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Anti-inflammatory and antinociceptive properties of blueberry extract (*Vaccinium corymbosum*)

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Abstract

Blueberries are among the edible fruits that are recognized best for their potential health benefits. The crude extract from *Vaccinium corymbosum* was assessed in anti-inflammatory and antinociceptive models. The crude hydroalcoholic extract was administered orally at doses of 100, 200 or 300 mg kg^{-1} for all the assays. In the carrageenan test, the crude extract reduced rat paw oedema by 9.8, 28.5 and 65.9%, respectively. For the histamine assay, the reductions of oedema were 70.1, 71.7 and 81.9%, respectively. In the myeloperoxidase (MPO) assay, 300 mg kg^{-1} crude extract produced a significant inhibition of the MPO activity, at 6 h and 24 h after injection of carrageenan, by 42.8 and 46.2%, respectively. With the granulomatous tissue assay dexamethasone displayed significant activity, whereas the blueberry extract was inactive. For the abdominal constriction test, inhibitions of 49.0, 54.5, 53.5%, respectively, were observed for the crude extract, and 61.4% for indometacin. In the formalin test, the crude extract (200 and 300 mg kg^{-1}) and indometacin inhibited only the second phase by 36.2, 35.3 and 45.8%, respectively. Considering that the crude extract of blueberry displayed antinociceptive and anti-inflammatory activity, its consumption may be helpful for the treatment of inflammatory disorders.

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Introduction

Blueberries are among the edible fruits that are recognized best for their potential health benefits. Many of the health-promoting properties are thought to be attributable to their main group of bioactive compounds, which belong to the proanthocyanidin and anthocyanin classes (Faria et al 2005). In this regard, the chemical profile of plants belonging to the genus *Vaccinium* could be characterized mainly by the presence of anthocyanin compounds in the fruits, which are called blueberry due to the high content of these compounds. As a result, there has been growing interest in the anthocyanin content of some *Vaccinium* species with regards to its pharmacological properties, particularly the effects on blood vessels, its use in ophthalmology, and the potential inhibition of HIV (Cabrita & Andersen 1999).

Anthocyanins are plant pigments responsible for the orange, red and blue colours of fruits, flowers, vegetables and other storage tissues in plants (Strack & Wray 1993). These compounds are involved in a wide range of biological activity (Kong et al 2003), including antioxidant (Mazza et al 2002), anti-inflammatory (Youdim et al 2002) and anticarcinogenic (Katsube et al 2003). Moreover, anthocyanins may also display neuroprotective action and the ability to reduce the risk of coronary heart disease (Renaud & De Lorgeril 1992) through vasoprotective activity, effects on arterial vasomotion, and inhibition of platelet aggregation (Colanttuoni et al 1991). Anthocyanins from *Vaccinium* are used to enhance vision and to increase capillary resistance (Peterson & Dwyer 1998).

In the literature there are many compounds of plant origin reported as possessing important biological activity, such as anti-inflammatory (Namiki 1990). The purpose of this study was to investigate the anti-inflammatory and antinociceptive effects of the hydroalcoholic extract of blueberry (*Vaccinium corymbosum*).

Materials and Methods

Plant material

Berries of the blueberry (*V. corymbosum*) were collected in the experimental field of "Pesquisa Agropecuária e Extensão Rural" company of the State of Santa Catarina (EPAGRI), Videira, in 2005. All berries were picked at the commercially ripe stage. The berries were selected by removing damaged, diseased, pest-infested fruits, stems and leaves. They were maintained in polyethylene bags at −20°C until extract preparation. Before extraction, frozen berries were crushed by using a food processor. The crushed berries (1.0 kg) were macerated with aqueous ethanol 70% (v/v) at room temperature for seven days. The crude extract was obtained by filtration, followed by concentration under reduced pressure, yielding 138.0 g (13.8%, w/w).

