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Evaluation of the Anti-Gout Potential of *Calluna vulgaris* L. (Ericaceae) in Rats

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Abstract: The present study evaluated the anti-gout potential of an ethanolic extract from *Calluna vulgaris* L. Hull (ECV). A preliminary phytochemical analysis of ECV was performed by spectrophotometric and HPLC-PDA-MS methods. Hypouricemic effect of ECV was tested *in vivo* using the oxonate-induced hyperuricemia model in rats, systolic blood pressure being also monitored in hyperuricemic animals. The anti-inflammatory and analgesic effects of ECV were investigated by the carrageenan-induced paw oedema and Randall-Selitto test in rats. The results of the phytochemical analysis of the extract obtained from *C. vulgaris* showed the main chemical constituents were flavonoids and chlorogenic acid. ECV showed significant and dose-dependent hypouricemic effects in oxonate-treated rats, increasing the urinary excretion of uric acid. The tested extract produced statistically significant anti-inflammatory and analgesic effects at the dose of 500 mg/kg. This study offers a new perspective regarding the anti-gout potential of *C. vulgaris*.

Keywords: *Calluna vulgaris* L.; flavonoids; chlorogenic acid; hypouricemic.

1. Introduction

Gout is a metabolic disease usually manifested as an inflammatory arthritis, affecting 1-2% of the general population, especially men above 40 years old and women above 65 year old [1]. Recent data indicate an increase in the incidence and prevalence of the disease due to the aging population, high levels of obesity and probably, dietary changes [2]. Although gout as a clinical manifestation of hyperuricemia is known from Antiquity, it was only in the 19th century when Sir Alfred Baring Garod

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identified uric acid as the cause of the disease [3]. The urate crystals formed inside the joints when serum concentration of uric acid is increased, are capable to initiate, amplify and sustain an intense inflammatory reaction, subsequently chronicized by immune mechanisms, which leads to chronic gout with cartillage and bone lesions [4]. Nowadays, gout is no longer regarded as a disease affecting strictly the joints, but as a disease which favours other co-morbidities such as arterial hypertension, chronic kidney disease (CKD) or ischemic heart disease [5].

The pharmacological intervention in gout is focused on the reduction of serum uric acid either by inhibiting its formation or by augmenting its urinary excretion, but also on the inhibition of inflammation and pain during the exacerbations of the disease. Unfortunately, the beneficial effects of the existing anti-gout drugs are oftenly accompanied by severe adverse reactions [6], thus the discovery of natural products with hypouricemic properties could be beneficial for large categories of patients.

Calluna vulgaris L. Hull (Ericaceae) or heather is a small perennial shrub, 15-80 cm tall, native to Europe, oftenly present in moors and heathlands in Northwestern Europe, but found also in the Central or Southeastern parts of the continent [7]. The aerial parts of *C. vulgaris* were traditionally used in Europe to treat urinary tract disorders and rheumatic disease [8] but the use of this species as a gout remedy in folk medicine is also mentioned in several countries [9,10]. Although a series of experimental studies demonstrated antibacterial [11], photochemopreventive [12], antioxidant [13] and MAO-A inhibitory effects [14] for *C. vulgaris*, there is little information regarding the possible biological effects who could explain the traditional use of this vegetal species as a gout remedy. Thus, our study was aimed at the assessment of hypouricemic, anti-inflammatory and analgesic effects of an ethanolic extract obtained from *C. vulgaris* (ECV), in several *in vivo* animal experimental models, with the additional purpose of studying the possible antihypertensive effect derived from the reduction of serum uric acid.

2. Materials and Methods

2.1. Plant Material and Preparation of the Extract

The aboveground parts (herba) of *C. vulgaris* were harvested from Sălaj County, Transylvania region, Romania, in July 2016. After identification, a voucher specimen (*85 A*) was stored in the Faculty Herbarium. The plant material was air dried and grinded to a fine powder which was extracted with 96% (v/v) ethanol for 24 h at room temperature using a powder:solvent ratio of 1:20 (w/v). Subsequently, the extract from *C. vulgaris* (ECV) was concentrated at reduced pressure in a Rotavapor (Buchi, Switzerland). The extract yield was 12.8%.

2.2. Phytochemical Analysis

A preliminary phytochemical analysis was performed on the extract from *C. vulgaris* (ECV). Total polyphenolic content and total flavonoid content were determined spectrophotometrically and a HPLC-PDA-MS analysis to determine the main phenolic acid was performed using previously described techniques [15,16,17]. Details of the experimental procedures are described in Supporting Information.

