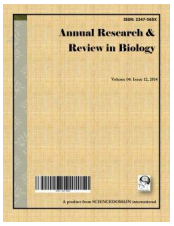

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Antioxidant, Antihemolytic, Antihyperuricemic, Anti-inflammatory Activity of Algerian Germander Methanolic Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Author IK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NB, HT and SO managed the analyses of the study. Authors BA and LA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The Germander (*Teucrium polium*) is commonly used as a medicinal plant in Algeria against a variety of human diseases. This study aims to investigate the antioxidant, anti-hemolytic and antihyperuricemic effects of the Algerian germander (*Teucrium polium* L.) extract. *T. polium* witch was collected from Bordj Bouarreridj, Algeria and extracted with methanol to give the methanolic extract (TPME). The objective of this work is to disassemble, at first, the antioxidant effect of TPME *in vivo* and *in vitro*, secondarily to evaluate the anti-inflammatory and finally to study for the first time the hypouricemic activity. The quantification of polyphenols and flavonoids showed that the TPME contains 160.72±0.78 µg EAG/mg of polyphenols and 37.96±0.317 µg EQ/mg of flavonoids.

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The antioxidant activities were carried out in mice by an *in vivo* assay, the plasma ability to inhibit DPPH radical and FRAP. TPME showed a protective effect against oxidative stress in erythrocytes. The total antioxidant defence system appears to be enhanced in the plasma, by increased FRAP levels probably due to higher levels of polyphenols in the *Teucrium polium* extract. The treated group showed an essential activity in the DPPH test compared to Vit C and control groups (28.64±5.84% vs 47.27±6.78% and 21.42±3.89%, respectively). The total antioxidant capacity of plasma and red blood cells was determined using the kinetics of hemolysis by the determination of HT_{50} (hemolysis half-life). The HT_{50} which was 179.6±10.53 min for treated group for, 158.2±3.85 for Vit C group and 146.5±1.78 min for the control, respectively. The present work demonstrated that *Teucrium polium* extract exerts a strong *in vivo* free radical scavenging and antioxidant activities. These activities are probably related to polyphenols and flavonoids. Hyperuricemia witch is induced by injection of potassium oxonate "PO", the uric acid, urea and creatinine were measured in plasma and supernatant of the liver. To evaluate their hypouricemic effect, TPME was administered intraperitoneally to potassium oxonate-induced hyperuricemic mice at a dose of 50 mg/kg body weight. TPME caused a decrease in plasma uric acid (3.3±0.18 mg/l) compared to control group (1.48±0.07 mg/l), almost the same value of uric acid of "PO" group. For "OP" group, value of uric acid in plasma is increased 4 times (6.33±1.22 mg/l) and almost 2 times for liver supernatant (31.36± 5.4 mg/l), the administration of 10 mg/kg of allopurinol decreased uric acid levels to normal (1.89±0.32 mg /l, 16.36±1.03 mg /l, respectively for plasma and supernatant). The findings data for the supernatant didn't show any significant decrease in plasma and liver uric acid comparing the urea level of "OP" group (0.48 g/l); we can conclude that the rate of urea and creatinine after treatment with plant extract is normal and that the results of this study indicate the absence of renal damage in mice. The present study was undertaken to evaluate the anti-inflammatory effect of TPME *in vivo* The administration of TPME (100 mg/kg body wt.), reduced ear edema induced by phorbol myristate acetate (PMA), achieving a low degree of anti-inflammatory activity (%I = 18.46±1.59%), the effect was comparable with that of diclofenac used as a reference drug (%I = 38.84±1.87 %). The histopathological analysis indicated that the treatment with TPME led to a moderate decrease of the inflammatory infiltrate with a persistence of the oedema, against the injection of diclofenac, led to a significant reduction of the leucocytes.
These results support the use of this plant in traditional medicine for inflammation disorder.

Keywords: TPME; antioxidant activity; DPPH; FRAP; hemolysis; *Teucrium polium;* hyperuricemia; anti-inflammatory.

1. INTRODUCTION

Free radicals are produced in standard and pathological cell metabolism. Oxidation is essential for the production of energy to fuel biological processes of many living organisms. Exogenous chemical and endogenous metabolic processes in the human body or the food system might produce highly reactive free radicals, mainly oxygen-derived radicals, which are capable of oxidising biomolecules, resulting in cell death and tissue damage [1].

Antioxidants are of great importance concerning preventing the oxidative stress that may cause several degenerative diseases. Flavonoids, tannins, anthocyanins and other phenolic constituents present in plants are potent antioxidants [2,3]. Food is rich in antioxidants which plays an essential role in the prevention of cardiovascular diseases, cancer [4] and neurodegenerative pathologies including Parkinson's and Alzheimer's diseases [5] as well as inflammation [6]. Inflammation is the first response of the immune system to infection or tissue injury, leading to the protection of the human body against these insults. But prolonged or chronic inflammation is detrimental and has an essential role in the development of diseases such as arthritis, diabetes and cardiovascular disease [7]. Acute inflammation, which is characterised by pain, heat, redness, and swelling involves a complex series of events including vasodilatation, increased permeability, fluid exudation, and migration of leukocytes to the site of inflammation [8]. In an inflammatory environment, activated neutrophils and macrophages produce large quantities of superoxide radical and other free radicals via the phagocytic isoform of NAD (P)H oxidase [9]. These free radicals witches are produced during inflammation, may lead to toxic effects, when produced at high levels during the oxidative burst [10]. The available anti-inflammatory drugs

(steroidal and non-steroidal) present a wide range of side effects. Therefore, many studies are being directed to find anti-inflammatory agents from natural sources [11].

