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Studies on the Anti-Inflammatory and Analgesic Effects of Extracts from Marine Sponges

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Abstract – Two specimens of sponges collected from Red Sea, Egypt, were investigated for their contents of secondary metabolites. The crude extracts of the sponges were tested for their anti-inflammatory and analgesic effects. The toxic effects of the extracts of the two marine sponges were studied. LD_{50} determination revealed that the investigated extracts of '*Igernella* and *Ircinia* spp' were 4.69 and 134.7 mg/100 g b.wt., respectively, when injected intraperitoneally in mice. The toxic signs were recorded within the first 24 hrs after injection. Also the two marine sponges extracts showed significant anti-inflammatory and analgesic effects.
Keywords – anti-inflammatory, analgesic, sponges, *Igernella*, *Ircinia*

Introduction

Among marine invertebrates, sponges have always been the preferred organisms for study. This is because they are rich in unusual secondary metabolites and are often easy to collect.

In addition, many metabolites isolated from these marine organisms were of interest both pharmacologically and ecologically and some of them have been applied practically in a wide range from health care to food additive (Bongiorni and Pietra, 1996).

Two specimens of sponges collected from the Red Sea, Egypt, were investigated for their contents of secondary metabolites. The studied sponges are *Igernella* sp. and *Ircinia* sp., the classification of sponges (class/order/genus/species) is quite difficult because almost all characteristics of these organisms are highly variable.

Isolation, purification and characterization of secondary metabolites from the sponges were carried out using different chromatographic and spectroscopic techniques.

The structures of the isolated compounds were established by applying the advanced spectroscopic techniques such as one-and two dimensional NMR spectroscopy; ^{1}HNMR, ^{13}C. NMR, DEPT, COSY, HSQC, HMBC, NOE difference, and spin decoupling experiments, in addition to mass spectroscopy.

Literature survey revealed that most studies centered on *Ircinia* sp. (Anglo *et al.*, 1999) and some other marine sponges (Gil *et al.*, 1995), while *Igernella* sp. has been neglected.

Our interest is focused on the chemistry of bioactive secondary metabolites of these sponges, for their unique structures and peculiarities of pharmacological activities in order to assess their possible utilization in local pharmaceutical industry. These two marine sponges are *Igernella* sp. and *Ircinia* sp.

Expermintal

Collection of the materials – The specimens of *Igernella* sp. and *Ircinia* sp. were collected from Hurghada, Red Sea, Egypt, using SCUBA-diving at a depth from 10 to 20 m. The samples were immediately frozen at –30 °C until extraction.

Animal material was identified at Istituto id Zoologia, Universita di Genova, Italy by Dr. M. Pansini, Professor of marine Taxonomy.

Extraction – The examined specimens were exhaustively extracted with acetone and using an ultrasonic bath for 10 minutes. The acetone extracts were used in this study as crude materials after evaporating the acetone (under vacuum).

Animals – Adult rats of both sexes weighing 150 - 200 g and adult mice weighing 18 - 20 g were used in the experiments. Animals were housed under standardized conditions for light and temperature and received standard rat chow and tap water *ad libitum*. Animals were randomly assigned to different experimental groups, each kept in

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separate cage. All animal procedures were performed after approval from the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No.85-23, revised 1985).

Approximate of median lethal dose (LD_{50}) – The approximate LD_{50} of the two tested extracts were determined using mice. Extracts dissolved in distilled water were given i.p. to groups of 10 mice each. Each group was given a different dose of the extract i.p., and the control group received the same volume of distilled water. Animals were observed for any toxic signs and the percentage mortality for each group was recorded after 24 h. Results were analyzed statistically and the approximate LD_{50} of the two extracts was calculated (Karber, 1931). The post mortem findings were recorded.

Anti-inflammatory effect – The carrageenan rat paw oedema model of inflammation was used to evaluate the anti-inflammatory properties of the extracts (Winter *et al.*, 1962). Rats were randomly assigned to treatment groups and sterile carrageenan lambda (100 µl of a 1% solution in saline) was injected sub-planter into the right hind paw of the rat. The contralateral hind paw received the same volume of saline and served as a normal control. Carrageenan caused visible redness and pronounced swelling that was well developed by 4h and persisted for more than 48hs (Vinegar *et al.*, 1976). Hind footpad thickness was measured with a micrometer caliber (Obukowics *et al.*, 1998; Meng *et al.*, 1999).

