Evaluation of the Anti-inflammatory, Analgesic and Antipyretic Activities of the Natural Polyphenol Chlorogenic Acid

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Received May 13, 2006; accepted August 23, 2006

Phenolic compounds are numerous and ubiquitous in the plant kingdom, being particularly present in health-promoting foods. Epidemiological evidences suggest that the consumption of polyphenol-rich foods reduces the incidence of cancer, coronary heart disease and inflammation. Chlorogenic acid (CGA) is one of the most abundant polyphenol compounds in human diet. Data obtained from *in vivo* and *in vitro* experiments show that CGA mostly presents antioxidant and anti-carcinogenic activities. However, the effects of CGA on the inflammatory reaction and on the related pain and fever processes have been explored less so far. Therefore, this study was designed to evaluate the anti-inflammatory, antinociceptive and antipyretic activities of CGA in rats. In comparison to control, CGA at doses 50 and 100 mg/kg inhibited carrageenin-induced paw edema beginning at the 2nd hour of the experimental procedure. Furthermore, at doses 50 and 100 mg/kg CGA also inhibited the number of flinches in the late phase of formalin-induced pain test. Such activities may be derived from the inhibitory action of CGA in the peripheral synthesis/release of inflammatory mediators involved in these responses. On the other hand, even at the highest tested dose (200 mg/kg), CGA did not inhibit the febrile response induced by lipopolysaccharide (LPS) in rats. Additional experiments are necessary in order to clarify the true target for the anti-inflammatory and analgesic effects of CGA.

Key words chlorogenic acid; analgesic; anti-inflammatory; formalin; carrageenin; lipopolysaccharide (LPS)

The term phenolic compound or phenolics is applied to a wide range of chemical compounds characterized by a benzene ring bearing one or more hydroxyl groups attached to it. Phenolics are numerous and ubiquitous in the plant kingdom, being particularly present in health-promoting foods, such as vegetables and fruits, as well as in some beverages prepared from plants, such as wine, tea and coffee.[2)] There has been a growing interest in the multiple biological activities of polyphenols and their ability in preventing some degenerative conditions.[3,4)] Epidemiological evidence suggests that the consumption of polyphenol-rich foods reduces the incidence of cancer, coronary heart disease and inflammation.[5—11)] The occurrence of chronic and acute pathological conditions is linked, at different degrees, with unbalanced redox states of the cells.[12,13)] Polyphenols, either isolated or as constituents of polyphenol-containing fractions, have been proven to act as potent antioxidants, protecting the body's tissues against oxidative stress and pathologies associated with this condition.[13,14)] Due to their physicochemical properties, these compounds are able to prevent oxidation by chelating metals and scavenging oxygen-free radicals (or reactive oxygen species).[9,15)] Therefore, most of the biological activities of polyphenols are associated to their antioxidant action. However, these compounds are also able to modify physiological and/or pathological conditions independently from an antioxidant mechanism.[16—18)]

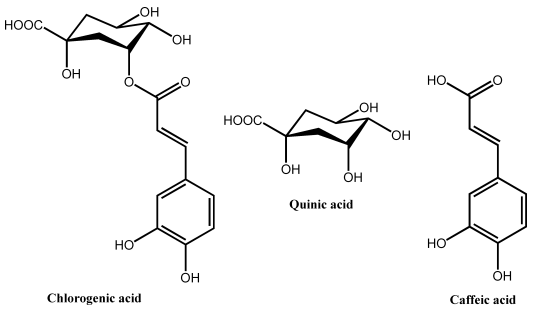

Fig. 1. Chemical Structures of Chlorogenic Acid (CGA) and Its Constituents, Quinic Acid and Caffeic Acid

Chlorogenic acid (CGA, Fig. 1), formed by esterification of caffeic and quinic acids, is one of the most abundant polyphenol in the human diet.[19)] In spite of that, some reports have focused on the metabolism of CGA by different study systems.[20—22)] CGA is highly bioavailable in nature and, according to Niggeweg *et al.*,[23)] its antioxidant activity is probably more accessible than that of many flavonoids. Due to the importance of CGA for human health, the same authors encourage the use of biotechnological approaches in order to increase CGA levels in food crops.[23)] As for other polyphenols, data obtained from *in vivo* and *in vitro* experiments show that CGA mostly presents antioxidant and anti-carcinogenic activities.[19,24—31)] However, despite presenting the various biological activities aforementioned, the effects of CGA on the inflammatory reaction and on the related pain and fever processes have not really been explored so far. Therefore, this study was designed to evaluate the anti-inflammatory, analgesic and antipyretic activities of CGA in rats.