Phytochemical studies carried out with *Vaccinium* berries have demonstrated the presence mainly of phenolic acid compounds, such as: gentisic, gallic, *o*-pyrocatechuic, protocatechuic, salicylic, syringic, vanillic, veratric, caffeic, *m*-coumaric, *o*-coumaric, *p*-coumaric, 3,4-dimethoxycinnamic, ferulic, hydroxycaffeic, sinapic, and *p*-hydroxyphenilacetic. In addition many anthocyanin compounds have been identified, such as: delphinidin-3-galactose, delphinidin-3-glucose, delphinidin-3-arabinose, cyanidin-3-galactose, cyanidin-3-glucose, petunidin-3-galactose, cyanidin-3-arabinose, petunidin-3-glucose, peonidin-3-galactose, petunidin-3-arabinose, peonidin-3-glucose, malvidin-3-galactose, peonidin-3-arabinose, malvidin-3-glucose and malvidin-3-arabinose (Blumenthal et al 2000; Faria et al 2005; Zadernowski et al 2005).

Quantification of total phenolics and anthocyanins

The concentration of phenolic compounds in the extract was determined by the Folin-Ciocalteu colorimetric method (Singleton & Rossi 1965). Analyses were carried out in triplicate, and the quantification was calculated from a calibration curve obtained with catequin. Total phenolics were expressed as catequin equivalents (mg (g extract)$^{-1}$).

Anthocyanidins were quantified using a Varian Pro Star HPLC system equipped with a type VA CP29257 C18 column (250 mm × 4.6 mm i.d., 5 μm, VARIAN), Pro Star 400 auto sampler and detector Pro Star 310 UV-vis at 524 nm. The mobile phase consisted of water:acetonitrile (85:15, v/v), acidified (pH 2.2) with phosphoric acid. The elution was undertaken using an isocratic mode at a flow-rate of 1.2 mL min^{-1}. Under analytical conditions standard samples were injected (10 μL; 5, 15 and 25 mg L^{-1}) obtaining standard curves with three levels, 10% of tolerance and r^2 coefficient = 0.999 for malvidin, cyanidin and delphinidin. Detection was performed at 280 nm. Samples of extract were filtered through a 0.45-μm syringe filter before injection of a 10-μL sample into the HPLC system. The concentrations of malvidin, cyanidin and delphinidin were determined and expressed as mg g^{-1}.

Animals

Male Wistar rats (200–250 g) and male Swiss mice (40–45 g) were provided by the Central Animal House of University of West of Santa Catarina (UNOESC). The animals were housed in groups of five in standard cages at room temperature (25 ± 3°C), under a 12-h dark/light cycle, the real period of light, with food and water freely available. Twelve hours before the experiments animals were transferred to the laboratory and were maintained only with water freely available. The experiments were authorized by the Ethical Committee for Animal Care of University of West of Santa Catarina, Brazil, in accordance with the Federal Government legislation on animal care. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Carrageenan-induced rat paw oedema

The method used was described by Winter et al (1962). Groups of rats (n = 6) were treated orally with blueberry crude extract dissolved in water (100, 200 or 300 mg kg^{-1}), indometacin (10 mg kg^{-1}) or vehicle (water), 30 min before the injection of the stimulus (carrageenan 1000 μg/paw) into the right hind paw plantar surface. Sterile saline solution (0.9%, 0.1 mL) was injected into the left paw as control reference for the tested paw. The foot volumes of the animals were determined by a plestimographic method described by Ferreira (1979). Foot volume was measured before and at hourly intervals for 3 h after the injection of the inflammatory stimulus into the right hind paw plantar surface. The inhibition of inflammation was calculated by measuring the volume difference between the right and left paws in comparison with the control group.

Histamine-induced rat paw oedema

The animals were treated in a manner similar to that of the carrageenan-induced paw oedema protocol. However, the paw oedema was induced by subplantar injection of histamine (50 μg/paw), and the oedema was measured as mentioned earlier at hourly intervals for 2 h. A positive control group was treated with hydroxyzine (10 mg kg^{-1}, p.o.).