Groups of rats were administered either saline (0.5 ml, n = 6, i.p.) and served as control or the *Igernella* sp. *extract* (4.69 mg kg^{-1}, 0.5 ml, n = 6, i.p.) or *Ircinia* sp. (134.7 mg kg^{-1}, 0.5 ml, n = 6, i.p.) or diclofenac (5 mg kg^{-1}, 0.5 ml, n = 6, i.p) were examined in the carrageenan model of inflammation. Marine sponge extracts or diclofenac were given either 1 h before carrageenan as pretreatment or as post treatment 30 min after carrageenan injection to study their preventive or therapeutic effects.

Analgesic effect – This effect was evaluated according to the method of (Charlier *et al.*, 1961) using electric current as a noxious stimulus. Electrical stimulation was applied to the rat tail by means of 515 Master shocker (Laffayette Inst. Co.) using alternative current of 50 cycles /sec. for 0.2 second. The minimum voltage required to emit a cry was recorded in all animal groups. Groups of rats were administered either saline (0.5 ml, n = 6, i.p. control group) or the *Igernella* sp. (4.69 mg kg^{-1}, 0.5 ml, n = 6, i.p.) or *Ircinia* sp. (134.7 mg kg^{-1}, 0.5 ml, n = 6, i.p.) or indomethacin (5 mg kg^{-1}, 0.5 ml, n = 6, i.p.). The reaction time was measured one and two hours after the administration of the extract or indomethacin (Charlier *et al.*, 1961).

Drugs – Carrageenan (Sigma USA), indomethacin (Kahira Pharm. and Chem. Ind. Co., Cairo, Egypt) and diclofenac sodium (Ciba-Geigy, Egypt). The doses of indomethacin and diclofenac used in this study were chosen according to Siham *et al.* (2002), and Jurna and Brune (2002). The therapeutic human dose was converted to rat dose according to Paget converting table (Paget and Barnes, 1964).

Statistical analysis – The results are expressed as percentage of change from control (Pre-drug) values and expressed as means ± S.E. Students t-test (Kapure and Saxena, 1972) was used for statistical analysis.

Results and Discussion

Metabolites of *Igernella* sp. – Analysis of the metabolic contents of *Igernella* sp. led to the isolation of three known bromotyrosine compounds; aerothionin (**1**), dienone acetamide (**2**), and 2-(3,5-dibromo-4-hydroxyphenyl) ethyltrimethylammonium chloride (**3**), together with a new compound which is the sodium phenate of the latter compound (**4**) (see Fig. 1).

Metabolites of *Ircinia* sp. – Further isolation and purification of the acetone extract of the metabolic contents of *Ircinia* sp. led to the identification of the sesterterpene (–)–wistarin (**5**) using advanced NMR and Mass spectroscopy (see Fig. 2).

Toxicological study – Signs of acute toxicity of marine

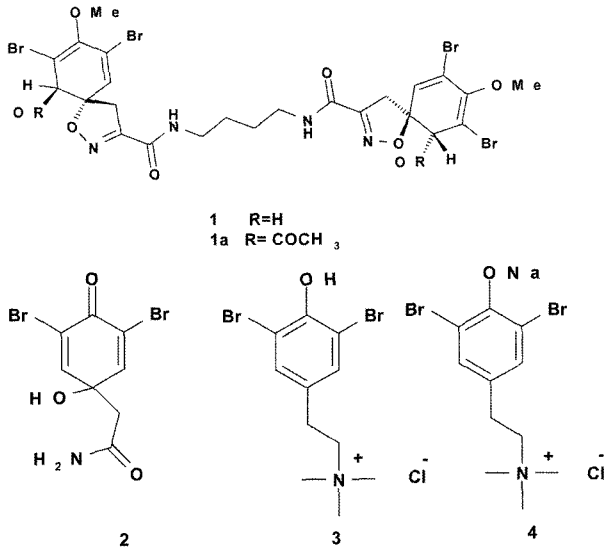

Fig. 1. Bromotyrosine compounds isolated from *Igernella* sp. **(1)** aerothionin. **(2)** dienone acetamide. **(3)** 2-(3,5-dibromo-4-hydroxyphenyl)ethyltrimethylammonium chloride. **(4)** sodium salt of **3**.

