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Quarterly reports

Biological Activities of Algerian *Bunium incrassatum* (Boiss.) Batt. & Trab. Against Sodium Fluoride Toxicity in Rats

Unique digital address (Digital object identifier [DOI] equivalent):
https://www.fluorideresearch.online/epub/files/262.pdf

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Accepted: 2024 Feb 16

Published as e262: 2024 Feb 18

ABSTRACT

Objective
Bunium incrassatum is one of the very important medicinal species grown in north of Algeria. In this work, the total phenolic, the flavonoid content and phytochemical screening of bioactive substance the species roots were investigated.
Material and methods
In order to evaluate the antioxidant and hepatoprotective activities of the species, the rats received sodium floride (NaF) concomitant with *B. incrassatum* root extract in a dose of 300 mg/kg. Important parameters such as transaminases (AST and ALT), blood Sucre, total bilirubin and hemoglobin were conducted, also, antibacterial and *antioxidant activity* were analyzed.
Results
The obtained results revealed remarkable antioxidant activities of *B. incrassatum* extract. Compared to the control group, the NaF-treated group showed significant increase in transaminases and bilirubin levels with concomitant decrease in blood sugar and hemoglobin levels. In the *B. incrassatum* co-treatment with NaF group, *B. incrassatum* restored the changes observed by NaF treatment.
Conclusion
These findings reveal that *B. incrassatum* supplementation has a protective effect against NaF induced hepatotoxicity in rats caused by the pollutant sodium fluoride.
Key words: *Bunium incrassatum, phenolic compounds, antioxidant and hepatoprotective activities, blood sugar, hemoglobin.*

INTRODUCTION

During the last years, attention has been drawn by pharmacologists and medical researches to the fact that plants contain chemical agents responsible for antioxidant activity. Furthermore, they are able to increase endogenous antioxidant defenses of the cell [1].

Increasing interest in plant-based medicinal resources has led to additional discoveries of many novel compounds, such as steroidal alkaloids, saponins, terpenoids, glycosides, in various Angiosperm and Gymnosperm species. [2]

The most diverse and original forests of the Mediterranean basin are found in Algeria, which has a flora of 3139 species [3] distributed in nearly 150 families among which 653 species are endemic with a rate of about 12.6% of endemism. [4]

Aromatic and medicinal plants in Algeria, with other forest species, constitute an important contribution to the development of the local and national economy, this is among the reasons that have prompted us to study this species widely used in phytotherapy and only few studies has been conducted on it.

Bunium is a genus of flowering plants of the family Apiaceae, it has about 50 species and is distributed in Asia, Europe and North Africa, the species of this genus are aromatic plants with medicinal properties, their grains and essential oil are often used in food and medicine. [5]

Bunium incrassatum (Boiss.) Batt. & Trab. is a 40-60 cm perennial plants, this species is an important endemic medicinal plant in the north of Algeria. [3]

Characterized by white flowers and tuberculous roots that are very nutritious and usually eaten like potatoes, *B. incrassatum* has been used as astringent, anti-diarrheal and are useful against inflammatory hemorrhoids, moreover, this plant is used for the treatment of bronchitis and cough[6]. In eastern Algeria, it is used to increase the weight and milk secretion in some farm animals [7], also it has various biological activities related to digestive and urinary tract disorders.

It has been reported [8] that this plant is used in chronic stomach diseases, colitis, jaundice, chronic cholangitis, swelling and kidney stones. Previous phytochemical studies on *Bunium incrassatum* growing in Algeria revealed the composition of essential oils [6]. Sodium fluoride (NaF) is an environmental pollutant that can cause many metabolic disorders, high level of fluoride acts as a potential pollutant with very high toxicity, associated with hematologic damage [9]. NaF can cause toxic effects on many tissues and organs, including soft tissues, causing a wide range of symptoms and pathological changes. Fluoride induces hematological abnormalities which include lower hemoglobin (Hb) content and anemia is well established. [10]

The aim of this study is to assess the antioxidant activity of *Bunium incrassatum* extract and to evaluate its in vivo biological activities against sodium fluoride (NaF) toxicity induced in rats which would be realized for the first time, because to the best of our knowledge it has not been previously carried out.