MATERIAL AND METHODS

Animals Male Wistar rats weighing 180—200 g were housed at 24±1 °C on a 12 : 12 h light dark cycle (lights on at 6 a.m.), and with free access to food and tap water. Eight hours before the experiment, only tap water was available to the rats. All experiments were performed between 10 a.m.

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and 5 p.m. All rats were killed shortly after the experiments to minimize suffering of the animal. The study was conducted in compliance with the ethical guidelines of the International Association for the Study of Pain[32] and the University of São Paulo Animal Care and Use Committee.

Surgery Rats were anesthetized with sodium penthobarbital (40 mg/kg, i.p.) and protected with antibiotic (oxytetracycline hydrochloride, 400 mg/kg, i.m.). A miniature battery-operated temperature-sensitive transmitter (Data Sciences, U.S.A.) was implanted through a medial laparotomy, and the surgical wound was sutured.

Induction and Measurement of Rat Paw Edema Rat paw edema was induced in the hind right paw by an intraplantar injection of 100 μl of freshly prepared carrageenan (1% solution in sterile saline—Marine Colloids). The left paw received the same volume of sterile saline and was used as the control. Edema was measured with the use of a plethysmometer (model 7150, Ugo Basile, Italy) at 1 h intervals up to 4 h after carrageenin injection. The results are expressed in milliliters as the difference between the right and left paws.

Formalin Test Formalin-induced paw flinching was determined as previously described.[33] The day before the experiment, rats were habituated to stay in open Plexiglas observation chambers for 2 h to allow them to acclimatize to their surroundings. The paw flinching was induced by a subcutaneous injection, into the plantar surface of the right hind paw, of 50 μl of a 1% formalin solution in saline (0.37% formaldehyde, Synth, Brazil) using a 30-gauge needle. The formalin injection produced specific pain behavior characterized as rapid and brief withdrawal or flexing of the injected paw. This behavior was called a flinching response. Such pain behavior was, therefore, quantified by periodically counting the number of flinches of the injected paw.[34] The number of flinches was counted at 5-min intervals from 0 to 60 min. Formalin-induced flinches were observed in a characteristic biphasic response. The early phase (phase 1) and late phase (phase 2) were defined as 0—14 and 15—60 min, respectively, after formalin injection, reflecting acute injury pain and facilitated state, respectively.

Fever Test Body core temperature (T_c) was measured by biotelemetry (Data Sciences) at 15-min intervals, during a period of 1 h before and 6 h after the treatments. Data were acquired and fed to a computer by using the Data Science software. Only animals whose initial T_c were between 36.8 and 37.4 °C were used in the experiments. Fever was induced by an intravenous (through the tail vein) injection of lipopolysaccharide (LPS, *E. coli* 0111:B4, Sigma Chemical Company, U.S.A.) at a dose of 5 μg/kg. Control animals received an i.v. injection of saline. T_c was measured for 6 h after the injection of LPS. The results are shown either as the changes from the basal values (ΔT_c), or fever indices. The ΔT_c was calculated for each rat by subtracting the temperature values after the treatments by its own initial T_c. Fever Indices were calculated for each rat, as areas under the ΔT_c curves (°C h).

Treatments CGA was obtained from Sigma Chemical Company (U.S.A.). Animals were orally treated (*p.o.*, 0.5 ml) with CGA (10, 50, 100 mg/kg; 200 mg/kg only for fever test) diluted in the vehicle (saline plus Cremophor RH40, BASF, 10%). Control animals received indomethacin (5 mg/kg, Merck, Sharp & Dohme) diluted in Tris–HCl buffer, pH 8.2, or vehicle.

Experimental Protocols In all protocols, rats were allowed to acclimate to the experimental conditions for at least 1 h before the experiments were initiated. In Experiments 1 and 2, ambient temperature was adjusted to be 24±0.5 °C. In Experiment 3, ambient temperature was set to be 27±1.0 °C.

Experiment 1: This experiment was performed to evaluate whether CGA presented anti-inflammatory activity in the rat paw edema. Rats were orally treated with CGA (10, 50 or 100 mg/kg), vehicle or indomethacin, 60 min before the subplantar injection of carrageenan.