Hyperuricemia is the most cited pathology involving Xanthine Oxidase (XO). It is a pathological state that arises from overproduction or underexcretion (renal tubule disorders) of uric acid (XO catalyses the oxidation of hypoxanthine and xanthine to uric acid). As a result of hyperuricemia, insoluble uric acid forms microscopic crystals in the capillary vessels of joints. These crystals cause inflammation and sharp pain, which is termed acute gouty arthritis or acute gout [12]. Therefore, XO inhibitors have been proposed as potential therapeutic agents for treating hyperuricemia as they could be used to block the biosynthesis of uric acid [13]. Allopurinol is the most commonly used xanthine oxidase inhibitor prescribed clinically for the treatment of gout [14]. However, the use of these agents is limited because of its undesirable effects [15]. The search for better agents antihyperuricemic is highly requested, this study represents an effort to find a new molecule hypouricemic.

The use of natural products isolated from medicinal plants represents a good source of novel and clinically essential drugs in connection with the treatment of some kinds of clinical disorders as hyperuricemia and gout. As part of our effort to find antihyperuricemic agents and antioxidants from herbs *Teucrium polium*.

The antioxidant potential of *T. polium* has been investigated. This plant, commonly known as 'mountain germander', is widely distributed in the Mediterranean region: southern west Asia, Europe and northern Africa [16]. *T. polium* is one of the medicinal plants primarily used in Algeria. In folk medicine, tea preparation of the aerial parts of the plant of *T. polium* is used for to treat abdominal colic, headache, diabetes and as an astringent [17]. In experimental animal models the aqueous extract of the plant exhibited antispasmodic, anorexic and hypolipidemic effects [18]. It shows various healthcare properties, especially diuretic antibacterial, anti-inflammatory, anti-nociceptive, antispasmodic, anti-ulcer, antidiabetic, hypolipidemic [19] and antioxidant [20]. Medicinal components from plants play an important role in conventional traditional medicine [21]. The *Teucrium* sp. contains a number of compound classes that include phenylpropanoid glycosides, iridoid glycosides [22,23], neoclerodane diterpenes [24], the most important compounds are polyphenols [25], flavonoids [26,27] and essential oil [28]. Medicinal value of *T. polium*, related to its phytochemical components suggests that the biological actions of these compounds relate to their antioxidant activity [29].

The present study aims to investigate *in vivo*, at first, the antioxidant and antihemolytic effects. Secondly, to demonstrate, the antihyperuricemic activity on plasma and liver uric acid levels in normal and potassium oxonate-induced hyperuricemic male mice; besides, the renal function of the mice after Germander administration was estimated by the determination of blood/liver urea and creatinine analysis. Finally, to evaluate the anti-inflammatory activity of this plant.

2. MATERIALS AND METHODS

2.1 Plant Materials

Teucrium polium L. was collected from Bordj Bouarreridj, northeast of Algeria during June 2011 and identified by Pr. Laouer H. (Faculty of Nature and Life Sciences, University Ferhat Abbas Setif 1, Algeria). Areal parts of the plant were dried in shadow and powdered using a mechanical grinder before the extraction. The powdered material was extracted at room temperature overnight with pure methanol (85%). The obtained extract was under reduced pressure on a rotary evaporator at 45°C, then air dried and stored at -20°C until use [3] and called TPME.

2.2 Animals

Adult male mice weighting 25–30 g were obtained from the 'Institut Pasteur d'Algérie'. They were kept under standardized conditions (temperature 21-24°C and a light/dark cycle of 12/12 hours) and fed a normal laboratory diet "*ad libitum*"(standard food provided by the National Office of Livestock Feed of Bejaia, Algeria). After one week of acclimatization, mice were divided into treated groups and one group was considered as control.

2.3 Determination of total flavonoids and polyphenol contents

The total flavonoids in the extract were determined by a colorimetric method as described by Bahorun et al. (1996) [30]. Each sample (1 ml) was mixed with 1 ml of Aluminium

chloride ($AlCl_3$) solution (2%) and allowed to stand for 15 min. Absorbance of the mixture was then determined at 430 nm versus prepared methanol blank. Results were expressed as µg of equivalent quercetin /mg dried extract (R^2 = 0.9841).

Total polyphenol content was determined by the Folin-Ciocalteu method [31]. Diluted sample (200 µL) of plant extract or standard (prepared in methanol or distillated water with suitable dilutions) were added to 1 mL of 1/10 diluted Folin-Ciocalteu reagent. After 4 min., 800 mL of saturated sodium carbonate solution (75 g/l) was added. After incubation for 1h at room temperature, the absorbance at 765 nm was measured in triplicate. Gallic acid (0–160 µg/ml) was used for calibration of standard curve. The results were expressed as µg gallic acid equivalent (GAE)/ mg of extract (R^2 = 0.999).