Myeloperoxidase activity

The anti-inflammatory activity of *V. corymbosum* was investigated by evaluating neutrophil infiltration indirectly by measuring myeloperoxidase (MPO) activity, which was determined at 6- and 24-h time points (De Young et al 1989). The crude extract (300 mg kg^{-1}) and vehicle (water) were administered to the animals as described above. Briefly, 6 and 24 h after carrageenan injection, the subcutaneous tissue of the injected paws was removed and homogenized in 5% (w/v) 80 mM phosphate buffer, pH 5.4, containing 0.5% hexadecyltrimethylammonium bromide. The homogenate was centrifuged at 12 000 *g* and 4°C for 20 min. Samples (30 μL) of each supernatant were mixed with 100 μL phosphate buffer 80 mM, 85 μL phosphate buffer 0.22 M and 15 μL 0.017% H_2O_2 in 96-well plates. The reaction was triggered with 20 μL tetramethylbenzidine (dissolved in dimethylformamide). The

plate was kept at 37°C for 3 min. It was then placed on ice, and the reaction was stopped by adding 30 μL sodium acetate 1.46 M, pH 3.0. The enzymatic activity was determined by measuring the optical density at 630 nm, and it was expressed as mOD (mg protein)$^{-1}$.

Granulomatous tissue induction

This assay was described by Niemegeers (1975). Pellets weighing approximately 40 mg each were made with 5 mm of dental cotton tampons and implanted into rats (n = 6). The pellets were sterilized and impregnated with 0.4 mL ampicillin water solution at the moment of implantation. Animals were anaesthetized with thiopental sodium, and four pellets were subcutaneously introduced through an abdominal skin incision. Each group was treated orally, daily for six consecutive days, with crude extract (300 mg kg^{-1}), dexamethasone (0.2 mg kg^{-1}) or vehicle (0.5 mL water). On the seventh day, the animals were killed, the pellets were dissected out, and the wet weights were immediately determined. After that, granulomas were dried at 60°C overnight to determine the dried weight. The difference between the initial and final weight was considered as the weight of the granulomatous tissue produced.

Abdominal constriction test

The abdominal constriction test was carried out as described by Koster et al (1959). Groups of mice (n = 8) were treated with crude extract (100, 200 or 300 mg kg^{-1}), indometacin (10 mg kg^{-1}) or vehicle (0.5 mL water), orally. The abdominal constrictions were induced by an intraperitoneal injection of 0.6% acetic acid solution (0.25 mL/animal), 30 min after the treatment. The number of abdominal constrictions was counted starting at 5 min after acetic acid injection for a period of 20 min. Data represented the average of the total abdominal constrictions observed.

Formalin test

The test was carried out as described by Hunskaar & Hole (1987). Animals were injected subcutaneously with 20 μL formalin into the dorsal hind paw. Groups of mice (n = 8) were treated with crude extract (100, 200 or 300 mg kg^{-1}), indometacin (10 mg kg^{-1}) or vehicle (0.5 mL water), orally, 30 min before formalin injection. The time spent by mice licking the injected paw was recorded. On the basis of the response pattern described by Tjolsen et al (1992), two distinct periods of intensive licking activity were identified and scored separately. The first period, early phase, was recorded for the first 5 min, after the injection of formalin, and the second period, late phase, was recorded between 20 and 30 min after the injection of the stimulus.

Statistical analysis

All the data were analysed for n ≥ 5. Data were reported as mean ± s.e.m., and it was analysed statistically by analysis of variance, followed by Dunnett's test (Sokal & Rohlf 1995). Results with $P < 0.05$ were considered significant.

Results

The total concentration of phenolic compounds in the extract was 5.66 ± 0.01 mg catechuin g^{-1}. Malvidin, cyanidin and delphinidin were present in the extract at concentrations of 0.131 ± 0.06, 0.099 ± 0.05 and 0.063 ± 0.06 mg g^{-1}, respectively (Figure 1).

Figures 2 and 3 show the effect of *V. corymbosum* crude extract on the carrageenan- and histamine-induced rat paw oedema, respectively. In the carrageenan protocol, the crude extract reduced the paw oedema, measured 3 h after carrageenan injection, which corresponded to the peak of oedema formation, by 9.8, 28.5 and 65.9%, at doses of 100, 200 and 300 mg kg^{-1}, respectively. For the group treated with indometacin, a 63.8% reduction of oedema was observed. These results were significant ($P < 0.05$) only for the groups treated with 300 mg kg^{-1} crude extract or indometacin (Figure 2). In the histamine protocol, a significant reduction of the oedema formation ($P < 0.05$) was observed at 1 h, which corresponded with the peak of oedema. For the groups treated with crude extract at 100, 200 or 300 mg kg^{-1}, inhibitions of 70.1, 71.7 and 81.9%, respectively, were observed. The positive control, hydroxyzine (10 mg kg^{-1}), reduced the oedema by 68.6% (Figure 3).