Experiment 2: This experiment was aimed at evaluating the effect of CGA treatment on inflammatory pain induced by formalin. Rats were orally treated with CGA (10, 50 or 100 mg/kg) or its vehicle 60 min before the plantar injection of formalin.

Experiment 3: This experiment was designed to address whether CGA treatment affects LPS-induced fever. Rats were orally treated with CGA (10, 50, 100 or 200 mg/kg) or its vehicle 60 min before the i.v. injection of LPS.

Statistical Analysis The responses were compared across treatments and time points by a two-way ANOVA for repeated measurements followed by the Holm–Sidak test (Sigma Stat 3.11, Systat Software Inc, Point Richmond, CA). The differences were considered significant at $p<0.05$. The data are reported as means±S.E.M.

RESULTS

Figures 2, 3 and 4 show the effect of CGA on animal models of inflammation, pain and fever, respectively.

Figure 2 shows the effect of CGA on the carrageenin-induced rat paw edema test. CGA at 50 and 100 mg/kg doses was able to significantly inhibit the carrageenin-induced edema beginning at 2nd hour of the experimental procedure, in comparison to control ($p<0.05$). On the other hand, CGA at 10 mg/kg inhibited the edema only at the 3rd and 4th hour ($p<0.05$).

Figure 3 shows the effect of CGA on the formalin-induced pain in rats. Injection of formalin in control animals induced a biphasic flinching response, with the early phase ranging from 0 to 14 min and the late phase from 15 to 60 min after

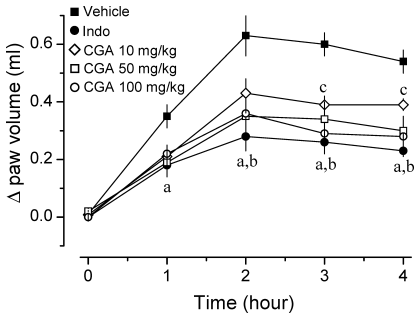

Fig. 2. Anti-edematogenic Effect of Chlorogenic Acid (CGA) on 1% Carrageenan-Induced Rat Paw Edema

CGA (doses indicated) or its vehicle (saline+Cremophor RH40 10%) were administered *p.o.* 1 h before subplantar carrageenan injection. Control animals were treated with Indomethacin (Indo, 5 mg/kg, *p.o.*) or vehicle. The values represent the mean± S.E.M. of the variation in the paw volume of 6—8 animals for each group. a, $p<0.05$ control *vs.* Indo; b, $p<0.05$ control *vs.* CGA at 50 and 100 mg/kg; c, $p<0.05$ control *vs.* CGA at 10 mg/kg.

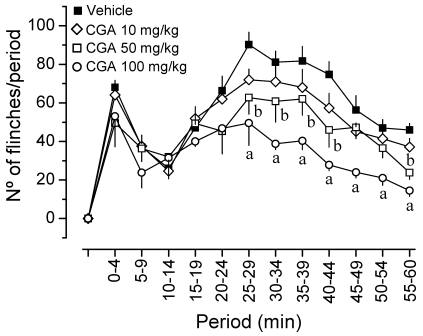

Fig. 3. Effect of Chlorogenic Acid (CGA) Treatment on the Behavioral Response to Formalin

The plantar injection of 50 μl of a 1% formalin solution elicited a biphasic behavioral response, characterized by flinching. CGA (doses indicated) or its vehicle (saline+Cremophor RH40 10%) were administered *p.o.* 1 h before the plantar injection of formalin. The values represent the mean±S.E.M. of the number of flinches/period of time. n=5—8 animals for each group. a, $p<0.05$ control *vs.* CGA at 100 mg/kg; b, $p<0.05$ control *vs.* CGA at 50 mg/kg.

