International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 5 (4): 251-256, 2009
ISSN 1811-7775

Studies on the Anti-Inflammatory, Analgesic and Antipyretic Properties of *Andrographis echioides* Nees.

[1]S.K. Basu, [2]M. Rupeshkumar and [3]K. Kavitha
[1]Department of Pharmaceutical Technology, Jadavpur University, Kolkatta-700 032, India
[2]Department of Pharmacology, Bharathi College of Pharmacy, Bharathi Nagara, Mandya Dist, Karnataka-571 422, India
[3]Department of Pharmaceutics, Bharathi College of Pharmacy, Bharathi Nagara, Mandya Dist, Karnataka-571 422, India

Abstract: The purpose of this investigation was to study the anti-inflammatory, analgesic and anti-pyretic properties of total extract and three fractions (ether, chloroform and ethyl acetate) from *Andrographis echioides* (Acanthaceae) in rats and mice. The plant material was extracted with methanol. In order to estimate the polarity of the active compounds, the total extract was successively partitioned between ether, chloroform and ethyl acetate. Dose of 200 and 400 mg kg^{-1} of each extracts were used in carrageenan-induced paw edema, cotton-pellet granuloma in rats, writhing nociception in mice and yeast induced hyperpyrexia in rats. All compounds reduced paw edema in comparison to the control group at 5 h post carrageenan injection. The total, ether and ethyl acetate extracts were similar to phenylbutazone ($p<0.001$), while the chloroform extract was weaker than phenyl butazone in reduction of paw edema and cotton-pellet granuloma. All extracts as well as Paracetamol induced antinociception in writhing test in comparison to control. Positive results for flavanoids and phenolic compounds were investigated by phytochemical analysis of total extract. Phenolic compounds were found in three fractions. The higher antinociception effects of total and ether extracts among different extracts tested, might back to the presence of flavanoids and phenolic compounds. The total, ether, ethyl acetate extract produced a significant dose dependent inhibition of temperature elevation. These data suggest that different extracts of *A. echioides* produce antinociceptive, anti-inflammatory and anti-pyretic activities that could be due to the effect of one or a combination of the bio active components in each extract.

Key words: *Anrographis echioides*, anti-inflammatory, antinociception, anti-pyretic, mouse and rats

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts (Acharya *et al*., 2008). Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many of the herbs and spices used by humans to season food yield useful medicinal compounds (Lai, 2004; Tapsell, 2006). Herbal therapy is used to treat a large variety of ailment and symptoms, e.g., inflammation, fever and pain; however, there are no adequate experimental evidences about their effectiveness (Kuhn *et al*., 2000; Golshani *et al*., 2004; Monsef-Esfahani *et al*., 2004). Some species of *Andrographis* like *Andrographis panniculata* have been reported to possess astringent, anodyne properties and is helpful in bronchitis, swellings anditches (Kiritikar and Basu, 2001). Plants belonging to the genus *Andrographis* have been shown to contain, a diterpenoid lactone and flavanoids (Handa and Anupam, 1990). The group of flavanoid is famous for its anti-inflammatory, anti-allergic, antithromtic, vasoprotective and protection of gastric mucosa properties. These properties have been attributed to influence of flavanoids on production of prostaglandins and their antioxidant effects (Evans, 2002). Till now *A. echioides* has not been the subject of any pharmacological research. Therefore, aim of this study was to carry out a pharmacological evaluation of different extracts of *A. echioides* for its anti-inflammatory, analgesic and antipyretic properties.

Corresponding Author: M. Rupeshkumar, Department of Pharmacology, Bharathi College of Pharmacy, Bharathi Nagara, Mandya Dist., Karnataka-571 422, India Tel: +91-9980002265 Fax: +91-8232-235111

MATERIALS AND METHODS

Plant material: The fresh aerial parts of *Andrographis echioides* were collected during the flowering stage from Siddha and Ayurvedic Medicines India Pvt. Ltd., Erode Dist., Tamilnadu, in Aug 2006 and identified by G.V.S. Murthy, Botanical Survey of India, Coimbatore, Tamil Nadu, voucher specimen (No. BSI/SC/5/23/06-07/TECH.835) has been deposited at the Herbarium of the Department of Pharmacology, Bharathi College of Pharmacy, Karnataka. The plant material was air dried, powdered and extracted twice with methanol (80%) in percolator. The combined methanol extracts were evaporated to dryness under reduced pressure. In order to estimate the polarity of the active compounds, the total extract was successively partitioned between ether, chloroform and ethyl acetate (yields 0.94.1.19 and 3.14% w/w, respectively).