With the myeloperoxidase assay, the crude extract of *V. corymbosum* (300 mg kg^{-1}) produced a significant inhibition of MPO activity in the treated animals, at 6 and 24 h after injection of carrageenan by 42.8 and 46.2%, respectively (Figure 4).

In the granulomatous tissue induction assay, on one hand dexamethasone displayed significant inhibition ($P < 0.05$) in both wet and dry granuloma weights, and on the other hand, the crude extract neither diminished the wet nor the dry granuloma weights (Table 1).

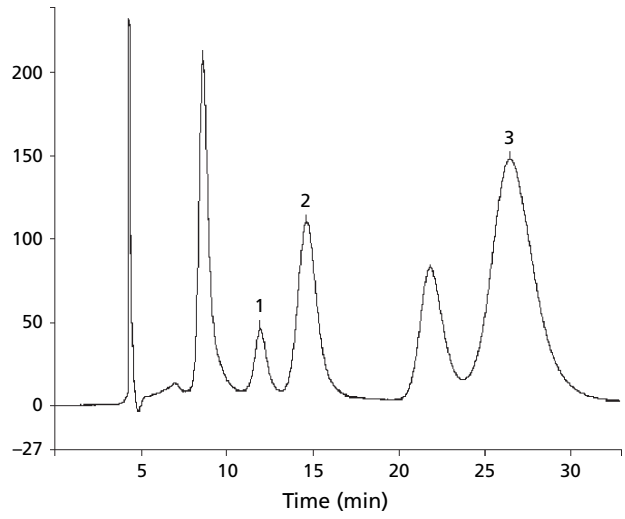

Figure 1 HPLC chromatographic profile of the blueberry hydroalcoholic crude extract, using a C18 column and an isocratic mobile phase consisting of water:acetonitrile (85:15, v/v), acidified (pH 2.2) with phosphoric acid. 1, Cyanidin; 2, delphinidin; 3, malvidin.

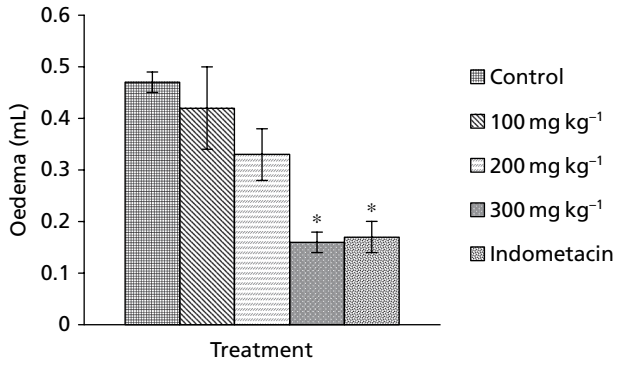

Figure 2 Effect of the administration of blueberry crude extract (100, 200 and 300 mg kg^{-1}) and indometacin (10 mg kg^{-1}) on carrageenan-induced rat paw oedema. The paw oedema volume was taken 3 h after carrageenan injection. Each column represents the mean ± s.e.m., n = 6. *$P < 0.05$, analysis of variance followed by Dunnett's test.

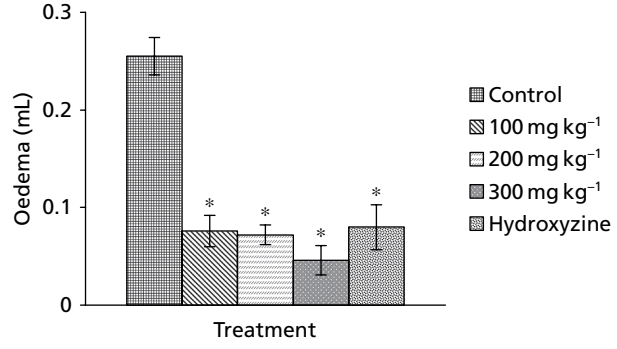

Figure 3 Effect of the administration of blueberry crude extract (100, 200 and 300 mg kg^{-1}) and hydroxyzine (10 mg kg^{-1}) on histamine-induced rat paw oedema. The paw oedema volume was taken 1 h after histamine injection. Each column represents the mean ± s.e.m., n = 6. *$P < 0.05$, analysis of variance followed by Dunnett's test.