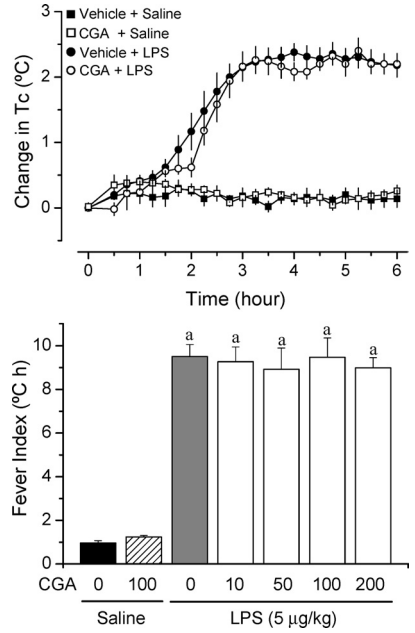

Fig. 4. Effect of Chlorogenic Acid (CGA) Treatment on Lipopolysaccharide (LPS) Fever

Top: Time course of the change in body core temperature (T_c) response in animals pre-treated with CGA or its vehicle (saline+cremophor RH40 10%) and injected with LPS (5 μg/kg, i.v.) or saline. CGA or its vehicle were administered *p.o.* 1 h before LPS or saline (injected at time zero). For clarity purposes, only one dose of CGA (200 mg/kg) treatment is shown in this figure. Bottom: Fever Indices (calculated as area under the ΔT_c curves) of the rats' response to the pre-treatment with CGA (doses indicated) or its vehicle, followed by LPS or saline injection. Values are represented as means±S.E.M. n=5—9 for each group. a, $p<0.05$ *vs.* control (vehicle+saline).

the injection. In animals treated with CGA, a dose–response effect was observed: at the 10 mg/kg dose, CGA did not affect the response in comparison to vehicle-treated animals (p=0.08). On the other hand, CGA at 50 mg/kg reduced the formalin-induced flinches from 25 to 45 min and, at 100 mg/kg, the compound inhibited the flinches from 25 to 60 min, when compared to the vehicle-treated group ($p<0.05$).

Figure 4 shows the effect of CGA on the LPS-induced fever in rats. In control animals (vehicle+LPS), 2 h after the LPS injection T_c started to increase, reached its maximum value *ca.* 3 h after injection (*ca.* 2 °C increase from basal values) and remained elevated until the end of the experiment. None of the evaluated CGA doses were able to affect the febrile response to LPS in comparison to control animals. As can be seen in Fig. 4, even at a highest dose, 200 mg/kg, CGA was unable to alter the LPS-induced fever.

DISCUSSION

Comprehensive data in the literature show that polyphenols present anti-oxidant, anti-carcinogenic and anti-inflammatory activities as their major biological features. Among these activities, the anti-inflammatory is the less explored by far. CGA, one of the most common polyphenols in human diet, has been regarded as a potent antioxidant and anti-carcinogenic agent, both *in vivo* and *in vitro*. However, relatively fewer studies have focused on the *in vivo* anti-inflammatory activity of pure CGA. Therefore, this study was designed to evaluate the anti-inflammatory and also the related analgesic and antipyretic activities of CGA in rats.

The inflammatory reaction is orchestrated by a large range of mediators able to promote vascular events, recruit cells to the site of inflammation and subsequently resolve the process. The literature has provided evidence showing that a vast array of inflammatory mediators (including prostaglandins (PGs), kinins, platelet-activating factor, leukotrienes (LTs), amines, purines, cytokines, adhesion molecules and chemokines) act on specific sites (*e.g.*, the microvasculature), leading to changes in vascular tonus and blood flow and to the local activation of leukocytes and endothelial cells.[35] Cytokines are regulatory proteins that are not constitutively produced under normal physiological conditions. However, inflammatory stimuli induce gene expression of cytokines, initiating the inflammatory response.[36] Tumor necrosis factor α (TNF-α) is a major cytokine involved in the initiation of the inflammatory response. Its actions include the induction of other cytokines such as interleukin 1 (IL-1) and interleukin 6 (IL-6), priming of PMN, up-regulation of adhesion molecules and activation of arachidonic acid (AA) metabolism.[36,37] AA metabolites include PGs and thromboxanes (*via* cyclooxygenases, COX) and LTs (*via* lipoxygenase). Prostaglandin E_2 (PGE_2), derived from COX metabolic pathway, is able to promote changes in vascular tonus and blood flow.