Animals: In breed Albino male Wistar rats (150-200 g) and Swiss Albino mice (20-25 g) were used for the experiments. All the animals were obtained from the laboratory animal centre, Bharathi College of Pharmacy, Karnataka. The animals were maintained under standard environmental conditions and fed with standard diet and water *ad libitum*. The experimental was approved by the Experimentation Ethics Committee (1135/a/07/CPCSEA).

Drugs and chemicals: The drugs and fine chemicals were purchased from Sigma Chemical Co., St. Louis, USA. All other chemicals and solvents were obtained from local firms (India) and were of highest purity and analytical grade.

Studies on inflammation

Acute inflammation study

Carrageenan-induced paw oedema in rats: Pedal inflammation in male Wistar rats (150-200 g) was produced according to the method described earlier (Winter *et al*., 1962). An injection (s.c.) was made of 0.1 mL of 1% carrageenan into the right paw of each rat under the sub plantar aponeurosis. The test groups of rats were administered intraperitonially with 200 and 400 mg kg^{-1} of the various serial extract of *A. echioides* 1 h before carrageenan injection.

At the same time, the control group received 5 mL kg^{-1} of 5% gum acacia and the reference group received 100 mg kg^{-1} phenyl butazone (i.p.). The paw value was measured immediately after carrageenan injection and at 1, 2, 3, 4 and 5 h intervals after the administration of the edematogenic agent using a plethysmograph-apparatus up to the anatomical hairline on lateral malleolus (Goldenberg and IIse, 1977) and compared with the control animals, which received only the vehicle. The inhibitory activity was calculated according to the following formula (Chu and Kovacs, 1977).

$$\text{Inhibition (\%)} = 100 - \frac{(\text{Oedema volume in the treated})}{(\text{Oedema volume in the control})} \times 100$$

Sub-acute inflammation study

Cotton-pellet granuloma in rats: This study was carried out by cotton-pellet implantation method in rats (Meier *et al*., 1950). This method used here was adopted from Sheth *et al*. (1972), with a slight modification of using only male rats (Lassman *et al*., 1977). Under light ether anaesthesia, sterile cotton-pellets (10 mg) were implanted subcutaneously in the axilla and groin regions of the rats. The animals were treated orally with various serial extracts at different doses (200 and 400 mg kg^{-1}) daily for 7 consecutive days. Animals in the control group received either normal saline or the vehicle gum acacia. Phenyl butazone (100 mg kg^{-1}, orally) was given to animals in the reference groups. They were sacrificed on day 8, the cotton-pellet removed, freed from extraneous tissue and dried overnight at 60°C and weighed. The percent inhibition of the dry weight of the granuloma were calculated and compared.

Antinociceptive activity

Effect on acetic acid-induced writhing in mice: Analgesic activity was evaluated on the acetic acid-induced writhing according to Koster *et al*. (1959). The writhes were induced by intra-peritoneal injection of 0.6% acetic acid (v/v) (10 mL kg^{-1}). Two different doses (200 and 400 mg kg^{-1}) of the various serial extracts were administered orally to different groups of six animals each, 30 min before chemical stimulus. The numbers of writhing movements were counted 10, 20, 30 and 40 min after acetic acid injection. Antinociception (analgesia) expressed as the reduction of the number of abdominal constrictions between control animals (acetic acid treated mice) and mice pretreated with the extract.

Antipyretic activity

Yeast induced hyperthermia: Ten groups of six rats each were injected subcutaneously with 10 mL kg^{-1} b.wt. Yeast

suspension (15% aqueous suspension) to induce pyrexia, after measuring the basal rectal temperature (0°C) of each animal. Nineteen hours after yeast injection, the rectal temperature was recorded again and animals showing a rise in temperature of <0.6°C were discarded (Mukherjee *et al*., 2002). Thereafter, treatment was carried out as follows:

- **Group I:** Distilled water (10 mL kg^{-1}, p.o.)
- **Group II-IX:** Various serial extracts of *A. echioides* (200-400 mg kg^{-1}, p.o.)
- **Group X:** Paracetamol (100 mg kg^{-1}, p.o.)