2.4 Antihemolytic Activity *In vitro*

Resistance of erythrocytes pre-treated with plant extracts and radical target was evaluated according to the protocol described by Takebayashi et al. (2010) [32] with a replacement of AAPH radical by tert-butyl hydroperoxide (t-BHP) [33]. The blood of the mice used in this test is obtained by decapitation. The blood is collected in a heparinized tube, and then diluted in phosphate buffer (300 mOs, pH 7.4) to obtain a hematocrit of 2%. The radical attack is induced by the addition of t-BHP (166 mM) to the erythrocyte suspension previously incubated for 15min with *Teucrium polium* extract. Four concentrations were tested: 10.59, 21.18, 42.37 and 84.74 µg/ml and vitamin C (Vit C) is used as standard. After three hours of incubation with the t-BHP at 37°C, the kinetics of disappearance of the red blood cells (RBC) are followed by the dynamic measurement of the decrease in absorbance at 620 nm. The resistance of the blood to the radical damage is expressed by the time required for 50% lysis of erythrocytes (hemolysis half-time; HT_{50}). The results are expressed as mean of HT_{50} ± SEM.

2.5 *In vivo* Antioxidant Activity

The antioxidant activities of TPME confirmed *in vivo via* intraperitoneal injection for 21 days. After one week of acclimatization, mice were divided into three groups of 8-9 and one group was considered as control. The control group1 received normal saline solution, group 2 received 50 mg/ kg of Vit C and the group 3 received 50 mg/ kg body weight, of TPME dissolved in normal saline. The total antioxidant capacity of blood was assessed by determining HT_{50}, plasma antioxidant capacity was also determined using the DPPH and FRAP scavenging assay.

2.5.1 Blood collection

Blood was collected into tubes containing heparin through direct heart puncture from anesthetized mice. An aliquot of 100 µl of whole blood is immediately transferred to another tube containing 2.4 ml of phosphate buffer saline (PBS: 125 mM NaCl, 10 mM sodium phosphate, pH 7.4) to obtain a dilution of 1/25. The remaining blood is centrifuged at 1500 g for 5 min at 4°C then the obtained plasma was aliquoted and stored at -20°C until use [34].

2.5.2 Total antioxidant capacity of plasma and red blood cells

Resistance to free radical damage was measured as the capacity of red blood cells (RBC) to withstand free radical induced hemolysis, as described by Girard et al. (2006) [35] with slight modifications. Briefly, the mice's blood was diluted 1/25 with PBS, then 50 µL of RBC suspension or whole blood were assayed using a 96-well microplates coated with a *t*-BHP as a free radical generator. The kinetics of RBC resistance to hemolysis were determined at 37°C by continuous monitoring of changes in absorbance at 620 nm. The time to reach 50% of total hemolysis was retained.

2.5.3 DPPH radical scavenging activity

In this test, the plasma ability to inhibit DPPH radical was measured according to Mansouri et al. (2005) [36]. DPPH is one of the few stable organic nitrogen radicals and has a maximum absorption at 517 nm. A volum of 25 µl mice's of plasma was added to DPPH solution (2.4 mg/100 ml of methanol) and the reaction mixture was shaken vigorously. After incubation at room temperature for 30 min, the absorbance of the solution was determined at 517 nm using the formula *% anti-radical activity* = $(A_{517}$ *control*– A_{517} *sample) x 100/* A_{517}*control*. The results were compared with the DPPH solutions in the absence of the plasma.

2.5.4 Ferric reducing ability of plasma

The total antioxidant capacity of plasma was determined by measuring its ability to reduce

Fe^{3+} to Fe^{2+} by using FRAP assay described by Benzie and Strain (1996) [37] and modified by Pulido et al. (2000) [38]. FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue-colored Fe (II)–TPTZ compound by the reaction of colorless $Fe^{3+}$and electron-donating antioxidants. FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer (pH 3.6) with 1 volume of 10 mM TPTZ in 40 mM HCl and 1 volume of 20 mM $FeCl_3$. Freshly prepared FRAP reagent was warmed to 37°C, and a reagent blank reading was taken at 593 nm. Subsequently, 30 µl of deionized water and 10 µl of sample or water as appropriate for the reagent blank, were added to the FRAP reagent. The final dilution of the sample in the reaction mixture was 1:34. The absorbance readings were taken every 15 s for 30 min. The initial blank reading with the FRAP reagent alone was subtracted from the final reading selected of the FRAP reagent with the sample to determine the FRAP value of the sample. Standard solutions of Fe^{2+} in the range of 0 to 2000 µM were prepared from ferrous sulfate ($FeSO_4$, 7 H_2O) in water (R^2 = 0.9983). The data were expressed as mM ferric ions reduced to the ferrous form (FRAP value).