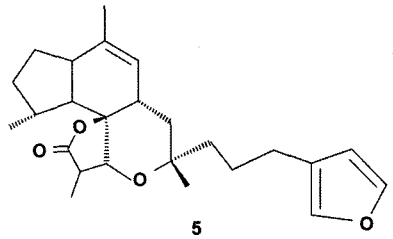

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Fig. 2. Chemical structure of (−)-wistarin **(5)** isolated from *Ircinia* sp.

Table 1. Determination of approximate LD_{50} for *Igernella* sp. by Karber method (1931) using constant (1.4)

dose used mg/100 g b.wt	M	reaction	Z	D	ZD
9.4	10	10	–	–	–
6.7	10	8	9	2.7	24.3
4.8	10	5	6.5	1.9	12.35
3.4	10	4	4.5	1.4	6.3
2.45	10	2	3.0	0.95	2.85
1.75	10	1	1.5	0.70	1.05
1.25	10	–	0.5	0.50	0.25

Σ ZD = 47.1

$$a\,M = DM - \Sigma\frac{ZD}{M}$$

$$= 9.4 - \frac{47.1}{10}$$

$$= 9.4 - 4.71 = 4.69$$

LD_{50} = 4.69 mg / 100 g b.wt

Where:

aM : arithmatic mean of LD_{50}

DM : the dose by which all the animal reacted

Z : half the sum of the positive reacted animals from 2 successive doses

D : difference between the number of 2 successive doses

M : number of animals in each group

sponge extracts (*Igernella* sp. or *Ircinia* sp.) were increased respiration, cyanosis of mucous membranes, loss of righting reflex, convulsions and death. The LD_{50} of the marine sponge extract of *Igernella* sp. was found to be 4.69 mg/ 100 g b.wt, LD_{10}, and LD_{100} were 1.75 mg and 9.4 mg/ 100 g b.wt., respectively, when intraperitonealy injected in mice (Table 1). Post mortem examination revealed general congestion of all internal organs. On the other hand, symptoms of acute toxicity of *Ircinia sp.* extract revealed convulsions, nervous disturbance, jumping, shallow and hurried respiration and finally death. The LD_{50} of *Ircinia* extract was found to be 134.7 mg/100 g b.wt., LD_{10} and LD_{100} were 60 mg and 268.9 mg/100 g b.wt., respectively, when intraperitoneally injected in mice (Table 2). Post mortem examination showed congestion of lungs and heart.

Table 2. Determination of approximate LD_{50} for *Ircinia* sp. by Karber method (1931) using constant (1.4)

dose used mg/100 g b.wt	M	reaction	Z	D	ZD
268.9	10	10	–	–	–
192.0	10	8	9.0	76.9	692.1
137.2	10	6	7.0	54.8	383.6
98.0	10	3	4.5	39.2	176.4
70	10	2.0	2.5	28.0	70.0
50	10	–	1.0	20.0	20.0

Σ ZD = 1342.1

$$aM = DM - \Sigma\frac{ZD}{M}$$

$$= 268.9 - \frac{1342.1}{10}$$

$$= 268.9 - 134.2$$

LD_{50} = 134.7 mg / 100 g b.wt

Where:

aM : arithmatic mean of LD_{50}

DM : the dose by which all the animal reacted

Z : half the sum of the positive reacted animals from 2 successive doses

D : difference between the number of 2 successive doses

M : number of animals in each group

Anti-inflammatory effect "pre-treatment" – Sub-plantar injection of carrageenan into the rat hind paw elicited an inflammation (swelling and erythema) and a time dependent increase in paw thickness. This represented a 133.52 ± 7.8% increase in paw thickness compared with the pre-carrageenan control value. The acute paw oedema response induced by sub-plantar carrageenan, was significantly reduced at 1, 2, 3, and 4 h by 39.9, 48.6, 56.0, and 68.0% respectively is rats receiving diclofenac 1 h before carrageenan. In rats treated with *Igernella* sp. 1 h before carrageenan injection the paw thickness increased by 53.5 ± 4.3% compared with pre-carrageenan values. The inflammatory oedema caused by carrageenan in *Igernella* sp. treated rats was significantly inhibited at 1, 2, 3, and 4 h by 36.4, 35.2, 41.4, and 59.9% respectively *vs* the control group. The effect of *Ircinia* sp. was also studied 1 h before carrageenan injection. The inflammatory oedema caused by carrageenan in *Ircinia* sp. treated rats was significantly reduced at 1, 2, 3 and 4h by 26.5, 22.8, 38.5 and 56.5% respectively *vs* the control group (Fig. 1).