2.3. Animals

For the biological tests, 120 male Wistar rats weighing around 200 g were obtained from the Animal Breeding Center of Iuliu Haţieganu University, Cluj-Napoca (Romania). The rats were kept in standard conditions with free access to food and water until the experiment day. The experimental protocol was approved by the Ethics Committee of the Iuliu Hatieganu University, Cluj-Napoca, Romania and was conducted according to the international regulations concerning laboratory animal welfare.

2.3.1. Experimental Design of Animal Groups

The experimental design of animal groups is presented in Table 1, with details of treatment and dose for each tested biological effect.

Table 1. Experimental design of animal groups

Group (n=6)	Hypouricemic effect (Treatment and dose)	Antihypertensive effect (Treatment and dose)	Anti-inflammatory effect (Treatment and dose)	Analgesic effect (Treatment and dose)
Control	250 mg/kg potassium oxonate (Oxo) single dose	250 mg/kg potassium oxonate (Oxo) daily for three weeks	Saline solution	Saline solution
ECV 125 mg/kg	Oxo+125 mg/kg ECV single dose	Oxo+125 mg/kg ECV daily for three weeks	125 mg/kg ECV single dose	125 mg/kg ECV single dose
ECV 250 mg/kg	Oxo+250 mg/kg ECV single dose	Oxo+250 mg/kg ECV daily for three weeks	250 mg/kg ECV single dose	250 mg/kg ECV single dose
ECV 500 mg/kg	Oxo+500 mg/kg ECV single dose	Oxo_500 mg/kg ECV daily for three weeks	500 mg/kg ECV single dose	500 mg/kg ECV single dose
Reference	Oxo+125 mg/kg probenecid single dose	Oxo+125 mg/kg probenecid daily for three weeks	20 mg/kg diclofenac	20 mg/kg diclofenac

2.4. Hypouricemic Effect

The hypouricemic effect of the ECV was determined by the oxonate-induced hyperuricemia model in rats [18,19]. A single dose of potassium oxonate (250 mg/kg) was administered orally to 5 groups of rats (n=6). After 1 h, the animals from the control group were treated with the vehicle, another group received a reference uricosuric drug, probenecid 125 mg/kg and the other three groups of rats were treated with ECV in graded doses (Table 1). All the substances were administered orally by gastric tube, the animals being individually placed in metabolic cages. After 24 h, samples of urine were collected from all the animals and blood samples were obtained by retroorbital sinus puncture under ketamine:xylazine anesthesia (100 mg/kg:10 mg/kg). Uric acid was spectrophotometrically determined in the serum and urine, at 670 nm, by the uricase peroxidase method with an automated biochemistry analyzer (Vitros 250, Johnson and Johnson Clinical Diagnostic, USA). Creatinine was spectrophotometrically determined in the serum and urine, also at 670 nm, with the same device, using a reaction which formed a triaryl imidazole leuco-dye [20].

Uric acid clearance (ClrUA) was calculated with the formula:

$ClrUA = (UA_U \times D)/UA_S$, where UA_U is the urine concentration of uric acid, D is the diuresis/min. and UA_S is the serum concentration of uric acid.

Fractional excretion of uric acid (FEUA) was calculated with the formula:

$FEUA = (UA_U \times CR_S)/(UA_S \times CR_U)$, where UA_U is the urine concentration of uric acid, UA_S is the serum concentration of uric acid, CR_S is the serum concentration of creatinine and CR_U the urine concentration of creatinine.

2.5. Antihypertensive Effect

The influence of high uricemia on systolic blood pressure and the possible beneficial effects of the ECV were investigated using a modified method of Mazzali and colleagues [21], hyperuricemia being produced in rats by a three-week administration of potassium oxonate (250 mg/kg) orally, by

gastric tube. Details of this experiment are presented also in Table 1. Control rats were treated only with oxonate 250 mg/kg, administered daily by oral route for 3 weeks. A reference uricosuric drug, probenecid (125 mg/kg) was administered orally for 3 weeks to another group of rats, in addition to oxonate. Three other goups of rats were daily treated, for 3 weeks with oxonate and graded doses of ECV, administered also orally. In all the animals, blood pressure was monitored each week, throughout the experiment, with a non-invasive, automated Blood Pressure Recorder 58500 (Ugo Basile, Varese, Italy). The animals were priorly placed in a heating box model 58000-845 (Ugo Basile, Varese, Italy) in order to cause a sufficient vasodilation of the caudal artery neccesary for optimal determinations.