The carrageenin-induced paw edema in rats is a common model to study inflammation and inflammatory pain.[38] The edema, or swelling, one of the cardinal signs of acute inflammation, is an important parameter to be considered when evaluating compounds with a potential anti-inflammatory activity.[39] The role of PGE_2 in the carrageenin-induced edema test has been well documented elsewhere[38,40—42] PGE_2 and bradykinin (BK) (which also induces the synthesis of this eicosanoid),[43] are responsible for the edema formation and also for the pain that accompanies the inflammatory reaction (both BK and PGE_2 are able to sensitize primary afferent neurons).[35,44] Therefore, the effect of CGA in inhibiting the edema could be attributed to a lowering effect in PGE_2 levels. However, some reports in the literature shows that CGA is ineffective, or poorly effective, in inhibiting PGE_2 synthesis in different models. In comparison to the control, CGA was unable to lower PGE_2 levels in RAW264.7 mouse macrophages stimulated with LPS.[45] In accordance, another report showed that CGA is ineffective in inhibiting PGE_2

synthesis by LPS-stimulated J774 macrophages.[46] Cunha *et al.*[47] demonstrated that the synthesis of COX products (such as PGE_2) is preceded by the release of a cascade of cytokines in carrageenin-evoked hyperalgesia. These authors showed that carrageenin stimulates the release of TNF-α, which in turn induces IL-1β and IL-6, which ultimately lead to the release of COX products. In agreement with these results, Griffiths[48] showed that the stimulation of macrophages/monocytes, fibroblasts and epithelial cells with IL-1β and TNF-α leads to PGE_2 production. Therefore, the impairment of TNF-α synthesis/release, and of other pro-inflammatory cytokines, represents an interesting alternative for the inhibition of PGE_2 and consequently of the edema. In this sense, the literature shows that CGA, in a concentration-dependent manner, is able to strongly inhibit the production of TNF-α and IL-6 by human peripheral blood mononuclear cells stimulated with staphylococcal exotoxins.[49] The author also showed that CGA inhibits the synthesis of other mediators such as IL-1β, interferon gamma, monocyte chemotactic protein-1, macrophage inflammatory protein (MIP)-1α and MIP-1α.[49] On the other hand, Jin *et al.* (2006)[45] showed that a single dose of CGA does not alter TNF-α levels in the supernatant of LPS-stimulated RAW cells. As can be noted, these studies[45,49] were conducted *in vitro*, and the literature still lacks data evidencing the *in vivo* actions of CGA on these mediators. However, the study by Krakauer (2002)[49] strengthens our hypothesis that CGA may inhibit TNF-α synthesis. Therefore, we suggest that the inhibitory effect of CGA in the carrageenin-induced rat paw edema (Fig. 2) might be, at least in part, due to its inhibitory action on both TNF-α and IL-6 synthesis/release. However, additional experiments are necessary to confirm this hypothesis. Besides PGE_2, NO is also a crucial mediator involved in the inflammatory and pain processes. Toriyabe *et al.*[50] investigated the effect of peripherally released NO on COX expression/activation and production of PGs in carrageenin-induced inflammation. The authors concluded that NO activates COX-1 and up-regulates COX-2, resulting in production of PGE_2 and PGI_2 at the site of carrageenin inflammation. Since the potent antioxidant activity of CGA is a well-established phenomenon, we cannot rule out the possibility that CGA also exerts anti-edematogenic activity through the inhibition of NO synthesis. Corroborating this hypothesis is the finding that a CGA-rich fraction from the medicinal plant *Saussarea costus* strongly inhibits NO formation.[51] Furthermore, it was also demonstrated that pure CGA suppresses the release of NO from LPS/IFN-γ-stimulated C6 astrocyte cells.[52]

The formalin test is a valuable tool in assessing the analgesic properties of drug candidates. Differently from other traditional pain-evaluating models, which consist on brief stimuli of threshold intensity, the formalin test involves moderate, long-lasting pain. Moreover, since the formalin nociception is associated with injured tissue, it is believed that it more closely resembles clinical pain in comparison to other tests that employ mechanical or thermal stimuli.[34,53] The subcutaneous injection of diluted formalin in the rat paw induces a biphasic response. The early phase is short-lived and initiates immediately after injection, being characterized by C-fiber activation due to peripheral stimuli. The late phase is a longer, persistent period caused by local tissue inflammation and also by functional changes in the dorsal horn of the spinal cord (DHSC). It is believed that these changes in the DHSC initiate by C-fiber barrage during the first phase.[34] Figure 3 shows that CGA, in a dose-dependent fashion, inhibits the number of flinches during the late (15 to 60 min) but not the early phase (0 to 14 min) of the formalin test in comparison to control. In general, TNF-α is the first cytokine detected in inflammatory sites since increased TNF-α levels are present as early as 30 min after inflammatory stimulus.[37,54] Accordingly, the participation of TNF-α in formalin-induced inflammatory pain was well documented by Granados-Soto *et al.*[55] The authors found that antibody anti-TNF-α significantly reduced, near 30%, the number of flinches in the second phase (15—60 min) of formalin test showing by the first time the involvement of this cytokine in this response. In addition, TNF-α, among other inflammatory mediators, contributes significantly to formalin-induced orofacial nociception.[56] These findings are in agreement with previous reports indicating that TNF-α is a mediator of inflammatory and nerve injury pain.[57,58] In view of that, it is possible that CGA inhibits the characteristic flinching behavior of the second phase of formalin test by inhibiting the synthesis of TNF-α.