2.6 Hypouricemic Effects on Potassium Oxonate-induced Hyperuricemia in Mice

Mice's were divided into groups of 9-10 animals. Induction of hyperuricemia was made by injection intraperitoneally of 200 mg/kg of potassium oxonate to induce hyperuricemia [39]. After one hour, the extracts were administered intraperitoneally (100 mg/kg). The mice were divided into 4 groups: group 1 (control): injected with the normal saline solution, group 2 (PO) injected with potassium oxonate, group 3 (PO + allopurinol) injected with potassium oxonate then treated with 10 mg/kg of allopurinol, and group 4 (PO + TPME): injected with potassium oxonate then treated with 100 mg/kg of TPME. Blood samples were collected on anesthetized mice and centrifuged at 1500 g for 5 min. The plasma obtained was stored at -20°C until use. Livers from each group were collected, rinsed with saline and homogenized in a phosphate buffer (50 mM, pH 7.8), and centrifuged at 8000 g for 15 min at 4°C. The supernatant is used for the determination of uric acid [40,41]. The concentration of uric acid in the plasma and supernatant of the liver is determined by an enzymatic method. To evaluate the effect of the extracts on renal function, the levels of urea and creatinine was determined. These assays were carried out at the Central Laboratory of the University Hospital of Setif using a Bechman device and kits purchased from Cypress Diagnostics.

2.7 Evaluation of Anti-inflammatory Activity

The anti-inflammatory effect was evaluated according to the method of Garrido et al., (2004) [42], on adult male mice, 4 µg/ear of PMA (phorbol myristate acétate), in 20 µl DMSO, was applied to both surfaces of the right ear of each mouse. The left ear (control) received the vehicle (20 µl DMSO). TPME was injected (100 mg/kg body wt. in normal saline 9‰) 1h before PMA application. Two control groups were used: a control group injected intraperitoneally (normal saline 10 ml/kg) with application of PMA to the right ear and a reference group treated with diclofenac (10 mg/kg body wt.). Six hours after PMA application, mice were killed by cervical dislocation and a 6-mm diameter disc from each ear was removed with a metal punch and weighed. Ear edema was calculated by subtracting the weight of the left ear (vehicle) from right ear (treatment), and was expressed as edema weight. Inhibition percentage was expressed as the reduction in weight with respect to the control group. Ear biopsies were then fixed in formol (10%) and processed for routine paraffin sections. Paraffin sections of right and left ears were made and stained with hematoxylin/eosin (H§E). Then, microscopic evaluation was carried out in the laboratory of Anatomo-pathology, CHU of Setif.

2.8 Statistical Analysis

All experiments were done in triplicate and results were reported as mean ± SD. Data were analyzed by one way ANOVA. Statistically significant effects were further analyzed and means were compared using Tukey's test for using GraphPad program version 5.0. Statistical significance was determined at p <0, 05.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic and Flavonoids Content

In order to characterize the different extracts, a total dosage of flavonoids and polyphenols was performed. Total polyphenols were assayed using Folin-Ciocalteau method [31] where gallic

acid was used as standard. Total flavonoids were measured [30] using as a standard quercetin. Present findings of the total phenolic and flavonoids dosage showed that TPME contains 160.72±0.78 µg GAE/ mg of extract and 37.96±0.31 µg QE per mg of extract, respectively.

3.2 Anti-hemolytic Activity *In vitro*

The antihemolytic activity of TPME was measured according to the protocol described by Takebayashi et al. (2010) [32]. The erythrocyte was submitted to oxidative attack. Under these conditions, the erythrocytes involved the enzymatic and molecular equipment to resist to the attack. The measurement of the decrease in absorbance at 620 nm used to monitor the progressive disappearance of the cells. The resistance of the blood to the radical damage is expressed by the time required for 50% lysis of erythrocytes (HT_{50}). This test allowed the monitoring of anti-radical power of natural molecules for use *in vitro* and *in vivo* drug in the system of erythrocytes. Theoretically, erythrocyte antiradical resistance should lead to a curve with a half-lysis higher in supplemented compared to control. A high HT_{50} value means that the extract effectively strengthened the radical anti-erythrocyte system. It appears from TPME treatment increases values of HT_{50} compared to the control group (HT_{50} = 56.01 ± 5.93); (Fig. 1; Table 1). A dose-dependent effect of the extract on the growth of overall antioxidant defenses has been noticed.

From the obtained kinetics of hemolysis (Fig. 1), it appears that treatment with the concentration of 21.18, 42.37, 84.74 µg/ml of TPME increased the values of HT_{50} compared to the control group (HT_{50} = 56.01±5.93) with dose-dependent manner. However, compared to the positive control "Vit C", these concentrations did not increase the values of HT_{50}. Consequently TPME caused an additional inhibition better than that of the standard antioxidant Vit C.

Table 1. Half-hemolysis (HT_{50}) for the different study groups treated with TPME

[C] µg/ml	TPME	Vit C
10.59 µg/ml	56.648 ± 2.38^{ns}	64.99 ± 4.46^{ns}
21.18 µg/ml	63.542 ± 4.12^{ns}	67.50 ± 3.91**
42.37 µg/ml	69.62 ± 1.67***	68.57 ± 4.43**
84.74 µg/ml	73.32 ± 3.21***	71.99 ± 7.79***

*Values are means ± SEM (n = 5). Comparisons are made with respect to the positive control group "Vit C". *: p ≤ 0.05, ** p ≤ 0.01, ns: not significant*

The results are expressed as delayed time of hemolysis (ΔT), which is calculated by: *ΔT (min)*