MATERIAL AND METHODS

Plant material

The plant material (roots) of *Bunium incrassatum* plant, commonly known as *Talghouda*, is collected from the region Mila in the North-east of Algeria, (Latitude: 36°27'01" Nord, Longitude: 6°15'51" Est) during February 2019. The roots were washed thoroughly under running tap water and were left slowly to welt at room temperature for a few weeks. The dried roots were then grounded into fine powder using an electric grinder.

Phytochemical screening

Phytochemical screening of bioactive substance in *B. incrassatum* roots was carried according to standard procedures [11]. The screening of flavonoids [12], tannins [13] and anthraquinones [14] were carried out.

Extraction

The extraction procedure was followed as described in [15] ; air-dried finely powdered roots of *B. incrassatum* (100g) are subjected to extraction in 70% ethanol/water mixture (30/70: v/v) at room temperature. The resulting extracts were combined and concentrated under vacuum to give the crude extract to be used in different biological activities.

Total phenolic and flavonoids contents

The total phenolic content (TPC) was determined by a spectrophotometer using the Folin-Ciocalteu method [16]. An amount of 20 µl of *B. incrassatum* extract was added to 100 µl of Folin-Ciocalteu reagent. After 8 min, 300 µl of saturated

sodium carbonate solution (25%) was added. The absorbance was measured at 765 nm. The calibration curve was prepared with gallic acid solutions, and the results are given as gallic acid equivalents (GAE) per 100 mg of extract (mg GAE/100 mg Ext).

The total flavonoids content (TFC) of *B. incrassatum* extract was determined. An amount of 1 ml of *B. incrassatum* extract was separately mixed with 1 ml of 2% aluminum chloride. The absorbance of the reaction mixtures was measured against blank at 420 nm. For TFC quercetin was used to make the standard calibration curve. TFC was expressed in quercetin equivalents (QE) per 100 mg of extract (mg QE/100 mg Ext) [17]

Flavonols contents

The reaction mixture, containing 0.5 ml of *B. incrassatum* extract and 1.5 ml of ethanol, 0.1 ml sodium acetate and 2.8 ml distilled water. After mixing, the solution was incubated for 30 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 415nm. Flavonones and flavonols contents were expressed in quercetin equivalents (QE) per 100 mg of extract (mg QE/100 mg Ext) [18].

In vitro biological activities

Antimicrobial activity

The anti-microbial activity for *B. incrassatum* extract was evaluated using a disc diffusion method [19]. The microorganisms, *Gram-negative bacteria*, used in this assay were the colonies of *Escherichia coli* ATCC 387 (American type culture collection). To prepare the inoculum, a suspension of colonies was realized in sterile physiological water (0.9%). 10 µl of *B. incrassatum* extract (0.03 g/ml), was added to a sterile paper disc of 6mm of diameter, placed in Petri dishes with agar nutrients containing the *E. coli* suspension. After 20min at room temperature, the Petri dishes were incubated at 37°C for 24 hours. The antibacterial activity of each extract was evaluated by measuring the diameters of the inhibition zones around the discs.

Antioxidant activities

DPPH° scavenging activity : The DPPH° scavenging capacity of *B. incrassatum* extract was measured as described in Sarikurkcu *et al.,* [20] with slight modifications. In brief, 1ml of methanolic extract at different concentration was mixed with 1ml of DPPH methanolic solution (0.004%). The reaction tubes were left in the dark at room temperature during 30 minutes. The absorbance (Abs) of different solutions is measured at 520 nm. Ascorbic acid was used as positive control. The scavenging activity was calculated as follows:

DPPH ° scavenging activity (%) = [(Abs $_{control}$ –Abs $_{sample}$)/Abs $_{control}$] × 100

Hydrogen peroxide scavenging activity: A solution of H_2O_2 (20 mM) was prepared in phosphate buffer saline (PBS, pH 7.4). 300µl of the extract at different concentrations were added to 600µl of H_2O_2 solution in PBS. After 10 min the absorbance was measured at 230 nm against a blank solution that contained H_2O_2 solution without the extract [21]. The percentage of H_2O_2 scavenging of *B. incrassatum* extract was calculated as follows:

H_2O_2 scavenging activity (%) = [(Abs $_{Control}$ – Abs $_{sample}$)/Abs $_{control}$] x 100.