Post-treatment – In the control group the paw thickness increased by 115.4 ± 6.5% 4 h after injection of carrageenan. Diclofenac showed significant reduction in the extent of their inflammation compared with control rats at 1, 2, 3, and 4 h after carrageenan (46.5, 42.6, 52.5, and 60.0% *vs* control values). On the other hand, the extracts of *Igernella* sp. and *Ircinia* sp. caused a significant decrease

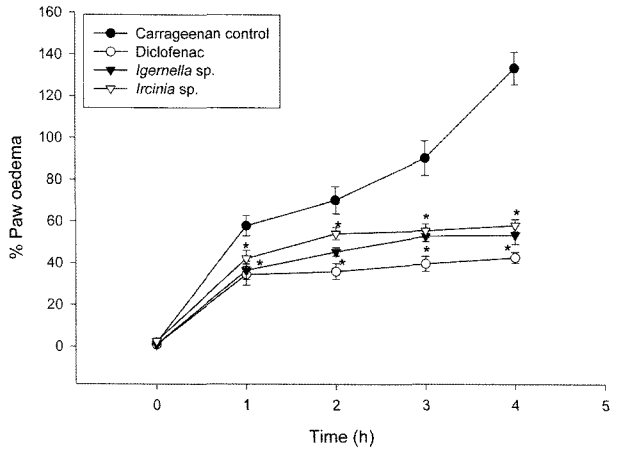

Fig. 1. Effect of the extract of the marine sponges (*Igernella* sp., *Ircinia* sp.) and diclofenac on the carrageenan-induced paw oedema. Sponge extracts or diclofenac were given 1 h prior to carrageenan (1%) and rats were evaluated for paw oedema at 1, 2, 3 and 4h post carrageenan. The results are expressed as percent change from control (pre-drug) values. Each point represents the mean ± SE of six rats.

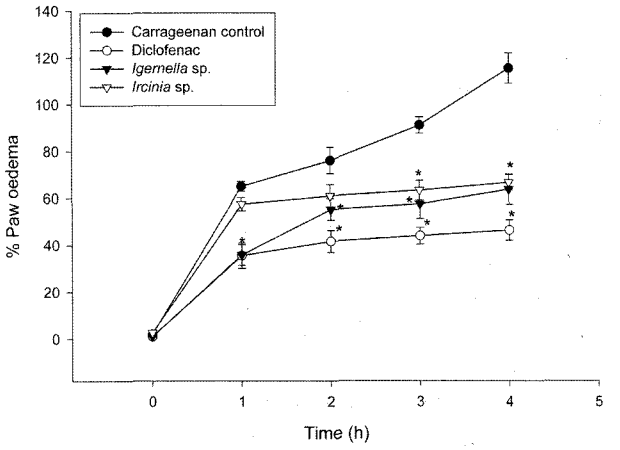

Fig. 2. Effect of the extract of the marine sponges (*Igernella* sp., *Ircinia* sp.) and diclofenac on the carrageenan-induced paw oedema. Sponge extracts or diclofenac were given 30 min after injection of carrageenan (1%). The results are expressed as percent change from control (pre-drug) values. Each point represents the mean ± S.E. of six rats.

in percent of oedema when compared with that of the control group (38.0, 44.8 and 31.6, 42.3%, respectively at 3 and 4 h) (see Fig. 2) .

Analgesic effect – From Table 3, the investigated marine sponge extract of *Igernella* sp. showed a rise in electrical current threshold in the tail stimulation test. After one and two hours post-treatment, *Igernella* sp. extract increased pain threshold by 34.3 and 58.6% relative to control values respectively. The electrical current threshold needed to induce rat cry was also significantly increased following the administration of *Ircinia* sp. at one and two hours in the test. The pain threshold relative to saline-treated control group was (33.3 and 56.8% at one and two hours post treatment, respectively). The two marine sponge extracts investigated exhibited their highest potency at 2 hours after single i.p. administration.