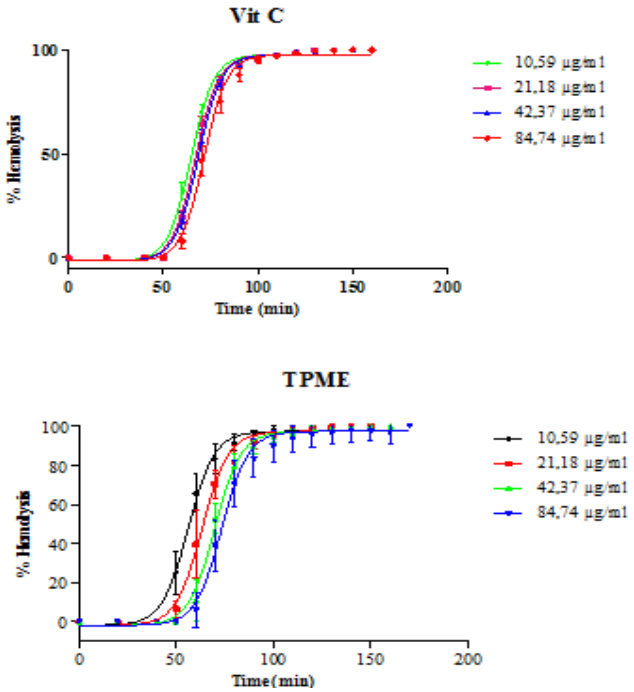

Fig. 1. Kinetics of hemolysis of erythrocyte in absence (Control) and in the presence of Vit C and TPME respectively. Values are means ± SEM (n = 5)

= HT_{50} *sample* - HT_{50} *blank*. The typical relationship between the concentration of the TPME and ΔT value was calculated. Good linearity was observed within the range of 10.59 –84.74 µg/ml (R^2= 0.8367). The inhibition duration produced by this extract was: 0.64, 7.53, 13.61 and 17.31 min. This extract at a concentration of 84.74 µg/ml delayed hemolysis almost 17 min. TPME increased the additional duration inhibition by comparing to the standard antioxidant "Vit C" which has a sequence ranging between 8.98 and 15.98 min. The aerial parts of *T. polium* are rich in flavonoids, in addition essential oils which have been reported as good antioxidants agents *in vitro* [28].

3.3 *In vivo* Antioxidant Activity

3.3.1 Total antioxidant capacity of plasma and red blood cells

In this context, the effect of treatment with TPME on blood defense mice towards the free radicals was evaluated. The half-life (HT_{50}), which corresponds to 50% of cell lysis, was calculated. From the kinetics of hemolysis obtained, the calculated HT_{50} reveal an extension of time for half hemolysis in all treated groups compared with the control group (Fig. 2). However, results showed an increase in HT_{50} value (HT_{50} = 179.6 ±10.39 min) of the treated group with TPME (50 mg / kg/$p \leq 0.05$) compared with the control group (HT_{50} = 146.5±1.77 min).The treated group with Vit C didn't significantly increase the HT_{50} (HT_{50} = 158.2±3.849 min).

The increase in plasma antioxidant capacity for the treated group is probably attributed to elevated levels of exogenous antioxidants such as ascorbic acid, carotenoids, vitamin E and flavonoids or phenolic compounds [43]. Dai et al., (2006) [44] have previously reported that flavonols and their glycosides derivatives are effective antioxidants protecting human red blood cells from free radical-induced oxidative hemolysis. In our study, the protective effect of flavonoids can be linked to their binding to the plasma membrane [45] and their ability to penetrate lipid bilayers [46]. In addition, these results showed that the anti-hemolysis effect could be linked not only on the polyphenols and flavonoids contents of the extract, but also to the nature (structure) of these compounds [47 , 48]. It was demonstrated that binding of the flavonoids to the erythrocytes membranes significantly inhibits lipid peroxidation, and at the same time enhances their integrity against lysis [49].

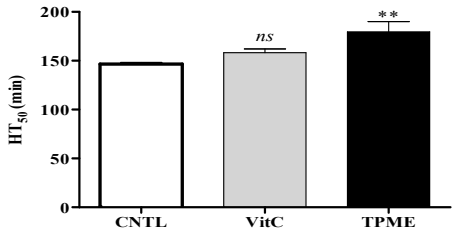

Fig. 2. The half live (HT_{50}) for the different group. Groups CNTL (control group treated with normal saline), Vit C (groups treated with a solution of Vit C 50 mg / kg), TPME (group treated with 50 mg/kg of TPME). Values are means ± SEM (n = 8-9). Comparisons are made with respect to the control group, **: p = 0.01, ns: not significant

3.3.2 DPPH radical scavenging activity

Antioxidant potential of *Teucrium polium* in mice blood using DPPH assay showed that the inhibition percentage (I %) of TPME at 50 mg/kg was: I% = 28.64 ± 5.84 (Fig. 3). This inhibition percentage wasn't statistically significant compared to the control group with I% = 21.42 ± 3.89. The administration of 50 mg / kg of Vit C increased significantly plasma capacity to 47.27 ± 0.017% ($p \leq 0.01$).

From the comparison of TPME results, the antioxidant activities *in vitro* and *in vivo* indicate that the plant extract retained the same properties. In comparison with the plasma antioxidant capacity using the DPPH radical with the test of the total antioxidant capacity of the blood, at the dose of 100 mg / kg of TPME caused an increase in the plasma antioxidant capacity (≈ 179 min) and the total antioxidant capacity of the blood (≈ 29%). This is consistent with published results of Hasani et al, (2007) [26] and Krishnaiah et al. (2011) [50].