2.6. Anti-inflammatory Effect

The anti-inflammatory effect of ECV was evaluated by the rodent paw oedema test induced by a phlogistic substance (λ-carrageenan), using the modified method of Winter [22-24]. In this experiment, ECV was orally administered to 3 groups of rats (n=6) in graded doses (Table 1), one hour before the subplantar injection of the phlogistic agent (1% w/v, 0.1 mL). The paw swelling was evaluated plethysmometrically (model 7140, Ugo Basile, Italy) before and after the administration of the phlogistic agent at predetermined time intervals. Oedema was calculated at each time point by subtracting the initial paw volume from the paw volume after the injection of the phlogistic agent. The inhibition of oedema was expressed as %.

2.7. Analgesic Effect

The analgesic effect of the ECV was assessed by the Randall Selitto test in rodents [25]. In this model, the injection of the phlogistic agent λ-carrageenan into the footpads of rats produces a persistent pain, closely mimicking the time course of some pain types in humans [26]. Animal groups used in this experiment are presented in Table 1. Initially, the animals received a subplantar injection of λ-carrageenan (1% w/v, 0.1 mL). Thirty minutes after the induction of inflammation, the substances were orally administered to the animals. At several time points (1 h, 2 h, 3 h and 4 h) the inflamed posterior member was mechanically compressed with an analgesimeter (Ugo Basile, Italy), the specific reaction of animal being considered sign of hypernociception. The device indicated the intensity of pressure (g) tolerated by the animals at the mentioned time points.

2.8. Statistical Analysis

Statistical analysis was performed by ANOVA and Dunett tests, using GraphPad Prism 6 software (GraphPad Software, USA), p values < 0.05 being considered statistically significant.

3. Results and Discussion

3.1. Phytochemical Analysis

The chemical composition of *C. vulgaris* has been investigated in previous studies. Virag and colleagues [27] showed the presence of hyperoside, quercetin, isoquercetin and kampferol in an extract from *C. vulgaris* originating from Romania. Another study which assessed several *Ericaceae* species originating from the Balkans, also found a high content of polyphenols in a dry extract from *C. vulgaris* [28]. The main chemical constituents from *C. vulgaris* identified in previous studies and this study are summarized in Figure 1.