Rectal temperatures were then recorded at 20, 21, 22, 23 and 24 h (T°C) after yeast injection.

Statistical analysis: The data were analyzed for significance using the unpaired two-tailed student's t-test.

RESULTS

Studies on inflammation

Acute inflammation study

Carrageenan-induced paw oedema in rats: Carrageenan-induced rat paw oedema was markedly inhibited by intraperitonial treatment with either the extracts (200 and 400 mg kg^{-1}) or phenyl butazone (100 mg kg^{-1}). Various serial extracts of *A. echioides* showed highly significant ($p<0.001$) acute inflammatory effect in a dose related manner, more or fewer equals to the effect were produced by phenyl butazone. The results were shown in the Table 1.

Sub-acute inflammation study

Cotton pellet granuloma in rats: In sub acute studies various serial extracts of *A. echioides* shows highly significant sub acute anti-inflammatory effect (Table 2).

Antinociceptive activity

Acetic acid induced writhing in mice: The various serial extracts (200-400 mg kg^{-1}, i.p.) were significantly reduced ($p<0.001$) acetic acid-induced abdominal constrictions and stretching of hind limbs in a dose-dependent manner (Table 3).

Antipyretic activity: As shown in Table 4 subcutaneous injection of yeast caused elevation of rectal temperature in control rats 19 h after administration. Oral administration of the extracts produced a significant ($p<0.001$) dose dependent inhibition of temperature elevation. Peak inhibitory effect was observed at 1 h post-therapy, i.e., 20 h post-yeast injection ($p<0.001$).

Table 1: Effect of *A. echioides* on carrageenan induced paw edema in rats

Treatments	Dose	Volume of paw edema (mL)	Inhibition (%)
Gum acacia	5 mL kg^{-1}	1.15±0.002	-
Met extract	200 mg kg^{-1}	0.46±0.003*	60.00
Met extract	400 mg kg^{-1}	0.36±0.002*	68.69
Pet extract	200 mg kg^{-1}	0.84±0.009*	26.95
Pet extract	400 mg kg^{-1}	0.71±0.005*	38.26
Chlor extract	200 mg kg^{-1}	0.52±0.004	54.78
Chlor extract	400 mg kg^{-1}	0.47±0.002	59.13
Ethyl acetate extract	200 mg kg^{-1}	0.67±0.002*	41.73
Ethyl acetate extract	400 mg kg^{-1}	0.56±0.004*	51.30

Values are expressed in Mean±SEM. n = 6. *p<0.001 vs. control (Student t-test)

Table 2: Effect of *A. echioides* on cotton-pellet granuloma in rats

Treatments	Dose	Weight of the cotton-pellet (mg)	Inhibition (%)
Gum acacia	5 mL kg^{-1}	46.66±0.22	-
Met extract	200 mg kg^{-1}	25.57±0.22*	45.19
Met extract	400 mg kg^{-1}	18.15±0.06*	61.10
Pet extract	200 mg kg^{-1}	41.22±0.26*	11.65
Pet extract	400 mg kg^{-1}	35.91±0.11*	20.03
Chloro extract	200 mg kg^{-1}	46.57±0.22	0.19
Chloro extract	400 mg kg^{-1}	46.52±0.21	30.00
Ethyl acetate extract	200 mg kg^{-1}	34.32±0.06*	26.44
Ethyl acetate extract	400 mg kg^{-1}	18.15±0.06*	45.19
Phenyl butazone	100 mg kg^{-1}	16.71±0.17*	64.18

Values are Mean±SEM. n = 6. *p<0.001 vs. control (Student t-test)