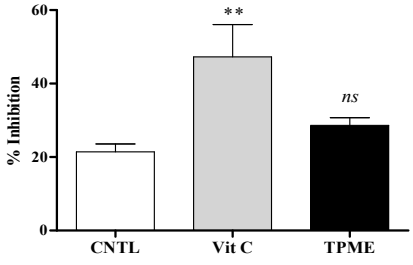

Fig. 3. Plasma antioxidant capacity (DPPH). Values are means ± SEM (n = 8-9). Comparisons are made with respect to the control group, **: p ≤ 0.01, ns: not significant

3.3.3 Ferric reducing ability of plasma

In order to assess the effect of polyphenols from methanol extract on plasma antioxidant status, variety methods have been employed. Commonly used is the FRAP assay. TPME and Vit C increased the plasma FRAP compared to the control (Table 2). The present data suggest that the antioxidant defense system appears to be enhanced in the plasma, by increased FRAP levels, probably due to the higher levels of polyphenols in the *Teucrium polium* extract.

Table 2. Reducing power by FRAP (mmol/µl) of groups treated with NaCl 0.9 % (CNTL), Vit C (50 mg/Kg) and TPME (100 mg/Kg)

	4 min	30 min
CNTL	3.83 ± 0.50	9.98 ± 0.16
TPME	4.05 ± 1.17^{ns}	11.07 ± 1.19^{ns}
Vit C	5.35 ± 0.34*	12.81 ± 0.76*

*Values are means ± SEM (n = 5). Comparisons are made with respect to the positive control group "Vit C". *: p ≤ 0.05, ns: not significant*

The FRAP assay gives fast, reproducible results with plasma, with single antioxidants in pure solution and with mixtures of antioxidants in aqueous solution and added to plasma [37]. The antioxidative properties of various flavonoids are well known. They are good chelators of metals ions, particularly iron or copper, the Fenton reaction catalyst. Polyphenol compounds have been reported to possess the higher reducing power, the presence of phenolics and flavonoids content in the extracts would have contributed towards FRAP [34]. However, other investigators have reported that the aerial parts of *T. polium* are rich in flavonoids. Therefore, it would be reasonable to assume that the antioxidant properties of an aqueous extract of *T. polium* can be attributed to presence of these bioactive components [28].

In comparison with the test of total antioxidant capacity of blood, we have observed that the dose 50 mg/kg hadn't given a significant effect neither on the plasma antioxidant capacity nor on the total antioxidant capacity of blood.

3.4 Hypouricemic Effects of TPME on Potassium Oxonate-induced Hyperuricemia in Mice

Hyperuricemia is induced by intraperitoneally injection of potassium oxonate "PO" which is a competitive inhibitor of liver uricase, it partially blocks the conversion of uric acid to allantoin and therefore it increase the levels of uric acid in the mice plasma giving an animal model hyperuricemic [39 , [41]. The uric acid, urea and creatinine were measured in plasma and supernatant of liver. The value of uric acid in the control group: 1.48 ± 0.07 mg/L l, for the "PO" group, the uric acid level is significantly increased: 6.33 ± 0.46 mg / l two hours after the intraperitoneal injection of potassium oxonate indicating that it has successfully induced hyperuricemia.

In the "PO" group, the uric acid levels increased 4 times for the plasma (6.33 ± 1.22) and to almost two times for the liver supernatant (31.36 ± 5.4 mg/l), the administration of 10 mg/kg of allopurinol decreased the rate of uric acid to the normal (1.89 ± 0.32 mg/l, 16.36 ± 2.31 mg/l, respectively for plasma and liver supernatant). From the results obtained shown in Fig. 4; it appears that treatment with TPME decreased uric acid value (3.3 ± 0.18 mg/L) comparing to the PO group (6.33 ± 1.22 mg/ml). This decrease can be explained by the significant inhibition of the XO by inhibition of synthesis pathways of uric acid [15].

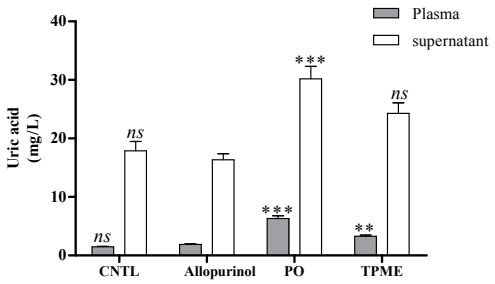

Fig. 4. Hypouricemic effects of TPME. Comparison of OP (200 mg/kg), OP+Allopurinol (10 mg/kg) and OP+TPME (100 mg/kg) groups against the control one (NaCl 0.9 %), **: p ≤ 0.01, *** p ≤ 0.001, ns: not significant

Several researchers study on gout disease, they have shown that flavonoids from Iranian *T. polium* méthanolique crude extract can influence the activity of XO and thus can reverse the disease of gout by reducing both of the concentrations of uric acid and inhibiting XO [14]. Huang and his collaborators (2011) [41] evaluated the effect of some flavonoid on the activity of XO *in vitro* and *in vivo* in plasma and liver. They found that these flavonoids have not a significant effect *in vitro*.