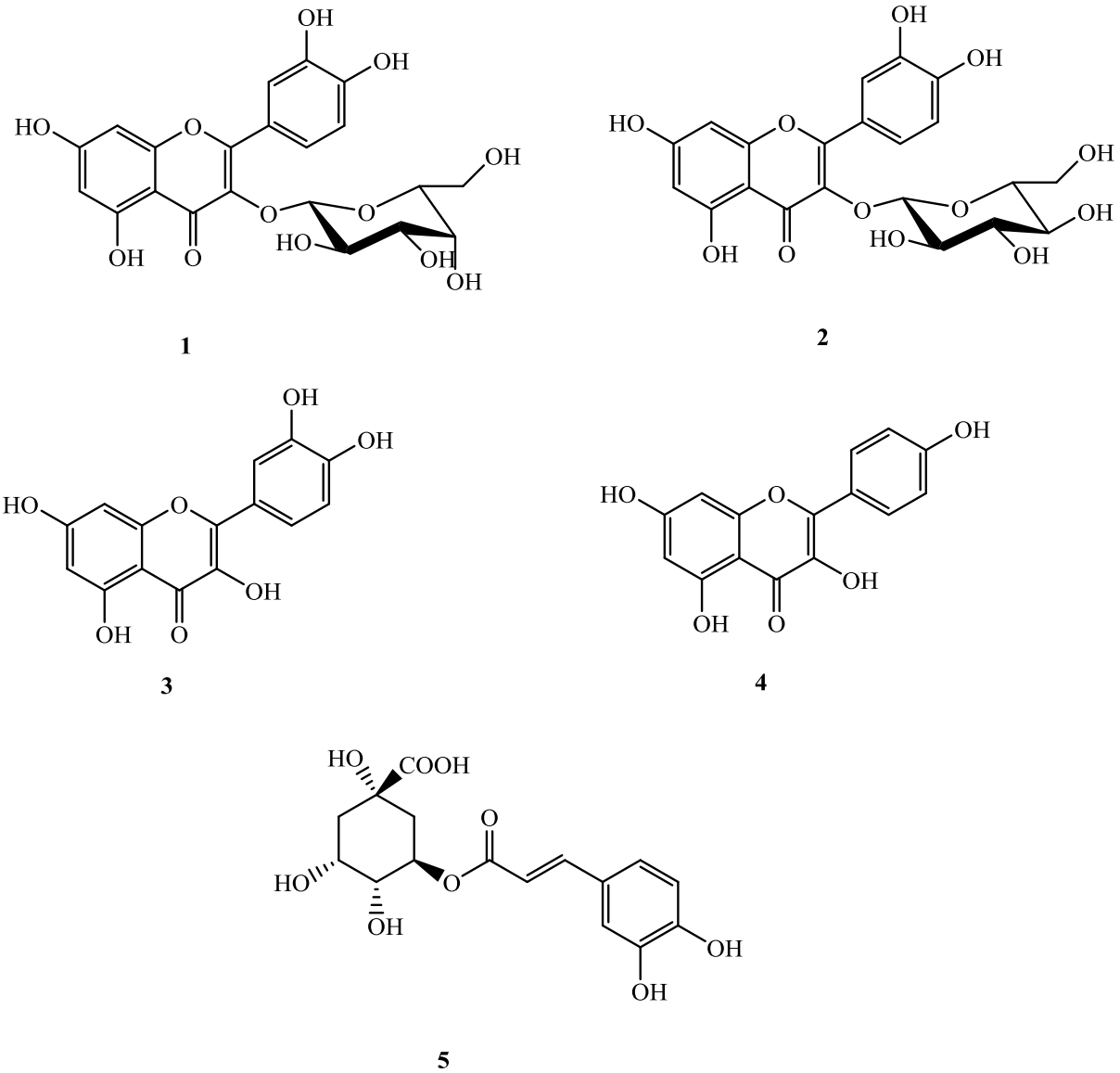


Figure 1. Chemical structures of reported constituents from *C. vulgaris*
1:hyperoside, **2**:isoquercetin, **3**: quercetin, **4:**kampferol, **5**: chlorogenic acid

The results of our preliminary phytochemical analysis of the tested extract from *C. vulgaris* (ECV) are shown in Table 2.

Table 2. Preliminary phytochemical analysis of the extract from *C. vulgaris* (ECV)

Analyzed extract	**Total phenolic content (mg GAE/g of extract)**	**Total flavonoid content (mg rutin/g of extract)**	**Chlorogenic acid (mg/g of extract)**
ECV	35.32±3.38 mg/g	20.15±1.64 mg/g	11.53±2.1 mg/g

Our data showed a high content of phenolic compounds (35.32±3.38 mg GAE/g and a total flavonoid content of 20.15±1.64 mg rutin/g) in the extract prepared from *C. vulgaris* (ECV). These findings are in accordance with the previously mentioned studies reporting significant amounts of polyphenolic compounds in this vegetal species.

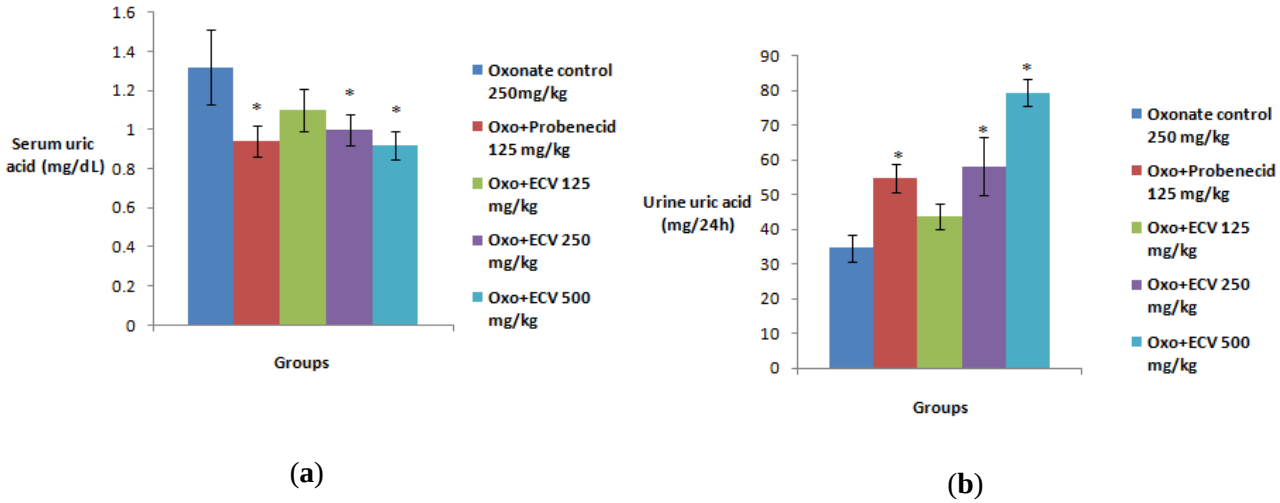
Flavonoids, represented by glycosides of quercetin ,isoquercetin and kampferol have been previously identified in *C. vulgaris*, but phenolic acids have been less studied, therefore our HPLC-PDA-MS analysis focused on the identification and dosing of these molecules in the tested ECV. Phenolic acid identification in vegetal extracts is difficult due to the presence of several isomers of