Total antioxidant capacity: The total antioxidant capacity (TAC) of *B. incrassatum* extract was estimated by the phosphomolybdenum method [22]. A 0.3 ml extract at different concentration was combined with 3 ml of the phosphomolybdenum solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated in water at 95°C for 90 min. After returning to room temperature, the absorbance was measured at 695 nm. After returning to room temperature, the absorbance was measured at 695 nm. The percentage of TAC of *B. incrassatum* extract was calculated as follows:

TAC (%) = (Abs $_{sample}$– Abs $_{control}$)/(Abs $_{sample}$) ×100

In vivo biological activities

Animals

Male Albino Wistar rats (200-210g) were used for this study; they were purchased from Pasteur Institute, Algiers (Algeria). All animals were kept in a temperature-controlled room with a 12/12 hours dark/light cycle and had free access to regular rat food and tap water.

The assay was conducted in accordance with the European Communities Council Directive (2010/63/EU) for animal experiments and the protocol employed was approved by the biology institute (Mentouri Brothers University Constantine1) under inscription number 06/SNL/14.

Animals were acclimatized for at least 1 week prior to the experiments. After the acclimation period, the rats were randomly divided into four groups (n=5) as follows:

Group 1: Normal control: rats received distilled water for 15 consecutive days.

Group 2: Negative control: (NaF): The rats received sodium floride (NaF) at 600 ppm in drinking water for 15 consecutive days. [23]

Group 3: Positive control: (curcuma +NaF): The rats received NaF as in group 2 concomitant with curcuma in a dose of 10mg/kg by intraperitoneal injection for 15 consecutive days. [23]

Group 4: (*B. incrassatum* extract+NaF): The rats received NaF as in group 2 concomitant with *Bunium incrassatum* root extract in a dose of 300 mg/kg orally by gavages for 15 consecutive days.

At the end of the experiment, all the rats were deprived of food, though not water, for 12 h before surgery. Rats were anesthetized and blood samples were collected from the portal vein. After blood centrifugation at 2300 g for 10 min (4°C), the plasma was collected and used to evaluate biochemical parameters.

Biochemical parameters

Hemoglobin levels were estimated according to Drabkin's method [24]. Serum transaminases, total bilirubin and glycaemia levels were carried out using commercial assay kits, according to the manufacturer's instructions.

Statistical analysis

All the *in vitro* determinations were carried out in triplicate (n=3). For *in vivo* study (n=5) the statistical comparisons were made by Student's t-test and one way ANOVA. Results are expressed as a mean value ± standard error (Mean ±SE).

RESULTS

Phytochemical screening

As shown in Table 1, the screening of *B. incrassatum* root extract indicates the presence of tannins, flavonoids and anthraquinones, also the presence of important total phenolic content (TPC) (3.65 mg GAE/100g Ext), total flavonoid content (TFC) (2.53 mg QE/100g Ext) and flavones and flavonols content (FFC) (0.28 mg QE/100g Ext).

Table 1. Phytochemical screening and quantification of phenolic compounds of *B. incrassatum*

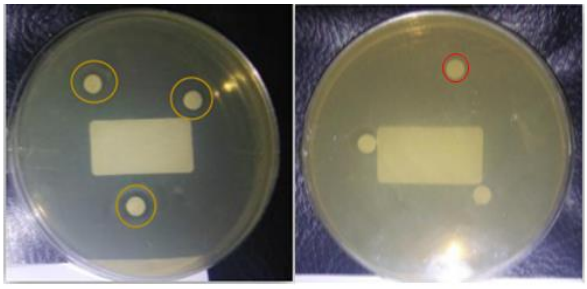
	TPC (mg GAE/ 100g Ext)	TFC (mg QE/ 100g Ext)	FFC (mg QE/ 100g Ext)
Flavonoïds (+) **Tannins (+++)** **Anthraquinones (+)**	3.65 ± 0.09	2.53 ± 0.02	0.28 ± 0.01

In vitro biological activities

Antimicrobial activity

The antimicrobial activity and antioxidant effectiveness of plant extracts are the most studied characteristics for their importance for the control of bacterial diseases affecting humans and animals. [25]

The results of anti-microbial activity of *B.incrassatum* root extract are presented in Figure 1. The visual observation of Petri dishes reveals a clear zone of 4 mm around the paper discs where our plant extract was used unlike the control (distilled water) which has no inhibition zone.