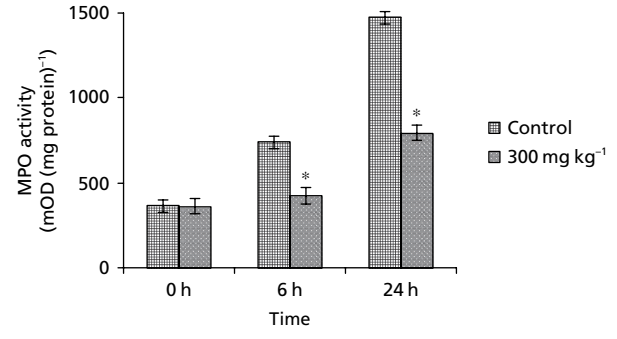

Figure 4 Effect of the administration of blueberry crude extract (300 mg kg^{-1}) on myeloperoxidase activity. The myeloperoxidase activity was measured at 0, 6 and 24 h after carrageenan injection. Each column represents the mean ± s.e.m., n = 6. *$P < 0.05$, analysis of variance followed by Dunnett's test.

In the abdominal constriction test, crude extract (100, 200 or 300 mg kg^{-1}) and indometacin were significantly effective in inhibiting the abdominal constrictions in mice, compared with the control group. Abdominal constriction inhibitions were 49.0, 54.5, 53.5 and 61.4% for the groups treated with 100, 200 or 300 mg kg^{-1} crude extract and indometacin, respectively (Table 2).

In the formalin test, the administration of crude extract and indometacin inhibited only the second phase of the test, which corresponded to inflammatory pain. The results were significant for the groups treated with 200 or 300 mg kg^{-1} crude extract and indometacin, displaying 36.2, 35.3 and 45.8% reduction in licking time, respectively (Table 3).

Discussion

This study demonstrated that the blueberry (*V. corymbosum*) hydroalcoholic extract could play a significant role in the inhibition of pain and inflammatory processes. Inflammation is an essential protective process to preserve the integrity of organisms against physical, chemical, and infective aggression (Walport & Duff 1993). However, the inflammatory response frequently leads to the damage of normal tissue (Majno & Joris 1996).

Among the protocols used to screen new anti-inflammatory agents, carrageenan-induced rat paw oedema is the most widely used primary test (Winter et al 1962). The development of oedema in rat paw, after the injection of carrageenan, has been described as a biphasic event (Brito & Antonio 1998). The initial phase, observed during the first hour, has been attributed to the release of histamine and serotonin, and the second phase to the release of prostaglandins (Crunkhorn & Meacock 1971). Therefore, it could be inferred that the significant activity observed in the suppression of the second phase may be explained by an inhibition of cyclooxygenase (Badilla et al 2003). Moreover, indometacin, the usual and non-selective inhibitor of cyclooxygenase, inhibits the second phase of the carrageenan-induced oedema (Tamura et al 2002). In addition, histamine is a basic amine related to the inflammatory and allergic process causing, among several effects, vasodilatation and an increase in vascular permeability (Rang et al 2001). The higher dose of the *V. corymbosum* extract (300 mg kg^{-1}) inhibited carrageenan and histamine oedema models, indicating anti-inflammatory and antihistaminic effects.

In the myeloperoxidase assay, a significant reduction in MPO activity for the group treated with blueberry extract was observed. The onset of the carrageenan oedema has been linked to neutrophil infiltration, release of other neutrophil-derived mediators and production of neutrophil-derived free radicals (Cuzzocrea et al 1999). MPO, a haeme protein, is an enzyme present in neutrophils and at a much lower concentration in monocytes and macrophages. It is a critical enzyme for the generation of hypochlorous acid and other toxic oxygen products. It is well known that the level of MPO activity is directly proportional to the neutrophil concentration in the inflamed tissue (Bradley et al 1982). Hence, the measurement of the enzyme activity has been considered a quantitative and sensitive tool to evaluate the chemotaxis and neutrophil infiltration in the inflammatory process (Smith 1994).

