into heparinized tubes. Whole blood was centrifuged at 4 000 rpm, for 10 min at 4 ^{0}C, washed three times with phosphate

buffered saline (0.2 M, pH 7.4) and re-suspended in the same buffer to the desired hematocrit level. Five fold serial dilution of the alkaloid extract were made in phosphate buffered saline. A total volume of 0,8ml for each dilution was placed in ependr off tube. A negative control tube (containing saline only) is also included in the analysis. Fresh Human erythrocytes were added to each tube, to give a final volume of 1ml. Solution were incubated at 37 ^{0}C for 30mn and all tubes were centrifuged for 5mn and then observed for haemolysis. Complete haemolysis was indicated by a clear red solution without any deposit of erythrocytes. Haemolysis was also checked microscopically and presence or absence of intact RBCS. Absorbance of the contents was measured at 540 nm. Increase in absorbance indicates greater haemolysis. The haemolysis percentage was calculated as follows: % haemolysis = [(Ac − At)/Ac] ×100, where Ac is the absorbance of control, and At is the absorbance of test (in the presence of alkaloids).

(v) Determination of reducing activity [6]

Potassium ferricyanide (2.5 ml, 10 mg/ml) was added to samples in phosphate buffer (2.5ml, 200 mM, pH 6.6) and the mixture was incubated at 50 ^{0}C for 20 min. Trichloroacetic acid (2.5 ml, 100 mg/ml) was added to the mixture, which was then centrifuged at 3000 g for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5 ml, 1.0 mg/ml), and then the absorbance was read at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing activity.

(vi) Determination of DPPH radical inhibition [7]

The reductive potential measures the ability of a sample to act as electron donor and, therefore, reacts with free radicals converting them to more stable products and thereby terminates radical chain reactions. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The samples were added to an ethanolic solution (1 ml) of DPPH radical (the final concentration of DPPH was 0.1 mM). The mixture was shaken vigorously and allowed to standard room temperature for 20 min; the absorbance of the resulting solution was then measured spectrophotometrically at 517 nm. The radical scavenging activity was calculated as follows: scavenging rate = [(Ac − At)/Ac] ×100, where Ac is the absorbance of pure DPPH and At is the absorbance of DPPH in the presence of extracts.

(vii) Determination of ABTS radical cation inhibition [8]

The ABTS radical cation was generated by reacting 1mM ABTS with 0.5 mM hydrogen peroxide and 10 units/ml horserad-ish peroxidase in the dark at 30 ^{0}C for 2h. After 1ml of ABTS radical cation was added to samples, the absorbance was recorded at 734 nm after 10 min. The radical scavenging activity was calculated as follows: Scavenging rate = [(Ac − At)/Ac] ×100, where Ac is the absorbance of pure ABTS and At is the absorbance of ABTS in the presence of extracts.

(Viii) Scavenging of hydrogen peroxide [9,10]

A solution of hydrogen peroxide (2mmol/l) was prepared in phosphate buffer (pH7.4). Alkaloids of *Fumaria capreolata* (100µg/ml) were added to a hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. The percentage scavenging activity of hydrogen peroxide by AFC was calculated using the following formula: scavenging rate = [(Ac − At)/Ac] ×100, where Ac is the absorbance of control and At is the absorbance of a solution of hydrogen peroxide in the presence of extracts.

RESULTS

1. Acute and cellular toxicity

The acute toxicity was conducted as per the OECD guidelines 420, where the limit test dose of 2000 mg/kg used. Mice administered with

total alkaloid of *Fumaria capreolata* did not show abnormal behavior except for mild sedation, for initial 4 h after drug administration. No test substance related mortality was observed at 2000 mg/kg during 14 days after treatment with alkaloid of *Fumaria capreolata*, and no changes attributable to treatment were found in body weight. The hemolytic activity of alkaloids was examined against human erythrocytes. A small percentage of haemolysis is observed with different concentrations of alkaloids, and it is almost the same percentage of haemolysis absence of alkaloids (Fig 1), so alkaloids have almost no toxicity to human erythrocytes even with the concentration of 1mg/ml. These results are confirmed by microscopic observation. No change in shape of erythrocytes with different concentrations of alkaloids (Fig 2).