(a) (b)

Figure 1. Antibacterial activity of *B. incrassatum* roots extract (a) and control (b)

Antioxidant activity

DPPH test is a fast and sensitive test to demonstrate the antioxidant activity of the plant species, results obtained are shown in Table 2, we can observe that the activity is proportionally related to the plant extract concentration, no matter the method used. Ascorbic acid presents a higher antioxidant activity.

Table 2. Antioxidant activity of B*. incrassatum* species

	Concentration (mg/ml)	*B. incrassatum*	Ascorbic acid
DPPH (%)	0.1	33 ± 0.19	85 ± 0.24
	0.5	51 ± 0.15	85 ± 0.13
	1	58 ± 0.14	86 ± 1.42
H_2O_2 (%)	0.1	4.61 ± 0.22	45.15 ± 2.13
	0.5	10.3 ± 1.81	65.02 ± 1.76
	1	24.52 ± 0.30	87.32 ± 2.01
TAC (%)	0.1	74.79 ± 4.57	49.47 ± 4.31
	0.5	92.02 ± 1.64	67.98 ± 3.05
	1	96.87 ± 0.48	87.43 ± 1.43

In vivo biological activities

Hepatoprotective activity

Serum transaminase (AST and ALT) are commonly used as hepatic markers to assess liver disorder. As depicted in table 3, NaF treatment significantly increased the serum transaminases levels comparing to the control. These changes reflected the occurrence of liver damage caused by NaF toxicity leading to the release of the transaminases enzyme from hepatic cytosol to circulation sanguine. [26]

Co-treatment with *B. incrassatum* (300 mg/kg) protected the liver against NaF toxicity and ameliorated its functioning as shown in table 3, by the significant decrease in these liver function bio-markers compared to the NaF treated group. This protective effect could be due to the ability of *Bunium incrassatum* to preserve the hepatocytes membrane integrity [26]. Data obtained in the table 3 also revealed that disturbed liver function in NaF group reflected by increased levels of total bilirubin (Bil) when compared with the control group. Co-treatment with *B. incrassatum* extract induced a decrease in bilirubin levels.

During our study we recorded also an increase in blood sugar (Gly) in rats treated with NaF (2 g/l) compared to control rats (1.30 g/l), this indicates impairment in glucose metabolism.

Results presented in Table 3 show that the hemoglobin (Hb) values are divided in the 3 different statistical groups, the highest values are registered for positive control rats and rats treated with *B. incrassatum* plant extract with 14.80 g/dl and 15.5 g/dl respectively.

Table 3. In vivo biological activity of Bunium *incrassatum* species

Groups	ALT (U/l)	AST (U/l)	Bil (mg/l)	Gly (g/l)	Hg (g/dl)
Control	55.7 ± 2.83 b	74.62 ±2.67 b	1.89 ± 0.13 b	1.30 ± 0.16 bc	12.12 ± 1.7 b
NaF treated	98.57 ± 2.98 a	104.7 ± 9.2 a	2.73 ± 0.18 a	2 ± 0.36 a	9.26 ± 1.3 c
Curcuma+NaF treated	45.51 ±3.45 b	62.64 ± 4.6 b	1.70 ± 0.19 b	1.35 ± 0.06 bc	14.80 ±0.8 a
B. incrassatum extract + NaF treated	58.37 ± 1.18 b	68.56 ± 9.7 b	1.59 ± 0.16 b	1.12 ± 0.24 b	15.5 ± 0.9 a

DISCUSSION

Phytochemical screening

B. incrassatum root extract indicates the presence of tannins, flavonoids and anthraquinones, which agree with a previous works realized on the same species [6], they demonstrated the presence of two coumarins, b-sitosterol, sucrose and oleic acid, and the presence of coumarins and sesquiterpenes. [27]

In comparison to data obtained from grains of a *Bunium bulbocastanum*, [28], 0.520 mg AGE/mg of TPC, 0.0016 mg QE/mg of TFC and 0.0039 mg QE/mg of flavonols, which are very lower values relative to our results. As we used root parts tubers, which are an accumulation organ, comparing to grains, it might explain our superior values. Hence, it has shown that geographical and climatic factors, genetic factors, and also the degree of maturation of the plant have a strong influence on the polyphenols content.