Table 3: Effect of *A. echioides* on acetic acid induced writhing in mice

		10	20	30	40
Treatments	Dose	(min)			
Gum acacia	5 mL kg^{-1}	17.50±0.42	36.16±0.47	47.33±0.42	35.33±0.49
Met extract	200 mg kg^{-1}	6.50±0.22*** (62.85)	26.50±0.22*** (26.71)	22.66±0.21*** (52.14)	17.50±0.22*** (50.41)
Met extract	400 mg kg^{-1}	4.11±0.30*** (76.51)	17.50±0.22*** (51.60)	12.66±0.21*** (73.25)	7.50±0.22*** (78.77)
Pet extract	200 mg kg^{-1}	20.50±0.42* (17.41)	29.50±0.42* (18.41)	31.00±0.44* (34.50)	24.66±0.33* (30.20)
Pet extract	400 mg kg^{-1}	15.50±0.34*** (11.42)	27.00±0.25*** (25.33)	30.16±0.30*** (36.27)	24.66±0.21*** (30.20)
Chloro extract	200 mg kg^{-1}	17.00±0.25	34.33±0.42***	45.66±0.42***	34.66±0.76
Chloro extract	400 mg kg^{-1}	15.66±0.33	34.16±0.47	44.66±0.42***	33.50±0.67
Ethyl acetate extract	200 mg kg^{-1}	10.66±0.21*** (39.08)	33.00±0.25*** (30.83)	38.00±0.36***	30.50±0.22*** (43.29)
Ethyl acetate extract	400 mg kg^{-1}	8.16±0.30*** (53.37)	30.83±0.16*** (14.71)	28.33±0.21*** (40.14)	20.00±0.25 (43.39)
Paracetamol	100 mg kg^{-1}	4.80±0.21 (96.22)	5.66±0.25 (75.11)	1.20±0.21 (83.81)	4.83±0.16 (86.32)

Values are expressed in Mean±SEM. n = 6. *p<0.001 vs. control (Student t-test), **p<0.01, ***p<0.05. Values in parenthesis indicate percentage of inhibition

Table 4: Effect of *A. echioides* on yeast induced hyperpyrexia in rats

Treatments	Dose	T0	T19	T20	T21	T22	T23	T24
Gum acacia	5 mL kg^{-1}	37.4±0.10	39.46±0.18	39.41±0.11	39.75±0.04	39.65±0.01	39.58±0.01	39.51±0.01
Met extract	200 mg kg^{-1}	37.58±0.01	39.50±0.02	39.56±0.02***	38.46±0.02***	37.86±0.02***	37.46±0.02***	37.46±0.02***
Met extract	400 mg kg^{-1}	37.50±0.04	39.61±0.14	39.66±0.02***	38.48±0.03***	37.70±0.03***	37.46±0.02***	37.46±0.02***
Pet extract	200 mg kg^{-1}	37.66±0.21	39.30±0.16	39.28±0.03*	39.05±0.02*	39.05±0.01	39.01±0.01*	39.06±0.21*
Pet extract	400 mg kg^{-1}	37.45±0.02**	39.50±0.12	39.68±0.01***	38.98±0.04***	38.60±0.02***	38.26±0.02***	37.83±0.03***
Chloro extract	200 mg kg^{-1}	37.50±0.03	39.30±0.12	39.35±0.13	39.63±0.03***	39.65±0.02	39.51±0.03***	39.45±0.02***
Chloro extract	400 mg kg^{-1}	37.40±0.02	39.08±0.10	39.11±0.04	39.55±0.02	39.45±0.02***	39.41±0.01***	39.41±0.05***
Ethyl acetate extract	200 mg kg^{-1}	37.78±0.01	39.70±0.12	39.75±0.02***	39.25±0.02***	38.76±0.02***	38.46±0.02***	38.16±0.02***
Ethyl acetate extract	400 mg kg^{-1}	37.35±0.04	39.60±0.10	39.66±0.02***	38.70±0.04***	38.31±0.02***	37.96±0.02***	37.95±0.03
Paracetamol	100 mg kg^{-1}	37.76±0.02	39.56±0.06	39.63±0.01	38.31±0.01	38.03±0.02	37.78±0.01	37.66±0.02

Values are Mean±SEM, *p<0.001 vs. Control (Students t-test) in respect of writhing response. **p<0.01, ***p<0.05

DISCUSSION

Among several traditional claims, the usefulness of *A. echioides* in fever, inflammation and pain have been emphasized more in literature. Hence, it was considered that investigations for these medicinal properties might give scientific authentication to the traditional claims. Moreover, this plant has not been subjected to above mentioned systemic pharmacological screening so far.