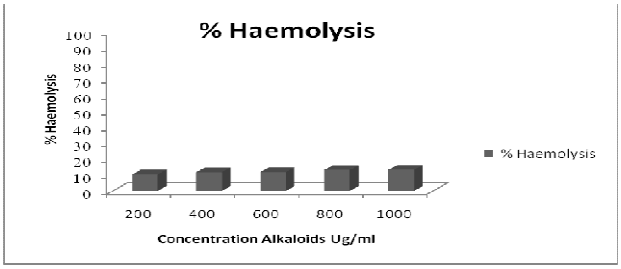

Figure 1
Percentage of haemolysis with different concentrations (200- 1000µg/ml) of total Alkaloids extract from Fumaria capreolata.

Morphology of erythrocytes under optical microscope (Gx100)

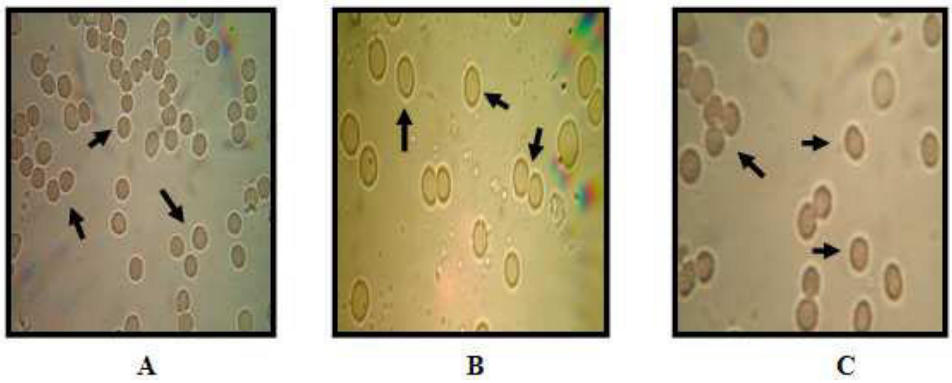

Figure 2
Picture of human erythrocytes observed under an optical microscope (Gx100) treated with alkaloids of F.capreolata. (A: Control; B: [Alkaloid] = 400µg/ml; C: [Alkaloid] = 1mg/ml).

2. Reducing power

The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts. It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used. The reducing power of a substance can be defined by its ability to transfer an electron or to give a hydrogen atom. In this test we measure this potential through the transformation of Fe^{3+} to Fe^{2+} by donating electrons. The best gear power obtained with the extract of *F. capreolata* (0.57 AU ± 0.005) with the concentration of 800µg/ml (Fig 3).

Reducing power

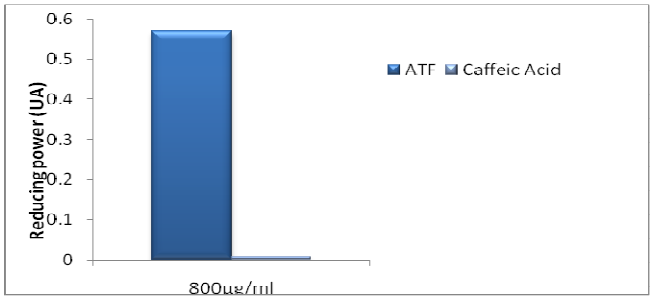

Figure 3
Reducing activity of alkaloids extract from F. capreolata (AFC) at 800µg/ml.

3. DPPH radical inhibition

The reduction ability of DPPH radicals formation was determined by the decrease in its absorbance at 517 nm induced by antioxidants. We evaluated the anti-radical activity of the total alkaloids from of *F. capreolata* by the use of a stable free radical of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). As shown in Fig 4, the anti-radical activity of alkaloids increases with the concentration in the range of 0-800 µg/ml. The best effect of DPPH radical scavenging is achieved by the alkaloid extract of *F. capreolata* (68.31 ± 0.35%) at a concentration of 100 µg/ml. The best IC50 was obtained by BHA (8, 21µg / ml), followed by the ATF extract (28, 87 µg/ml).

DPPH radical inhibition

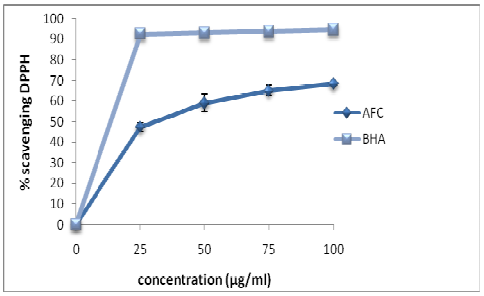

Figure 4
Effect of AFC on DPPH scavenging. (AFC: Alkaloid extract of F. capreolata).