Fever is defined as an elevation in body temperature characteristically exhibited by most species in response to an invasion of infectious agents. When a pyrogenic agent, such as LPS, enters the body through a break in its natural barriers, it will interact with immune cells, and promote the synthesis and release of endogenous mediators, such as cytokines (*e.g.* TNF-α, IL-1β, IL-6), PGs and endothelins.[59—61] In the preoptic area of the anterior hypothalamus, PGE_2 seems to be crucial for the induction of fever, at least to LPS.[60] Although presenting inhibitory activities on the carrageenin-induced paw edema (Fig. 2) and on formalin pain (Fig. 3), in the present study CGA was not able to reduce the febrile response to LPS, even when a higher dose of CGA (200 mg/kg) was employed (Fig. 4). It is possible that CGA lacks antipyretic activity because the compound was shown to be ineffective, or poorly effective, in inhibiting PGE_2 in different experimental models.[45,46] Furthermore, besides TNF-α, other mediators such as IL-1 (α, β), IL-6 and chemokines, which depend or not on PGE_2 synthesis to produce fever, are also involved in the fever to LPS. Thus, even though CGA would inhibit TNF-α synthesis, other mediators or pathways, for instance those that do not depend on PGs synthesis such as endotelin-1 and macrophage inflammatory protein-1α,[62—64] can work in fever development. It is also possible that, due to its relatively high polarity, no effective amount of CGA passively crosses the blood brain barrier to exert its inhibitory effects on the synthesis of all of these pyrogenic mediators.

CONCLUSION

We demonstrate here that CGA presents anti-edematogenic and antinociceptive activities in animal models of carrageenin-induced inflammation and formalin-induced pain, respectively. Such activities may be derived of the inhibitory action of CGA in the peripheral synthesis/release of inflammatory mediators involved in these responses, such as TNF-α and NO. On the other hand, CGA did not inhibit the febrile response induced by LPS in rats. We suggest that this may be related to the lack of effect of CGA in inhibiting PGE_2 syn-

thesis/release and/or, because its relative high polarity, to CGA's inability to cross the blood brain barrier to exert inhibitory effects on mediators involved in the febrile response.

Additional experiments are necessary in order to confirm these hypotheses and to clarify the true target for the anti-inflammatory and analgesic effects of CGA.

Acknowledgements The authors thank Miriam C. C. de Melo and Juliana A. Vercesi for their helpful technical assistance and CNPq, CAPES and FAPESP for the financial support. The authors also thank Dr Paul Gates, School of Chemistry, University of Bristol, for the English language revision.