caffeoylquinic acid, but the use of mass spectrometry allow the characterization of each individual compound. In our experimental conditions detailed in Supplementary Information section, only one phenolic acid was unambiguously identified and quantified in the tested extract: chlorogenic acid (11.53±2.1 mg/g), with a retention time of 7.65 minutes. The presence of chlorogenic acid (3-caffeoylquinic acid) in ECV was further confirmed via mass spectrometry as well.

The differences in chemical composition could be explained by variations in temperature, humidity or soil composition [29]. The study of Monschein and colleagues [30] highlighted the influence of altitude on the chemical composition of *C. vulgaris*, a species widely spread from lowlands to mountain areas, stating that the percentage of flavonol-3-O-glycosides increased with altitude. Our study which used an extract prepared from *C. vulgaris* harvested from a relatively low altitude area (300-500 m), confirmed these data, the amount of phenolic acids being significant in the tested samples. Nevertheless, due to the limitations of our experimental model, other unidentified active substances could be present in the tested extract as described previously.

3.2. Hypouricemic Effect

The hypouricemic effect of the ECV was tested *in vivo* using the experimental model of oxonate-induced hyperuricemia in rats. The experimental results are presented in Figure 2.



(**a**) (**b**)

Figure 2. Hypouricemic effect of the extract from *C. vulgaris* (ECV) in rats, in the experimental model of oxonate-induced hyperuricemia: (**a**) Serum levels of uric acid in ECV-treated animals (mg/dl); (**b**) Urine levels of uric acid in ECV-treated animals (mg/dL).

The oral administration of ECV produced a statistically significant reduction of serum uric acid at the doses of 250 and 500 mg/kg. Also, ECV at doses of 250 mg/kg and 500 mg/kg produced an increased urinary excretion of uric acid, the results being statistically significant and superior to the reference hypouricemic drug acting by a uricosuric mechanism, probenecid.

Furthermore, in our experimental model, the assessment of blood/urine concentration of uric acid and creatinine, allowed the calculation of two key parameters of uric acid renal handling: uric acid clearance (ClrUA) and the fractional excretion of uric acid (FEUA). As seen in Table 3, urinary levels of uric acid and creatinine are considerably reduced in hyperuricemic animals, treated only with oxonate (control group). The low uric acid clearance (3.40±0.17) and FEUA (22.46±2.78) clearly showed a net reabsorptive flux of uric acid in the renal tubules of the rats. The administration of the extract from *C. vulgaris* produced a significant increase of both uric acid clearance and FEUA (11.21±0.25 and 45.15±4.01, respectively, for the dose of 500 mg/kg ECV), suggesting that the hypouricemic effect is probably due to the augmentation of urinary uric acid excretion (uricosuric effect).

Table 3. Uric acid excretion parameters in rats treated with the extract from *C.vulgaris* (ECV), in the experimental model of oxonate-induced hyperuricemia.

Group (Dose)	Serum uric acid (mg/dL)	Urine uric acid (mg/dl)	Uric acid clearance (mL/kg min)	Serum creatinine (mg/dL)	Urine creatinine (mg/dL)	Fractional excretion of uric acid (%)
Oxonate control (250 mg/kg)	1.32±0.19	34.60±3.84	3.40±0.17	0.60±0.05	70±6.13	22.46±2.78
Oxo+ECV (125 mg/kg)	1.10±0.15	43.60±6.02	5.15±0.48	0.61±0.10	79±5.44	30.59±4.52
Oxo+ECV (250 mg/kg)	1.00±0.18*	58.20±3.53*	7.56±0.28*	0.48±0.02*	83±7.21*	33.65±3.81*
Oxo+ECV (500 mg/kg)	0.92±0.13*	79.40±4.56*	11.21±0.25*	0.45±0.03*	86±4.48*	45.15±4.01*
Oxo+Probenecid (125 mg/kg)	0.94±0.08*	54.80±4.02*	7.57±0.32*	0.53±0.07*	85±6.29*	36.34±2.14*

*Statistically significant, $p \leq 0.05$; Data are expressed as Mean±SD

Our data showed that the ECV manifested significant and dose-dependent hypouricemic effects in oxonate-treated rats, by an uricosuric effect (by increasing the urinary excretion of uric acid).