4. Determination of ABTS radical cation inhibition

The activity is expressed as alkaloids the Trolox Equivalent Antioxidant Capacity (TEAC) which corresponds to the concentration of Trolox (similar hydrophilic vitamin E) giving the same antioxidant capacity that concentration of test compound. Over the TEAC value, the higher the antioxidant activity is effective. The best antioxidant capacity is obtained by ATF (728.12 mmol Trolox equivalent /g of extract).

5. Scavenging of hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a strong oxidant formed in living tissue by several metabolic processes and detoxification is essential for cell protection. The test results of scavenging H_2O_2 alkaloids are presented in Fig 5. At a concentration 100 µg/ml of alkaloid extract of *F. capreolata* has a scavenging effect of (78.89 ± 0.48%). This effect is similar to that exerted by caffeic acid (95.21 ± 0.01%).

Scavenging of H_2O_2

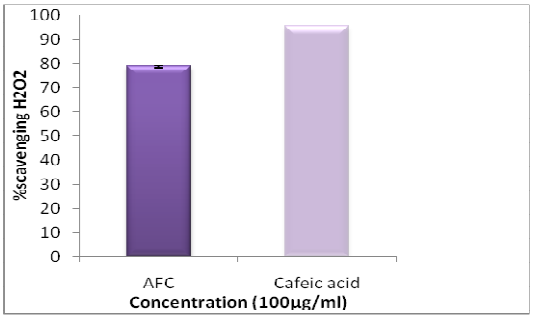

Figure 5
Effect H_2O_2 Scavenging of alkaloids extract and cafeic acid.

DISCUSSIONS

Herbal medicines have received greater attention as an alternative to clinical therapy and demand for these remedies has currently increased. Experimental screening method is important in order to ascertain the safety of traditional and herbal product and also to establish the active component of the herbal products. In acute toxicity study, there was no any mortality observed up to the maximum dose level of 2000 mg/kg of the alkaloids of *Fumaria capreolata*. The use of herbal preparations as a treatment of diseases is very common, in Algeria, rural communities used herbs as food and traditional medicine. *Fumaria capreolata* has been used traditionally by some population as digestive agent, food and skin healing. Some of these usages have been studied *in vitro* and in animal model. Therefore it is essential to evaluate the toxicity of the total alkaloids of *Fumaria capreolata* in animals and erythrocytes as cell model to ensure of its safety. The AFC did not affect the body weight of the treatment rats when compared to the control rats. Food and water intake of the treatment and control group were similar. In a normal healthy organism or human body, the generation of pro-oxidants in the form of ROS is effectively kept in check by various levels of antioxidant defense. Antioxidants present in various dietary supplements offered their beneficial effects by neutralizing these ROS during various disease conditions [11]. A great number of *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plants extracts. The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, and prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts [12]. Hydrogen peroxide occurs naturally at low concentration in the air, water, human body, plants, microorganisms, food and beverages. Human beings are continuously exposed to H_2O_2 indirectly via the environment which is estimated as 0.28 mg/kg/day. Hydrogen peroxide enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH) that can initiate lipid peroxidation and cause DNA damage [10]. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines [13]. The alkaloid extract of *Fumaria capreolata* was evaluated by using various *invitro* antioxidant models such as DPPH radical, hydroxyl radical, reducing power, superoxide anion scavenging activities. The reducing power (0.57 AU ± 0.005) with the concentration of 800µg/ml, and H_2O_2 radical scavenging activity (78.89 ± 0.48%) at a concentration 100 µg/ml alkaloids *F. capreolata* suggests the presence of reducing molecules. These results suggest that an alkaloid extract

of *Fumaria capreolata* has several antioxidant properties. The antioxidant property may be due to the protopine which are major chemical constituents and is shared by berberine, which are previously reported as major constituents responsible for antioxidant activity [1].

CONCLUSION

It was concluded that the acute toxicity study of alkaloid of *Fumaria capreolata* at 2000 mg/kg administered orally to mice did not caused any death. This result was confirmed by the haemolysis test with different concentrations. Haemolysis of the erythrocytes was not observed at any dilution of extracts. Our study showed that alkaloid of *F. capreolata* has an effective antioxidant potential in various *in vitro* assay systems. Antioxidant properties of *Fumaria capreolata* could be beneficial in pathological condition involving oxidative stress.

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