REFERENCES AND NOTES

1) Present address: *São Paulo State University at Rio Claro, Department of Zoology. Avenida 24A, 1515, Rio Claro-SP 13506–900, Brazil.*
2) Stoclet J., Chataigneau T., Ndiaye M., Oak M., Bedoui J. E., Chataigneau M., Schini-Kerth V. B., *Eur. J. Pharmacol.*, **500**, 299—313 (2004).
3) Laranjinha J. A., Almeida L. M., Madeira V. M., *Biochem. Pharmacol.*, **48**, 487—494 (1994).
4) Soleas G. J., Grass,L., Josephy P. D., Goldberg D. M., Diamandis E. P., *Clin. Biochem.*, **35**, 119—124 (2002).
5) Mukhtar H., Ahmad N., *Toxicol. Sci.*, **52**, 111—117 (1999).
6) Ruf J. C., *Drugs Exp. Clin. Res.*, **25**, 125—131 (1999).
7) Duthie G. G., Gardner P. T., Kyle J. A., *Proc. Nutr. Soc.*, **62**, 599—603 (2003).
8) Birrell M. A., McCluskie K., Wong S., Donnelly L. E., Barnes P. J., Belvisi M. G., *FASEB J.*, **19**, 840—841 (2005).
9) Sang S., Hou Z., Lambert J. D., Yang C. S. *Antiox. Redox Signal.*, **7**, 1704—1714 (2005).
10) Yoon J. H., Baek S. J., *Yonsei Med. J.*, **46**, 585—596 (2005).
11) Valko M., Rhodes C. J., Moncol J., Izakovic M., Mazur M., *Chemicobiol. Interactions*, **160**, 1—40 (2006).
12) Rice-Evans C., *Curr. Med. Chem.*, **8**, 797—807 (2001).
13) Sies H., Stahl W., Sevanian A., *J. Nutr.*, **135**, 969—972 (2005).
14) Clifford M. N., *Planta Med.*, **70**, 1103—1114 (2004).
15) Bravo L., *Nutr. Rev.*, **56**, 317—333 (1998).
16) Srivastava R. C., Husain M. M., Hasan S. K., Athar M., *Cancer Lett.*, **153**, 1—5 (2002).
17) Waddington E., Puddey I. B., Croft K. D., *Am. J. Clin. Nutr.*, **79**, 54—61 (2004).
18) Kitagawa S., *Biol. Pharm. Bull.*, **29**, 1—6 (2006).
19) Feng R. T., Lu Y. J., Bowman L. L., Qian Y., Castranova V., Ding M., *J. Biol. Chem.*, **280**, 27888—27895 (2005).
20) dos Santos M. D., Martins P. R., dos Santos P. A., Bortocan R., Iamamoto Y., Lopes N. P., *Eur. J. Pharm. Sci.*, **26**, 62—70 (2005).
21) Lafay S., Gil-Izquierdo A., Manach C., Morand C., Besson C., Scalbert A., *J. Nutr.*, **136**, 1192—1197 (2006).
22) Nardini M., Cirillo E., Natella F., Scaccini C., *J. Agric. Food Chem.*, **50**, 5735—5741 (2002).
23) Niggeweg R., Michael A. J., Martin C., *Nature Biotechnol.*, **22**, 746—754 (2004).
24) Huang M. T., Smart R. C., Wong C. Q., Conney A. H., *Cancer Res.*, **48**, 5941—5946 (1988).
25) Kono Y., Shibata H., Kodama Y., Ueda A., Saway Y., *Biochem. Biophys. Res. Commun.*, **217**, 972—978 (1995).
26) Tsuchiya T., Suzuki O., Igarashi K., *Biosci. Biotechnol. Biochem.*, **60**, 765—768 (1996).
27) Kono Y., Kashine S., Yoneyama T., Sakamoto C., Matsui Y., Shibata H., *Biosci. Biotechnol. Biochem.*, **62**, 22—27 (1998).
28) Shibata H., Sakamoto Y., Oka M., Kono Y., *Biosci. Biotechnol. Biochem.*, **63**, 1295—1297 (1999).
28) Zang L. Y., Cosma G., Gardner H., Castranova V., Vallyatahn V., *Mol. Cell. Biochem.*, **247**, 205—210 (2003).
30) Jin U. H., Lee J. Y., Kang S. K., Kim J. K., Park W. H., Kim J. G., Moon S. K., Kim C. H., *Life Sci.*, **77**, 2760—2769 (2005).
31) Lee W. J., Zhu B. T., *Carcinogenesis*, **27**, 269—277 (2006).
32) Zimmermann M., *Pain*, **16**, 109—110 (1983).
33) Dubuisson D., Dennis S. G., *Pain*, **4**, 161—174 (1977).
34) Tjolsen A., Berge O. G., Hunskaar S., Rosland J. H., Hole K., *Pain*, **51**, 5—17 (1992).
35) Calixto J. B., Otuki M. F., Santos A. R. S., *Planta Med.*, **69**, 973—983 (2003).