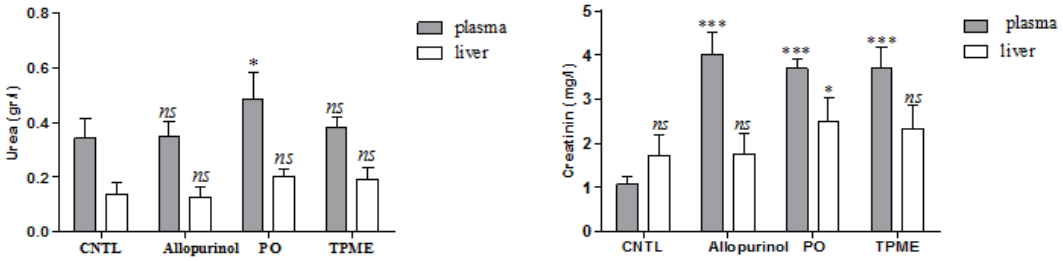

Fig. 5. Renal function evaluation by the determination of urea and creatinine. Comparison against the control group, *** p ≤ 0.001, ns: not significant

Renal failure is the cessation of renal function and it can be acute or chronic. In acute renal failure there is rapid loss of renal function within hours or days, although the condition is potentially reversible and normal renal function can be regained. The deterioration is sudden, with increases in the concentrations of urea and creatinine.

3.5 Renal Function Evaluation

The objectives of this study is to evaluate the effect of the extracts on renal function. The rate of urea and creatinine levels can be indicators for the assessment of renal function. Kidney injury may be accompanied by an increase of creatinine and urea.

Our results are in agreement with the results obtained by [51] ; they have found that induction of hyperuricemia by PO caused a renal dysfunction and thus it increased the rate of uric acid and creatinine. TPME and allopurinol did not decrease the rate of creatinine (above Fig. 5). However, the level of urea decreased comparing to the normal control mice. Comparing urea group "OP" (0.48 g/l) but this decrease is not significant.

3.6 Anti-inflammatory Effect of TPME

Topical application of PMA induces a long-lasting inflammatory response, resulting from protein kinase C (PKC) activation associated with a transient increase in prostanoid production and marked cellular influx [52]. This response reaches a maximum by 6h and subsides by 24h; multiple applications of PMA have been shown to produce a more prolonged inflammatory response characterized by ear edema, inflammatory cell influx and epidermal cell hyperplasia [53]. In the present study, the inhibition activity of TPME is significant ($p \leq 0.001$) compared to the diclofenac group (Fig. 6). However, plant extract present a low degree inhibition of inflammation of (%I= 18.46 ± 1.59%) comparable with that of diclofenac used as a reference drug (%I = 38.84 ± 1.87 %).

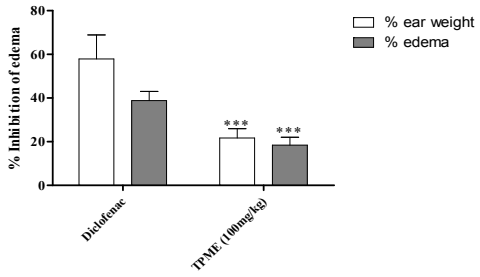

Fig. 6. Anti-inflammatory effect of TPME (100 mg / kg body wt., respectively), TPME reduced ear edema induced by PMA in mice. Values are means ± SEM (n = 8). Comparisons are made with respect to the control group ***: $p \leq 0.001$

The histological sections showed that all the right ears that received the vehicle (DMSO) have no signs of inflammation (Fig.7 a, b and c). However, histological evaluation of acute PMA challenged ears revealed prominent polynuclear leukocyte recruitment in the dermis with accompanying connective tissue disruption and edema. Treatment with TPME caused a non-significant decrease of the inflammatory infiltrate and the persistence of the edema was noticed, against the injection of diclofenac has led to a significant decrease in leukocyte.

Phenolic compounds such as flavonoids, phenolic acid and tannins possess diverse biological activities including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions. These activities might be related to their antioxidant activity [54]. Plants with high phenolic content have been popularly used for the treatment of many diseases [12].

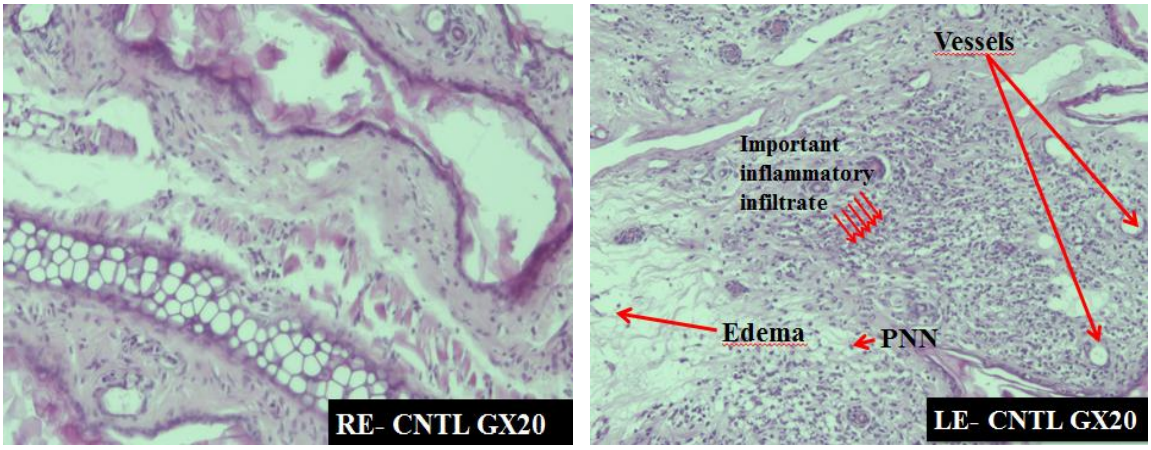

Fig. 7 a. Representative photomicrographs of H&E stained control mice ear cross sections in the acute PMA model (G×20). PNN: polynuclear neutrophils