Table 1 Effect of the administration of blueberry crude extract and dexamethasone on cotton pellet-induced granuloma weights

Groups	Dose (mg kg^{-1})	Mean weight of wet granuloma (mg)	Inhibition (%)	Mean weight of dry granuloma (mg)	Inhibition (%)
Control	–	714.2 ± 27.8	–	133.2 ± 5.0	–
Crude extract	300	570.2 ± 16.8	20.1	112.4 ± 3.6	15.6
Dexamethasone	0.2	392.0 ± 6.5*	45.1	79.6 ± 2.1*	40.2

The results are expressed as mean ± s.e.m. (n = 6). *$P < 0.05$, analysis of variance followed by Dunnett's test.

Table 2 Effect of the administration of blueberry crude extract and indometacin on acetic acid-induced abdominal constrictions

Groups	Dose (mg kg^{-1})	Number of constrictions (mean ± s.e.m.)	Inhibition (%)
Control	–	92.7 ± 5.7	–
Crude extract	100	49.8 ± 2.7*	49.0
	200	42.1 ± 2.3*	54.5
	300	43.1 ± 2.5*	53.5
Indometacin	10	35.7 ± 4.7*	61.4

The results are expressed as mean ± s.e.m., n = 8. *$P < 0.05$, analysis of variance followed by Dunnett's test.

Table 3 Effect of the administration of blueberry crude extract and indometacin on formalin-induced nociception

Groups	Dose (mg kg^{-1})	Licking time (s) (mean ± s.e.m.)		Inhibition (%)	
		Early phase (0–5 min)	Late phase (20–30 min)	Early phase	Late phase
Control	–	66.5 ± 3.2	114.2 ± 4.5	–	–
Crude extract	100	55.3 ± 4.9	93.0 ± 6.1	16.8	18.5
	200	63.0 ± 6.6	72.8 ± 8.9*	5.2	36.2
	300	72.3 ± 9.3	73.8 ± 2.5*	–8.7	35.3
Indometacin	10	66.8 ± 7.5	61.8 ± 9.1*	–0.4	45.8

The results are expressed as mean ± s.e.m., n = 8. *$P < 0.05$, analysis followed by Dunnett's test.

Nevertheless, it is also known that inflammation sites present high concentrations of free radicals and oxidants, which play an important role in different inflammation processes. Therefore, antioxidant compounds may be helpful to prevent this process (Salvemini et al 1996). In this regard, antioxidant activity has been described for several flavonoids and anthocyanins (Peterson & Dwyer 1998; Moyer et al 2002), which are present in high concentrations in the fruits of *Vaccinium* species, such as malvidin, cyanidin, delphinidin, astragalin, hyperoside, isoquercitrin and quercitrin (Blumenthal et al 2000). Hence, these compounds may play an important role in the anti-inflammatory activity of blueberry extract, not only because of its actions on inflammatory chemotatic mediators, but also its reported antioxidant activity. It is known that the inflammatory granuloma is a typical response of a chronic inflammatory process. It has been established that the dry weight of the pellets is well correlated with granulomatous tissue (Olajide et al 2000). Anti-inflammatory steroidal drugs show higher activity in this model (Swingle & Shideman 1972). However, the crude extract of *V. corymbosum* was not able to significantly inhibit this process. Regarding the antinociceptive activity, the intraperitoneal administration of acetic acid irritates serous membranes, provoking a stereotypical behaviour in mice characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles, and a reduction in motor activity and coordination (Bars et al 2001). Acetic acid causes algesia by liberating endogenous substances that excite pain nerve endings, and it is a sensitive method for screening peripheral and central analgesic agents (Collier et al 1968). The administration of crude extract of *V. corymbosum* reduced the number of mouse abdominal constrictions after acetic acid administration, indicating analgesic activity for this extract at the assayed doses. The obtained results for the abdominal constriction test alone did not allow the determination as to whether the antinociceptive effect was either central or peripheral. Thus, to clear the mode of the inhibitory effects of this extract on the nociceptive responses, the formalin test was used. In the formalin test, there was a distinct biphasic response. The initial pain, early phase, was explained as a direct stimulation of nociceptors. The late phase is thought to be secondary to the inflammatory reactions (Hunskaar & Hole 1987). Therefore, the test can be used to clarify the possible mechanism of antinociceptive effect of a proposed analgesic (Tjolsen et al 1992). On one hand, centrally acting drugs, such as opioids, inhibit both phases equally (Shibata et al 1989), but on the other hand, peripherally-acting drugs such as aspirin, indometacin and dexamethasone inhibit only the late phase. The crude extract of *V. corymbosum* inhibited only the second phase of the formalin test, suggesting peripheral analgesic activity.