36) Thomson A. W., Lotze M. T., "The Cytokine Handbook," 4th ed., Academic Press, New York, 2003.
37) Santos D. R., Calixto J. B., Souza G. E., *Br. J. Pharmacol.*, **139**, 271—278 (2003).
38) Nantel F., Denis D., Gordon R., Northey A., Cirino M., Metters K. M., Chan C. C., *Br. J. Pharmacol.*, **128**, 853—859 (1999).
39) Morris C. J., *Methods Mol. Biol.*, **225**, 115—121 (2003).
40) Zanin M. T., Ferreira S. H., *Agents Actions*, **8**, 606—609 (1978).
41) Mnich S. J., Veenhuizen A. W., Monahan J. B., Sheekan K. C., Lynch K. R., Isakson P. C., Portanova J. P., *J. Immunol.*, **155**, 4437—4444 (1995).
42) Portanova J. P., Zhang Y., Anderson G. D., Hauser S. D., Masferrer J. L., Seibert K., Gregory S. A., Isakson P. C., *J. Exp. Med.*, **184**, 883—891 (1996).
43) Williams T. J., Peck M. J., *Nature* (London), **270**, 530—532 (1977).
44) Gallin J. I., Snyderman R., Fearon D. T., Haynes B. F., Nathan C. N., "Overview in inflammation: basic principles and clinical correlates," 3rd ed., Lippincott, Willians and Wilkins, Hagerstown, 1999.
45) Jin X. H., Ohgami K., Shiratori K., Suzuki Y., Koyama Y., Yoshida K., Ilieva I., Tanaka T., Onoe K., Ohno S., *Exp. Eye Res.*, **82**, 860—867 (2006).
46) Rossi A., Ligresti A., Longo R., Russo A., Borrelli F., Sautebin L., *Phytomedicine*, **9**, 530—535 (2002).
47) Cunha F. Q., Poole S., Lorenzetti B. B., Ferreira S. H., *Br. J. Pharmacol.*, **107**, 660—664 (1992).
48) Griffiths R. J., "Prostaglandins and Inflammation," eds. by Gallin J. I., Snyderman R., Fearon D. T., Haynes B. F., Nathan C. N., "Overview in inflammation: basic principles and clinical correlates," 3rd ed., Lippincott, Willians and Wilkins, Hagerstown, 1999, pp. 349—360.
49) Krakauer T., *Immunopharmacol. Immunotoxicol.*, **24**, 113—119 (2002).
50) Toriyabe M., Omote K., Kawamata T., Namiki A., *Anesthesiol.*, **101**, 983—990 (2004).
51) Pandey M. M., Govindarajan R., Rawat A. K., Pushpangadan P., *Acta Pharm.*, **55**, 297—304 (2005).
52) Soliman K. F., Mazzio E. A., *Proc. Soc. Exp. Biol. Med.*, **218**, 390—397 (1998).
53) Capone F., Aloisi A. M., *Ann. Ist. Sanita*, **40**, 223—229 (2002).
54) Miller A. J., Luheshi G. N., Rothwell N. J., Hopkins S. J., *Am. J. Physiol.*, **272**, R857—R861 (1997).
55) Granados-Soto V., Alonso-López R., Asomoza-Espinosa R., Rufino M. O., Gomes-Lopes L. D., Ferreira S. H., *Proc. West. Pharmacol. Soc.*, **44**, 15—17 (2001).
56) Chichorro J. G., Lorenzetti B. B., Zampronio A. R., *Br. J. Pharmacol.*, **141**, 1175—1184 (2004).
57) Opre A., Kress M., *J. Neurosci.*, **20**, 6289—6293 (2000).
58) Reeve A. J., Patel S., Fox A., Walker K., Urban L., *Eur. J. Pain*, **4**, 247—257 (2000).
59) Kluger M. J., *Physiol. Rev.*, **71**, 93—127 (1991).
60) Romanovsky A. A., Almeida M. C., Aranoff D. M., Ivanov A. I., Konsman J. P., Steiner A. A., Turek V. F., *Frontiers Biosci.*, **10**, 2193—2216 (2005).
61) Fabricio A. S. C., Rae G. A., D'Orleans-Juste P., Souza G. E., *Brain Res.*, **1066**, 92—100 (2005).
62) Fabricio A. S. C., Veiga F. H., Cristofoletti R., Navarra P., Souza G. E. P., *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **288**, R671—R677 (2005).
63) Fabricio A. S. C., Rae G. A., Zampronio A. R., D'Orleans-Juste P., Souza G. E. P., *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **290**, R164—R171 (2006).
64) Soares D. M., Veiga-Souza F. H., Fabrício A. S. C., Minano F. J., Souza G. E. P., *Brain Res.*, **1109**, 83—92 (2006).