Discussion

The marine environment has been, and continues to be, a fruitful source of novel chemical compounds that are not found in terrestrial and fresh water organisms. These molecules differ from those of terrestrial organisms in both chemical structure and peculiarities of biological activities (Kilagawa and Kobayash, 1989). In the present work, the toxic effects of the marine sponge extracts of both *Igernella* sp. and *Ircinia* sp. were studied. It was found that *Ircinia* sp was of low acute toxicity (as indicated by LD_{50}) than *Igernella* sp. Post mortem examination revealed congestion of the internal organs and change of blood colour to dark brown, that may suggest an oxidative effect of a toxic constituent of the extract, thus changing oxyhemoglobin to methemoglobin which is brown in colour (Liener, 1980). *Igernella* sp. and *Ircinia* sp. extracts,

Table 3. Analgesic effect

group	dose mg/100 g b. wt.	after one hour			after two hours		
		volts needed mean ± S.E.	% of change	potency[e]	volts needed mean ± S.E.	% of change	potency
control	saline	82.5 ± 5.0	–	–	92.5 ± 4.8	–	–
indomethacine	0.5	123.3 ± 3.6[c]	+49.5	1.0	149.2 ± 2.7[c]	+61.3	1.0
Igernella sp.	0.47	110.8 ± 5.1[b]	+34.3	0.7	146.7 ± 4.4[c]	+58.6	0.96
Ircinia sp.	13.5	110.0 ± 5.2[b]	+33.3	0.67	145.0 ± 6.6[c]	+56.8	0.93

[a]$p > 0.05$, [b]$p > 0.01$, [c]$p > 0.001$ vs control.
[e]Potency is considered as the percent of change of the different treatments. The % change denotes % increase over the control group divided by the percent of change of indomethacine.

at the tested dose levels, induced a significant anti-inflammatory effect. These findings are in harmony with those reported on some marine sponge metabolites, including manoalide, which inhibits phospholipase A_2 activity from different sources, *in vitro*. This property could be responsible, at least in part, for the anti-inflammatory effects shown in mice using ear oedema test (De Carvalho and Jacobs, 1991; Marshall *et al.*, 1994). Gil *et al.* (1995) reported that phospholipase A_2 activation represents the point of regulatory control for arachidonic acid release and subsequent eicosanoid synthesis (Proinflammatory mediators). Thus a number of marine sponges inhibit an enzyme activity possessing a crucial role in the pathogenesis of the inflammatory processes (Vadas and Pruzanski, 1986; Angel *et al.*, 1993; Pfeilschifter *et al.*, 1993).

Furthermore, in the present study, electrical stimulation of the tail test was used to evaluate pain perception in rats acutely treated with marine sponge extracts (*Igernella* sp., *Ircinia* sp.) and indomethacin was used as a standard non-steroidal anti-inflammatory drug for comparative purposes. The two sponge extracts as well as indomethacine displayed a powerful analgesic effect as they increased the pain threshold significantly. Ferrandiz *et al.* (1994) reported an anti-inflammatory activity of two natural products of marine origin, avarol and avarone, isolated from the sponge *Dysidea avara*. These diterpenoids possess a hydroquinone (avarol) or quinone (avarone) moiety. Other sponge metabolites contain a hydroquinone moiety attached to a terpene residue, like three 2-polyprenyl-1,4-hydroquinones isolated from the Mediterranean sponge *Ircinia spinosula* which had shown analgesic activity in mice (De Pasquale *et al.*, 1991) These compounds also showed moderate antimicrobial effects *in vitro* (De Rosa *et al.*, 1994).

In conclusion, the findings of the present study indicated that the two marine sponge extracts (*Igernella* sp. and *Ircinia* sp.) exerted powerful anti-inflammatory and analgesic effect (approximating standard NSAIDs). These results may highlight the importance of these marine sponge extracts in therapeutic purposes.

References

Angel, J., Colard, O., Chevy, F., and Fournier C., Inter leukin-1-mediated phospholipid breakdown and arachidonic acid release in human synovial cells. *Arthritis Rheum.* **36**, 158-167 (1993).

Anglo, F., Issa, F., Ernesto, M., and Guido C., (−)-Wistarin from the marine sponge *Ircinia* SP.: the first case of enantiomeric sesterterpenes. *Tetrahedron Assymetry.* **10**, 3869-3872 (1999).

Bongiorni, P.R., and Pietra, F., Marine Natural Products for Industrial Application. *Chem.& Ind.* **2**, 54-58 (1996).

Charlier, R., Prost, M., Binon, F., and Deltour, G., Pharmacology of an anti-tussive, -1- phenethyl (-2-propynyl) -4-propinoxypipridine acid fumarate. *Arch. Intern. Pharma- codynamic* **134**, 306-327 (1961).

De Carvalho, M.S., and Jacobs, R.S., Two-step inactivation of bee venom phospholipase A_2 by Scalaradial. *Biochem. Pharmacol.* **42**, 1621-1626 (1991).