Conclusion

The crude extract of berries from *V. corymbosum* displayed antinociceptive and anti-inflammatory activity. Considering that blueberries are rich in phenolic acids, flavonoids and anthocyanins, and that there are several works reporting the anti-inflammatory and antinociceptive activity for compounds belonging to these classes, it is suggested that the reported activity might have been, at least in part, due to these

compounds. Moreover, considering that blueberries are edible fruits, their consumption may be helpful for the treatment of inflammatory disorders. In addition, this work has corroborated the traditional indication of different species of *Vaccinium* to treat inflammatory conditions, which may contribute to the understanding of the role of this edible fruit in promoting health.

References

Badilla, B., Arias, A. Y., Arias, M., Mora, G. A., Poveda, L. J. (2003) Anti-inflammatory and antinociceptive activities of *Losa speciosa* in rats and mice. *Fitoterapia* **74**: 45–51

Bars, D., Gozariu, M., Cadden, S. W. (2001) Animal models of nociception. *Pharmacol. Rev*. **53**: 597–652

Blumenthal, M., Goldberg, A., Brinckmann, J. (2000) Herbal Medicine - expanded commission E monographs. 1st edn, American Botanical Council, Newton, MA, USA, p. 519

Bradley, P. B., Pribat, D. A., Christensen, R. O., Rothstein, G. (1982) Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J. Invest. Dermatol*. **78**: 206–209

Brito, A. R. M. S., Antonio, M. A. (1998) Oral anti-inflammatory and anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turneraceae). *J. Ethnopharmacol*. **61**: 215–228

Cabrita, L., Andersen, O. M. (1999) Anthocyanins in blue berries of *Vaccinium padifolium. Phytochemistry* **52**: 1693–1696

Colanttuoni, A., Bertuglia, S., Magistretti, M. J., Donato, L. (1991) Effects of *Vaccinium myrtillus* anthocyanosides on arterial vasomotion. *Arzneimittelforschung* **41**: 905–909

Collier, H. O. J., Dinneen, L. C., Johnson, C. A., Schneider, C. (1968) The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmacol*. **32**: 295–310

Crunkhorn, P., Meacock, S. C. (1971) Mediators of the inflammation induced in the rat paw by carrageenin. *Br. J. Pharmacol*. **42**: 392–402

Cuzzocrea, S., Constantino, G., Mazzon, E., Zingarelli, B., De Sarro, A., Caputi, A. P. (1999) Protective effects of Mn (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP), a superoxide dismutase mimetic, in paw oedema induced by carrageenan in the rat. *Biochem. Pharmacol*. **58**: 171–176

De Young, L. M., Kheifets, J. B., Ballaron, S. J., Young, J. M. (1989) Edema and cell infiltration in the phorbol ester-treated mouse ear are temporally separate and can be differentially modulated by pharmacological agents. *Agents Actions* **26**: 335–341

Faria, A., Oliveira, J., Neves, P., Gameiro, P., Santos-Buelga, C., Freitas, V., Mateus, N. (2005) Antioxidant properties of prepared blueberry (*Vaccinium myrtilus*) extracts. *J. Agric. Food. Chem*. **53**: 6896–6902

Ferreira, S. H. (1979) A new method for measuring variations of rats paw volumes. *J. Pharm. Pharmacol*. **31**: 648

Hunskaar, S., Hole, K. (1987) The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* **30**: 103–114

Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K., Kobori, M. (2003) Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *J. Agric. Food. Chem*. **51**: 68–75

Kong, J. M., Chia, L. S., Goh, N. K., Chia, T. F., Brouillard, R. (2003) Analysis and biological activities of anthocyanins. *Phytochemistry* **64**: 923–933