In the present study, the anti-inflammatory, analgesic and anti-pyretic activity of the various serial extracts of *A. echioides* has been established. The test extracts at two different doses (200-400 mg kg^{-1}) were found to significantly inhibit the Carrageenan-induced rat paw oedema, a test, which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa *et al*., 1995). Oedema formation due to carrageenan in the rat paw is the biphasic event (Vinegar *et al*., 1969). The initial phase is attributed to the release of histamine and serotonin (Crunkhorn and Meacock, 1971). The second phase of oedema is due to release of prostaglandins, protease and lysosome (Vinegar *et al*., 1969; Crunkon and Meacock, 1971). The second phase is sensitive to most clinically effective anti-inflammatory drugs (Vineger *et al*., 1969; Di Rosa *et al*., 1971). Besides in the carrageenan-induced rat paw oedema model the production of protanoides has been through the serum expression of COX-2 by a positive feedback mechanism (Nantel *et al*., 1999). Therefore, it is suggested that the mechanism of action of test extracts may be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of non-steroidal anti-inflammatory drugs in the inhibition of inflammatory process induced by carrageenan (Di Rosa *et al*., 1971).

Like wise, the granulomatous tissue formation is related to the chronic inflammatory process, which is characterized by several phases (Swingle and Shideman, 1972). In this regard, the oral treatment with 200 and 400 mg kg^{-1} of the various serial extracts of *A. echioides* and 100 mg kg^{-1} of phenyl butazone lead to 54.78 and 68.69% reduction of the granulomatous tissue formation, respectively (p<0.001).

In addition, the classification of antinociceptive drugs is usually based on their mechanism of action either on the central nervous system or on the peripheral nervous system (Planas *et al*., 2000). With respect to the writhing test the research group of Derardt *et al*. (1980), described the quantification of prostaglandins by radio immuno assay in the peritoneal exudates of rats, obtained after intra peritoneal injection of acetic acid. They found high levels of prostaglandins, PGE2 and PGF2 alpha during the first 30 min after acetic acid injection. Nevertheless, it was found that the intra peritoneal administration of acetic acid-induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators (Hokansan, 1978; Duarte *et al*., 1988). Thus, the results obtained for the writhing test using acetic acid are similar to those obtained for the odematogenic test using carrageenan. Therefore, anti-inflammatory substances may also be involved in the peripheral analgesic activity.

Indeed, Non-Steroidal Anti-Inflammatory Drugs (NSAIDP), like paracetamol, exert their antipyretic action by largely inhibiting prostaglandin (E-type) protection in the hypothalamus (Rang *et al*., 1999). Consequently, elevated plasma prostaglandin level, as observed in fever, is suppressed. Acetyl salicylic acid, another reference anti-pyretic drug (not used in this study), also brings about the same effect by a selective action on a specific cyclo-oxygenase (COX) isoenzyme in the CNS. The various serial extracts of *A. echioides* demonstrated effective anti pyretic activity as evident in the inhibition of the temperature elevation in the yeast model. The antipyretic action of the extract may possibly be through inhibition of prostaglandin production, leading to suppression of elevated plasma level, especially since the extract had been shown to possess analgesic and anti-inflammatory activities.

CONCLUSION

From these investigations, it may be concluded that the various serial extracts of *A. echioides* showed analgesic, anti-inflammatory and antipyretic effects, similar to those observed for non-steroidal drugs such as, phenyl butazone and paracetamol. It is important to point out that the phytochemical analysis showed the presence of flavonoids and this might be responsible for anti-inflammatory and analgesic activity. Further investigations are under process in our laboratory to isolate and characterize the specific active components of the plant extract which is responsible for observed pharmacological actions.

REFERENCES

Acharya, D. and A. Shrivastava, 2008. Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices. 1st Edn., Aavishkar Publishers, Distributors, Jaipur, India, ISBN-13: 9788179102527.

Chu, D. and B.A. Kovacs, 1977. Anti-inflammatory activity in Oakgall extracts. Arch. Int. Pharm. Ther., 230: 166-176.

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

Derardt, R., S. Jougney, F. Delevalcee and M. Falhout, 1980. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol., 61: 17-24.

Di Rosa, M., J.P. Giroud and D.A. Willough, 1971. Studies of the acute inflammatory response induced in rats in different sites by Carrageenan and Turpentine. Pathology, 104: 15-29.

Duarte, J.D.G., M. Nakamura and S.H. Ferreia, 1988. Participation of the Sympathetic system in acetic acid induced Writhing in mice. Braz. J. Med. Res., 21: 341-343.