A limited number of studies investigated hypouricemic effect of natural products in laboratory animals. In rodents and most mammals, uric acid is rapidly converted into soluble allantoin by the enzyme uricase, therefore the inhibition of uricase with potassium oxonate is a necessary precondition in order to study the hypouricemic effect. Chen and colleagues [31] found that the total saponin from *Dioscorea* reduced hyperuricemia in rodents by influencing xantine-oxidase (XOD). Additionally, another study showed that procyanidins from grape seeds reduced hyperuricemia in mice by decreasing XOD activity in the liver [32]. The uric acid lowering capacity of flavonoids has been rarely tested in laboratory animals. The study of Kondo and colleagues [33] showed that aspalathin, a C-glycosyl-dihydrochalcone from *Aspalathus linearis* showed hypouricemic effects in mice by inhibition of XOD.

Hence, we report for the first time a hypouricemic effect for a vegetal extract (ECV) containing mainly falavones and their glycosides (**1**-**4**) and chlorogenic acid (**5**). Thus, our research supports the reported ethnopharmacological use of *C. vulgaris* as a traditional remedy for gout.

Our work demonstrated a different mechanism for the hypouricemic effect of ECV: the augmentation of uric acid urinary excretion in oxonate-treated rats (uricosuric effect). Sugino and Shimada found that a probable mechanism for a uricosuric drug is represented by the inhibition of postsecretory reabsorption of urate in the renal tubules [34]. Another study showed that potassium oxonate was able to impair the expression of several organic anion transporters (mURAT1, m GLUT9, mOAT1) involved in the excretion of uric acid in the renal tubules, leading to hyperuricemia, thus a normalizing effect on the expression of these transport proteins could be responsible for the increased excretion of uric acid observed in rodents after the administration of natural products with uricosuric effect [35]. However, due to the complexity of physiological processes involved in the regulation of uric acid metabolism, other mechanisms of lowering uric acid concentration are possible. Nevertheless, the uricosuric effect is a validated anti-gout mechanism and according to the study of Bach and Simkin [36], an inhibition of renal tubular reabsorption of uric acid with increased urinary excretion should provide relief for millions of patients suffering from hyperuricemia and gout.

3.3. Antihypertensive Effect

The influence of high uricemia on systolic blood pressure and the possible beneficial effects of the ECV were investigated *in vivo*, using the oxonate-induced chronic hyperuricemia in rats. Hyperuricemic rats from the control group treated daily with oxonate only, by oral route, developed a significant increase in systolic blood pressure (SBP) after 3 weeks of treatment, from 110.6±6.65 mm Hg at the start of the experiment to 145.4 ±4.33 mm Hg at the end of week 3. In these animals, an increase of 1.45 mg/dl in serum uric acid concentration was directly correlated with an augmentation

of SBP of 34.8 mm Hg. The daily oral administration of a reference hypouricemic drug, probenecid (125 mg/kg) reduced the increase of systolic blood pressure from 111.4±7.16 mm Hg (week 0) to 129.8±3.70 mm Hg (week 3). The daily oral administration of the ECV throughout the experiment reduced the progression of arterial hypertension in oxonate-treated animals. The results were statistically significant ($p \leq 0.05$) for the doses of 250 and 500 mg/kg ECV. The highest dose of ECV, produced an antihypertensive effect which was superior to the reference drug, limiting the increase of SBP from 112.4±3.50 mm Hg at the start of the experiment to 123.8±2.77 at the end of week 3, as shown in Table 4.

Table 4. The effect of the extract from *C. vulgaris* (ECV) on systolic blood pressure (SBP) in rats, in chronically oxonate-induced hyperuricemia

Group (Dose)	SBP[a] week 0 (mm Hg)	SBP[a] week 3 (mm Hg)	UAs[b] week 0 (mg/dl)	UAs[b] week 3 (mg/dl)
Oxonate control (250 mg/kg)	110.6±6.65	145.4±4.33	0.73±0.06	2.18±0.31
Oxo+ECV (125 mg/kg)	113.4±6.65	140.2±2.68	0.69±0.08	1.83±0.61
Oxo+ECV (250 mg/kg)	114.6±4.56	131.6±3.36*	0.78±0.13	1.46±0.22*
Oxo+ECV (500 mg/kg)	112.4±3.50	123.8±2.77*	0.75±0.09	1.32±0.19*
Oxo+Probenecid (125 mg/kg)	111.4±7.16	129.8±3.70*	0.81±0.11	1.41±0.25*