Koster, R., Anderson, M., DeBeer, E. J. (1959) Acetic acid analgesic screening. *Fed. Proc*. **18**: 418–420

Majno, G., Joris, I. (1996) *Cells, tissues and disease: principles of general pathology*. Blackwell Science, Cambrigde, MA, p. 293

Mazza, G., Kay, C. D., Cottrell, T., Holub, B. J. (2002) Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *J. Agric. Food. Chem*. **50**: 7731–7737

Moyer, R. A., Hummer, K. M., Finn, C. E., Frei, B., Wrolstad, R. E. (2002) Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: Vaccinium, Rubus and Ribes. *J. Agric. Food. Chem*. **50**: 519–525

Namiki, M. (1990) Antioxidants/antimutagens in food. *Crit. Rev. Food. Sci. Nutr*. **29**: 273–300

Niemegeers, C. J. E. (1975) The activity of suprofen on nystatin-induced paw oedema in rats. *Arzneimittelforschung* **23**: 1516–1519

Olajide, O. A., Awe, S. O., Makinde, J. M., Ekhelar, A. I., Olusola, A., Morebise, O., Okpako, D. T. (2000) Studies on the anti-inflammatory, antipyretic and analgesic properties of the *Alstonia boonei* stem bark. *J. Ethnopharmacol*. **71**: 179–186

Peterson, J., Dwyer, J. (1998) Flavonoids: dietary occurrence and biochemical activity. *Nutr. Res*. **12**: 1995–2018

Rang, H. P., Dale, M. M., Ritter, J. M. (2001) *Pharmacology*. 4th edn, Guanabara-Koogan, Rio de Janeiro, pp 174–188, p. 691

Renaud, S., De Lorgeril, M. (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **339**: 1523–1526

Salvemini, D., Wang, Z. Q., Bourdon, D. M., Stern, M. K., Currie, M. G., Manning, P. T. (1996) Evidence of peroxynitrite involvement in the carrageenan-induced rat paw oedema. *Eur. J. Pharmacol*. **303**: 217–220

Shibata, M., Ohkubo, T., Takahashi, H., Inoki, R. (1989) Modified formalin test: characteristic biphasic pain response. *Pain* **38**: 347–352

Singleton, V. L., Rossi, J. R. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic*. **16**: 144

Smith, J. A. (1994) Neutrophils, host defense, and inflammation: a double-edged sword. *J. Leuk. Biol*. **56**: 672–686

Sokal, R. R., Rohlf, F. J. (1995) *Biometry: the principles and practice of statistics in biological research*. 2nd edn, W. H. Freeman & Co., NY, Campbell, R.C., pp 175–205, 404–486

Strack, D., Wray, V. (1993) The anthocyanins. In: Harborne, J. B. (ed) *The Flavonoids, advances in research since 1986*. Chapman & Hall, London, p. 1

Swingle, K. F., Shideman, F. E. (1972) Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. *Pharmacol. Exp. Ther*. **183**: 226–234

Tamura, T., Shirai, T., Kosaka, N., Ohmori, K., Takafumi, N. (2002) Pharmacological studies of diacerein in animal models of inflammation, arthritis and bone resorption. *Eur. J. Pharmacol*. **448**: 81–87

Tjolsen, A., Berge, O. G., Hunskaar, S., Rosland, J. N., Hole, K. (1992) The formalin test: an evaluation of the method. *Pain* **51**: 5–17

Walport, M. J., Duff, G. W. (1993) Cells and mediators of inflammation. In: Maddison, P. J., Isenberg, D. A., Woo, P., Glass, D. N. (ed) *Oxford text book of rheumatology*. Vol. 1, Oxford University Press, Oxford, NY, pp 285–295

Winter, C. A., Risley, E. A., Nuss, G. W. (1962) Carrageenan-induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. *Exp. Biol. Med*. **111**: 544–547

Youdim, K. A., McDonald, J., Kalt, W., Joseph, J. A. (2002) Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *J. Nutr. Biochem*. **13**: 282–288

Zadernowski, R., Naczk, M., Nesterrowicz, J. (2005) Phenolic acid profiles in some small berries. *J. Agric. Food. Chem*. **53**: 2118–2124