Evans, W.C., 2002. Trease and Evans Pharmacognosy. 15th Edn., Bailliere Tindall, London, ISBN-10: 0702026174.

Goldenberg, M.M. and A.C. Ilse, 1977. Anti-inflammatory activity in EU-2972, 2-(3,4-di chlorophenyl amino) quinazolinium bromide. Arch. Int. Pharm. Ther., 228: 153-161.

Golshani, S., F. Karamkhani, H.R. Monsef-Esfehani and M. Abdollahi, 2004. Antinociceptive effects of the essential oil of Dracocephalum Kotschyi in the mouse writhing test. J. Pharm. Pharm. Sci., 7: 76-79.

Handa, S.S. and S. Anupam, 1990. Hepatoprotective activity of *Andrographis panniculata* against Carbon tetrachloride. Ind. J. Med. Res., 92: 276-283.

Hokansan, G.C., 1978. Acetic acid for analgesic screening. J. Natural Prod., 41: 497-498.

Kiritikar, K.R. and B.D. Basu, 2001. Indian Medicinal Plants. 2nd Edn., Oriental Enterprises, Dehradun, India.

Koster, R., M. Anderson and E.J. Beer, 1959. Acetic acid for analgesic screening. Fed. Proc., 18: 412-418.

Kuhn, M.A. and D. Winston, 2000. Herbal Therapy and Supplements a Scientific and Traditional Approach. Lippincott, New York.

Lai, P.K. and J. Roy, 2004. Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem., 11: 1451-1460.

Lassman, H.B., R.E. Kirby, J.C. Wilkeret, A.R. Fadden and D.E. Aultz, 1977. Pharmacology of a new non-steroidal anti-inflammatory agent HP-549. Arch. Int. Pharm. Ther., 227: 142-154.

Meier, R., W. Schuler and P. Desaulles, 1950. On the mechanism of cortisone inhibition of connective tissue proliferation. Experientia, 6: 469-471.

Monsef-Esfahani, H.R., A. Ghobadi, M. Iranshahi and M. Abdollahi, 2004. Antinociceptive effect of harmala. L alkaloid extract on mouse formalin test. J. Pharm. Pharm. Sci., 7: 65-69.

Mossa, J.S., S. Rafatullah, A.M. Galal and M.A. Al-Yahya, 1995. Pharmacological studies of rhus retinorrhaea. Int. J. Pharmacognosy, 33: 242-246.

Mukherjee, K., B.P. Saha and P.K. Mukherjee, 2002. Evaluation of antipyretic potential of *Leucas lavandulaefolia* (Labiatae) aerial part extract. Phytother. Res., 16: 686-688.

Nantel, F., D. Denis, R. Gordon, A. Northey, M. Cirino, K.M. Metters and C.C. Chan, 1999. Distribution and regulation of cyclo-oxygenase-2 in carrageenan-induced inflammation. Br. J. Pharmacol., 128: 853-859.

Planas, E., S. Sanchez, L. Rodriguez, O. Pol and M.M. Puig *et al*., 2000. Antinociceptive, anti-edema effects of liposomal morphine during acute inflammation of the rat paw. Pharmacology, 60: 121-127.

Rang, H.P., M.M. Dale and J.M. Ritter, 1999. Anti-Inflammatory and Immunosuppressant Drugs: Pharmacology. 4th Edn., Churchill Levingstone, Edinburgh.

Sheth, U.K., N.K. Dadkar and U.G. Kamat, 1972. Selected Topics in Experimental Pharmacology. The Kothari Book Depot, Bombay, India.

Swingle, K.F. and F.E. Shideman, 1972. Phases of inflammation response to subcutaneous implantation of Cotton-pellet and other modifications by certain anti-inflammatory agents. J. Pharm. Exp. Ther., 13: 226-234.

Tapsell, L.C., I. Hemphill, L. Cobiac, C.S. Patch and D.R. Sullivan *et al*., 2006. Health benefits of herbs and spices: the past, the present, the future. Med. J. Aust., 185: S4-24.

Vinegar, R., W. Schreiber and R. Hugo, 1969. Biphase development of carrageenan oedema in rats. J. Pharm. Exp. Ther., 166: 96-103.

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