De Pasquale, R., Circosta, C., Occhiuto, F., De Rosa, S., and De Stefano, S., Pharmacological studies on terpenoids from marine sponges: analgesic and muscle relaxant effects. *Phytother. Res.* **5**, 49-53 (1991).

De Rosa, S., De Giulio, A., and Iodice, C., Biological effects of prenyl-hydroquinones: Structure activity relationships studies on antimicrobial, brine shrimp and fish lethality assays. *J. Nat. Prod.* **57**, 1711-1716 (1994).

Ferrandiz, M.L, Sanz, M.J., Bustos, G., Paya, M., Alcaraz, M.J., and De Rosa, B., Avarol and avarone, two new anti-inflammatory agents of marine origin. *Eur. J. Pharmacol.* **253**, 75-82 (1994).

Gil, B., Jesus Sanz, M., Carmen Terencio, M., De Giulio, A., De Rosa, S., Jose Alcaraz, M., and Paya, M., Effect of marine 2-polyprenyl-1,4-hydroquinones on phospholipase A_2 activity and some inflammatory responses. *Eur. J. Pharmacol.* **285**, 281-288 (1995).

Jurna, I. and Brune, K., Central effect of the NSAI agent, indomethacin, ibuprofen, and diclofenac, determined in C fiber-evoked activity in single neurons of the rats thalamus. *Pain* **95**, 289-90 (2002).

Kapure, J.N. and Saxena, H.C., Mathematical Statistics, S Chand and Co Ltd., Ram Nagar, New Delhi (1972).

Karber, G., Bietrag Zur Kollectiven behandlung pharmakologischem, Rainhen Versuche. *Arch. Exp. Pathol. Pharmakol.* **162**, 480-482 (1931).

Kilagawa, I. and Kobayash, M., Anti-tumor natural products isolated from marine organisms Ed by Gan To, K.R.; **169**, 1-8 (1989).

Liener, I.E., Toxic constituents of plant food stuffs, 2nd Ed., Academic Press, New York, 1980, p. 286.

Marshall, L.A., Winkler, J.D., Griswold, D.E., Bolognese, B., Roshak, A., Sung, C.M., Webb, E.F., and Jacobs, R.S., Effects of scalaradial, a type II phospholipase A_2 inhibitor, on human neutrophil arachidonic acid mobilization and lipid mediator formation. *J. Pharmacol. Exp. Ther.* **268**, 709-717 (1994).

Meng, L., Mohan, R., Kwok, B.H.B., Elofsson, M., Sin, N., and Crews, C.M., Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo anti-inflammatory activity. *Pro. Matl. Acad. Sci. USA* **96**, 10403-8 (1999).

Obukowics, M.G., Welsch, D.J., Salsgiver, W.J., Martin-Berger, C.L., Chinn, K.S., Duffin, K.L., Ras, A. and Needleman, P., Novel, Selective 6 or 5 fatty acid desaturase inhibitors as anti-inflammatory agents in mice. *J. Pharmacol. Exp. Thera.* **287**, 157-66 (1998).

Paget, G.E. and Barnes, J.M., in: Evaluation of drug activities, Pharmacometric, Vol., D.R. Laurence (eds.), Academic Press, London-New York, 1964.

Pfeilschifter, J., Schalkwijk, C., Briner, V.A., and Van den Bosch, H., Cytokine-stimulated secretion of group II phospholipase A_2 by rat mesangial cells. Its contribution to arachidonic acid release and prostaglandin synthesis by cultured at glomerular cells. *J. Clin. Invest.* **92**, 2516-2523 (1993).

Siham, M.E., Omar, M.A., Ayman, R.B.,Seham, E., and Mahmoud, S.A., Studies on the anti-inflammatory and anti-nociceptive effect of melatonin in rats. *Pharmacological Research* **46**, 235-243 (2002).

Vadas, P. and Pruzanski, W., Biology of disease, Role of secretory phospholipases A_2 in the pathobiology of disease. *Lab. Invest.* **55**, 391-404 (1986).

Vinegar, R., Truax, J.F., and Selph, J.L., Quantitative comparison of the analgesic and anti-inflammatory activities of aspirin, phenacetin and acetaminophen in rodents. *Eur. J. Pharmacol.* **37**, 23-30 (1976).

Winter, G.A., Rislfy, E.A., and Nuss, G.W., Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drug. *Proc. Soc. Exp. Biol. Med.* **111**, 544-547 (1962).

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