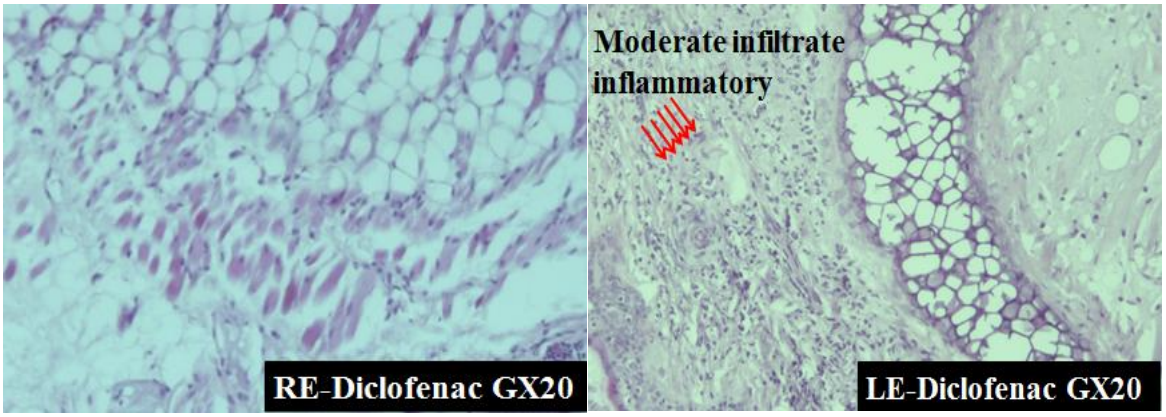

Fig. 7 b. Representative photomicrographs of H&E stained mice ear treated with diclofenac in the acute PMA model (G×20)

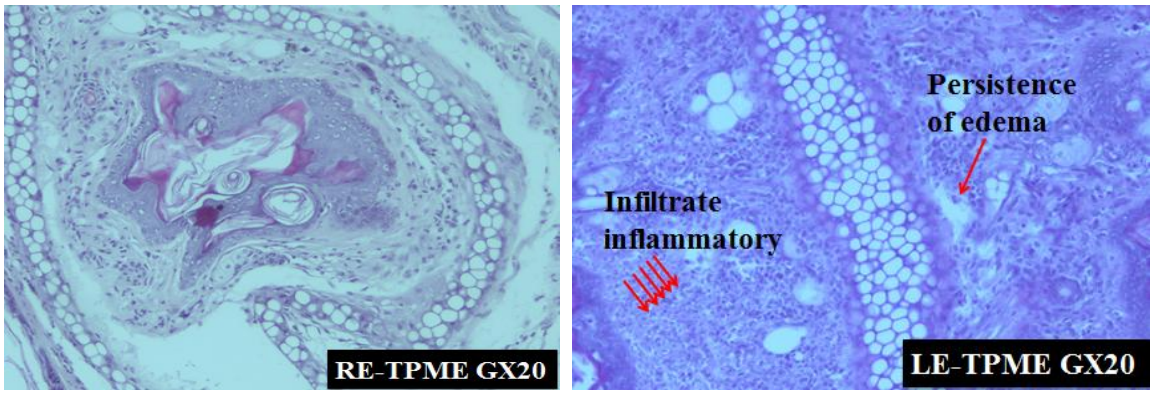

Fig. 7 c. Representative photomicrographs of H&E stained mice ear treated with TPME in the acute PMA model (G×20). RE: Right ear that received vehicle (DMSO), LE: left ear that received PMA. Ears were harvested 6 h post-treatment with DMSO vehicle, 20µl of a solution of PMA (a) or PMA plus injection of diclofenac (b) and PMA plus injection of 100 mg / Kg of TPME (c). Note persistence of the edema and of the inflammatory in the treated mice with TPME

4. CONCLUSION

The medicinal plant *Teucrium polium* is among the plants widely used today in traditional medicine in Algeria. In this present study, TPME seems to be of interest and potential for their antioxidant activities that have been established *in vivo*. In tests conducted in the present study, a significant correlation between flavonoids content and antioxidant activity of the extract was observed, but this in no way excluded the contribution of other compounds in this activity. These results could represent an essential contribution to the understanding of the anti-inflammatory and anti-hyperuricemic effects reported by the use of *T. polium*. Further work on the isolation and identification of more bioactive compounds, combined with an investigation on other biological activity of Algerian Germander exacts will be helpful to understand the benefits of this important medicinal plant. People should consider about the use *T. polium*, particularly if they are not officially informed of their possible unfavourable reactions.

ETHICAL APPROVAL

All animal experiments and treatments were conducted according to the directive of university ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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