[a] SBP is systolic blood pressure (mm Hg), [b]UAs is the serum concentration of uric acid (mg/dl); Results are expressed as mean±SD; *Statistically significant, $p \leq 0.05$

Our data indicated a strong correlation between increased serum uric acid concentration and the development of arterial hypertension in rats. The daily administration of the tested extract produced a statistically significant antihypertensive effect at the doses of 250 and 500 mg/kg in hyperuricemic rats, due to the reduction of serum uric acid concentration.

The relationship between serum level of uric acid and arterial hypertension is a much debated topic in medical journals. Several preclinical studies showed that hyperuricemia could induce endothelial dysfunction with subsequent arterial hypertension by reducing nitric oxide production in endothelial cells [37] and by promoting afferent arterial thickening [38]. In humans, these data were confirmed by the studies of Agarwal and colleagues [39] which showed that elevated uric acid is an independent risk factor for the development of arterial hypertension and Uedono and colleagues [40] which showed that higher serum uric acid levels may cause impaired perfusion of the glomeruli with the possible development of chronic kidney disease (CKD) and arterial hypertension, due to the increase of the resistance of the afferent arterioles. Our data showed that the 3-week oral administration of ECV at 250 and 500 mg/kg to hyperuricemic rats reduced the development of arterial hypertension, further research being necessary to elucidate the mechanism of this promising antihypertensive effect.

3.4. Anti-inflammatory Effect

The anti-inflammatory potential of ECV was evaluated *in vivo* by the rodent paw oedema test induced by a phlogistic agent: λ-carrageenan.

As seen in Table 5, a net anti-inflammatory effect was recorded in animals treated with ECV 500 mg/kg, at all time intervals after the induction of inflammation and ECV 250mg/kg, 3 h and 4 h after the induction of inflammation. The anti-inflammatory effect of ECV at the highest dose (500 mg/kg) was only slightly inferior to the standard anti-inflammatory drug, diclofenac, at all time intervals.

The apparition of rodent paw oedema in this experimental model is a two-stage phenomenon. Firstly, several pro-inflammatory mediators (histamine, 5-hydroxy-tryptamine, complement fractions) are liberated and secondly, an augmented synthesis of eicosanoids is seen at the inflammatory site.

Table 5. Effect of the extract from *C. vulgaris* (ECV) on carrageenan-induced paw oedema in rats

Group	Dose	Oedema 1h mL) (% inhib.)	Oedema 2h (mL) (% inhib.)	Oedema 3h (mL) (% inhib.)	Oedema 4h (mL) (% inhib.)
Control (vehicle)	-	0.89±0.16	1.18±0.33	2.35±0.30	2.82±0.45
ECV	125 mg/kg	0.88±0.13 (1.12%)	1.16±0.09 (1.69%)	2.17±0.35 (7.65%)	2.30±0.59 (18.43)
ECV	250 mg/kg	0.76±0.11 (14.60%)	1.08±0.18 (8.47%)	1.90±0.15* (19.14%)	2.16±0.21* (23.40%)
ECV	500 mg/kg	0.61±0.20* (31.46%)	0.84±0.16* (28.81%)	1.35±0.31* (42.55%)	1.48±0.41* (47.51%)
Diclofenac	20 mg/kg	0.59±0.04* (33.70%)	0.77±0.13* (34.74%)	1.04±0.25* (55.74%)	1.38±0.39* (51.06%)

*Statistically significant, $p \leq 0.05$; Data are expressed as Mean±SD

A series of other studies investigated both *in vitro* and *in vivo* the anti-inflammatory and antinociceptive effects of active compounds identified in our work. Flavonoids are a well-known class of compounds capable of inhibiting inflammation by the reduction of concentration of multiple pro-inflammatory factors: eicosanoids, cytokines or adhesion molecules [41]. Also, the main phenolic acid component, chlorogenic acid is considered to be an antioxidant, anti-inflammatory and antinociceptive agent. In an *in vitro* study, chlorogenic acid significantly inhibited NO production and also the expression of COX-2 and iNOS, without any cytotoxicity in lipopolysaccharide (LPS)-stimulated murine RAW 264.7 macrophages and BV2 microglial cells [42]. In an *in vivo* model, chlorogenic acid administered orally in the rat inhibited the T cell count and the levels of IL-1 and TNF-α, two prominent pro-inflammatory cytokines [43]. Furthermore, another study found that *in vivo* administration of chlorogenic acid in the rat at doses of 50 and 100 mg/kg inhibited the inflammatory process in carrageenan-induced paw oedema and reduced the number of flinches in the formalin test [44].