In vitro biological activities

Antimicrobial activity

According to an earlier study [29], the strain of *E.coli* we used is resistant to our plant extract, because the inhibition diameter is less than 8 mm. a previous test showed that *B. incrassatum* has a good antibacterial activity with an inhibition zone diameter of 8 mm [6]. It could be explained by the concentration of the plant extract used or the yield obtained after extraction.

Antioxidant activity

Recent studies realized on *B. incrassatum* roots essential oils, reported that species has a very good antioxidant activity [30]. The ability of the *B. incrassatum* extracts to scavenge hydrogen peroxide is demonstrated in table 2, which is related to the extract concentration.

The total antioxidant activity of *B. incrassatum* roots extract was evaluated by using the phosphomolybdate method. We can observe an increase in antioxidant capacity with an increase in extract concentration with a very good total antioxidant capacity (92.02% and 96.87% at 0.5mg/ml and 1mg/ml respectively) due to its richness in polyphenol and aromatic compounds. These values are higher than the values of ascorbic acid activity used as control

In vivo biological activities

Hepatoprotective activity

The improvement exhibited by extract was near to that induced by curcuma. In this line, turmeric supplementations protect against NaF induced hematological perturbations and hepatic injuries in rats, plausibly by the up-regulation of antioxidant enzymes to confer the protective effect. [31]

The liver is the target organ of sodium fluoride (NaF) toxicity. It was assumed that NaF would induce both pathomorphological and metabolic changes in the liver. [32]

Fluoride-induced necrosis, disturbance of the metabolic processes and detoxication capabilities of the liver and are associated with oxidative stress. [33]

Transaminases elevated levels are indicative of cellular leakages and loss of functional integrity of cell membrane in the liver are indicative of cellular leakages and loss of functional integrity of cell membrane in the liver. [34]

Hyperbilirubinaemia is characteristic of impaired bilirubin metabolism involving metabolic disturbances in the liver, this could be explained by overproduction of bilirubin caused by an excessive breakdown of red blood cells due to the toxins from NaF. [35]

Treatment of exposed rats with *Bunium incrassatum* resulted in a reduction in serum ALT and AST activities and total bilirubin which confers a hepatoprotective activity to this plant. The products

which possess antioxidant nature are useful in reducing the production of free radicals from NaF, thus provide beneficial effects against NaF toxicity .[36]

The present study revealed that *B. incrassatum* restored biochemical markers in NaF exposed rats probably via enhancement of liver antioxidant defense as it is confirmed in several studies where NaF target liver function [33]. We believe that improvement of liver toxicity with *B. incrassatum* extract may be linked to a direct and/or indirect antioxidant activity of their polyphenolic compounds.

Blood sugar

A correlation between exposure to NaF and decreased insulin secretion has been highlighted, with a development of glucose intolerance [37]. Co-treatment of rats with curcuma or *B. incarssatum* induced a significant in blood sugar levels as compared to NaF treated rats, this could mean the ability of the plant to correct blood sucre level disturbance following NaF administration. Some medicinal plants with antioxidant capacity exhibited ability to blood sucre normalization after Naf exposure (Perez-Torres *et al*., 2013). [38]

Hemoglobin

The group treated with NaF has recorded the lowest value which corresponds to a previous study on rabbits and rats [39]. The possible explanation for this decrease is that fluoride affects the formation of hematopoietic cells due to decrease production of bone marrow resulting in anemia and low hemoglobin production [39]. More recently, it was confirmed that NaF enhances generation of free radical in red blood cells , increased the oxidation of hemoglobin, which inactive its oxygen transport capacity [17].

Co-treatment of rats with *B. incrassatum* root extract ameliorates Hb levels compared to NaF treated rats. Our findings corroborated with a recent research [7], where treatment with *B. incrassatum* increases the level of Hb in the blood of rabbits.

CONCLUSIONS

The Algerian *Bunium incrassatum* species is a very rich plant in polyphenols compounds, has a considerable antimicrobial activity and antioxidant activity. This species seems to have the ability to counteract sodium fluoride liver toxicity and to protect rats against its harmful effect on hemoglobin, and to have a very appreciable hepatoprotective activity. The overall results of this work showed that *Bunium incrassatum* is a rich natural source of antioxidants. This finding is useful for further advancements in the fields of food supplements, food additives and drug synthesis in the future.

FUNDING

Not applicable.

CONFLICT OF INTERESTS

None.

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