Our data suggest that a series of potential mechanisms could generate the anti-inflammatory effect of ECV. A decrease of eicosanoid synthesis in the inflamed tissues would be the most likely mechanism, but ECV may also reduce the first stage of oedema generation, probably by diminishing the liberation of other inflammatory substances. Further studies are necessary to elucidate the molecular mechanism of the anti-inflammatory effect.

3.5. Analgesic Effect

The analgesic effect of ECV was investigated *in vivo* by applying the Randall-Selitto test in rats, the results being presented in Figure 3. The oral administration of ECV produced a net analgesic effect at the dose of 500 mg/kg, with a maximal amount of pressure supported by the animals of 95 g, 4 h after the administration. The magnitude of the effect was equal to the reference analgesic drug, diclofenac.

In Randall-Selitto test, the injection of the noxious substance triggers an increased expression of cyclooxygenase 2 in the affected tissue, with a subsequent PGE2 overproduction which stimulates nociceptors, causing pain. Kupeli and Yesilada showed that flavonoids represented by glycosides of quercetol and kampferol could have antinociceptive effects by peripheral mechanisms in the *p*-benzoquinone induced writhes experimental model in rodents [45]. More specifically, Orhan and colleagues [46] demonstrated that an extract from *C. vulgaris* containing kaempferol-3-O-β-D-galactoside presented strong anti-inflammatory and antinociceptive properties.

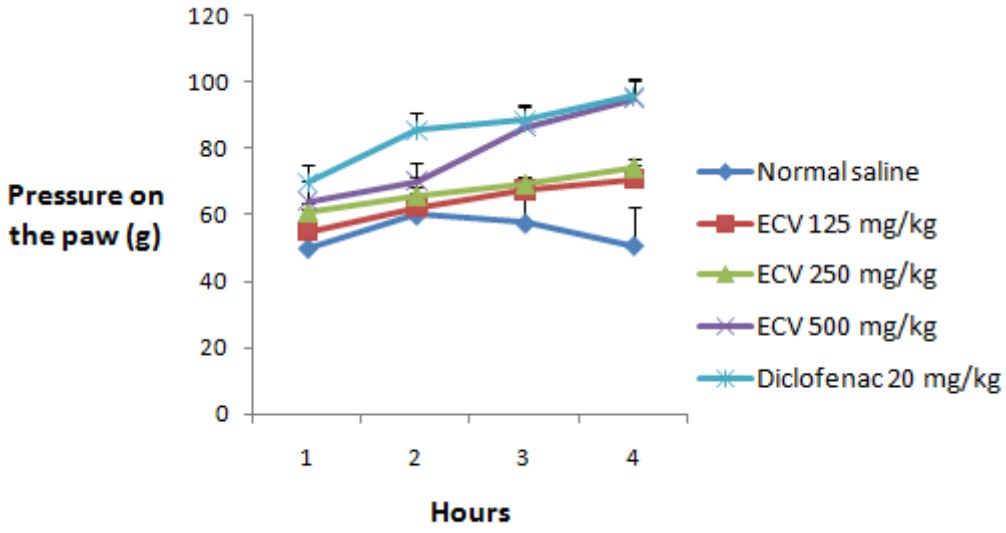


Figure 3. Antinociceptive effect of the extract from *C. vulgaris* (ECV) in Randall-Selitto test in rats

Our data confirmed those studies, suggesting that anti-inflammatory and antinociceptive effects of the tested ECV (containing flavonol glycosides) may have peripheral mechanisms represented by an inhibition of eicosanoid synthesis. A reduction of liberation of other pro-inflammatory substances is also have a possible mechanism.

In conclusion, our study reports for the first time a dose-dependent hypouricemic effect for an ethanolic extract of *C. vulgaris* (ECV), due to the augmentation of uric acid urinary excretion in oxonate-treated rats. ECV showed also an additional effect of preventing arterial hypertension caused by hyperuricemia in rats. These effects were accompanied by anti-inflammatory and analgesic effects, reported also by other studies investigating polyphenols. The co-existence of these valuable biological effects of the tested extract from *C. vulgaris* could provide relief in acute gout attacks but also in the chronic treatment of this disease, using an easily available natural resource.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP

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