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RESEARCH ARTICLE

ANTI-INFLAMMATORY, ANALGESIC AND ANTI-PYRETIC EVALUATION OF N, N'-PROPYLENEBIS(SALICYLAMIDE) TRANSITION METAL COMPLEXES IN RAT

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ABSTRACT: A salicylic acid derivative, N, N' - propylenebis(salicylamide) and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were evaluated for anti-inflammatory, analgesic and anti-pyretic activities. Anti-inflammatory activity was evaluated using carrageenan-induced paw edema method. Activities of Co(II) and Cu(II) complexes (200 mg/kg, po) are equal to the standard drug, diclofenac (100 mg/kg, po) at the experimental dose level. The Zn(II) complex also exhibits enough activity (p=0.001). Tail immersion method was employed for analgesic activity. The Co(II), Cu(II) and Zn(II) complexes (200 mg/kg, po) show significant activity but lesser than the standard drug, pentazocine (4mg/kg, ip). Anti-pyretic activity was evaluated using the Brewer's yeast-induced pyrexia in rats. Co(II) and Cu(II) complexes show significant activity ($p < 0.05$). The activities of complexes are greater than ligand in all experiments and among the complexes Co(II) and Cu(II) complexes exhibit superior activity.

KEYWORDS: propylene bis-(salicylamide), anti-inflammatory, analgesic, anti-pyretic, metal complexes

INTRODUCTION

Inflammation is a protective response of body's immune system to harmful stimuli such as pathogen, irritants, allergens and trauma. The classic symptoms of inflammation are heat, redness, swelling, pain and dysfunction of the organs involved [1,2]. While inflammation may be a normal response, chronic inflammation results in destruction of normal connective tissue due to the activities of catalytic enzymes and cytokines[3,4]. Anti-inflammatory refers to a drug that reduces inflammation. Anti-inflammatory drug can help improve sense of well being by relieving pain, reducing swelling, slowing down or stopping joint damage, and increasing ability to function. Anti-inflammatory includes non-steroidal anti-inflammatory drugs (NSAIDs), enzymes, and steroids. Non-steroidal anti-inflammatory drugs are the one kind of pharmaceuticals used for the relief of mild to severe pain. Inhibition of the COX-I and COX-II enzyme systems, and subsequent down-regulation of PG synthesis, is the well-accepted mode of action of NSAIDs[5,6]. Aspirin, ibuprofen, IndoH, flurbiprofen and meloxicam etc., are some of the well known COX inhibitors. NSAIDs act at additional sites in the inflammatory cascade, e.g. they may block tumor necrosis factor-alpha ((TNF)-α), which also controls the PGs[7].

Many of today's anti-inflammatory drugs are associated with problematic renal, gastrointestinal and cardiovascular side effects. Salicylamide[8] and its simple derivatives encompass significant biological activities like antifungal,

anti-inflammatory, analgesic, antipyretic activities. The derivatives with active structural moieties like N, N-dimethyl-3-phenyl[9], N-benzyl, N-chlorobenzyl[10] and N-heterocyclicles[11] have improved activity. Synthesis and study of metal complexes with anti-inflammatory drugs as ligands is a research area of considerable interest, particularly complexes which exhibit synergistic activity between metal and drug[12-14]. Anti-inflammatory drugs with metal centers show improved activity and lesser side effect[2]. Motivated by above mentioned findings the anti-inflammatory, analgesic and anti-pyretic properties of N, N' - propylenebis(salicylamide) metal complexes were screened.

MATERIALS AND METHODS

The N, N' - propylenebis(salicylamide) (ligand, **BA**) and its Co(II), Ni(II), Cu(II) and Zn(II) complexes were prepared as per the literature procedure[15], and were washed thoroughly with ether and chloroform. The purified compounds were used for pharmacological studies.

Animal: The pharmacological studies were carried out at Department of Pharmacology, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi. Adult albino rats of either sex weighing 100-140 grams were used for the study. All animals were kept in polypropylene cages in the animal house facility of Department of Pharmacology and maintained under standard laboratory conditions. They were fed with a standard diet and water *ad libitum*. All experiments were conducted after overnight fasting but there was free access to water. All the experimental protocols were approved by the Institutional Animal Ethical Committee.

Evaluation of Anti-inflammatory activity — Carrageenan induced paw edema method: The anti-inflammatory activity was assessed by the method suggested by Winter *et al*, using carrageenan as phlogestic agent[16]. Albino rats of either sex weighing between 100-140 g were used for all studies. The animals were divided into seven groups with each group having four animals. The animals were fasted over night prior to the experimental procedure but had free access to water. The solid samples were suspended in 0.5% w/v Sodium carboxy-methyl cellulose and administered orally 30 min before injection of carrageenan (0.1 mL of 1% w/v solution) in normal saline into subplanter region of right hind paw of each rat. The contalateral paw was injected with an equal volume of saline. The control group, standard group and test groups received normal saline, diclofenac sodium (100 mg/kg) and the test samples (200 mg/kg) respectively. The effective dose of the test compounds was assigned as per the similar works carried out with salicylamide and related compounds[17,14]. The left and right paw volumes were measured plethysmographically at each hour, for 4 h. The percentage inhibition of edema has been calculated by the following formula.

$$\% \text{ inhibition} = 100 \times \frac{(V_c - V_t)}{V_c}$$

where, Vc is volume of the edema in control and Vt is volume of the edema in animals treated with standard drugs/samples.

Evaluation of Analgesic activity — Tail immersion test: Wister albino rats were screened for its sensitivity by placing the tip of the tail (last 1−2 cm) gently in warm water maintained at 55°C (±2°C). Any albino rats flicking the tail within 5 sec were selected for the study. The selected rats were divided into seven groups of four animals each. The control, standard and test groups received normal saline, pentazocine (4 mg/kg, ip) and test samples (200 mg/kg, po) suspended in 0.5% w/v CMC respectively. After drug treatment, the basal reaction time of all groups of animals was noted at different time intervals viz., 1, 2, 3 and 4 h.

Evaluation of Anti-pyretic activity — Brewer's yeast induced pyrexia: The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats. The initial rectal temperatures were recorded using digital tele thermometer. Fever was induced by subcutaneous injection of 15% aqueous suspension of sterile Brewer's yeast (10 mL/kg). The animals that developed satisfactory pyrexia (0.5 or more raise in rectal temperature) after 18 h were used. Control group received normal saline and paracetamol (100 mg/kg, po) served as standard drug. The test samples were administered to different groups (200 mg/kg) orally as a suspension in 0.5% w/v CMC. After drug administration the rectal temperature was recorded at 0, 1, 2, 3 and 4 h.

Statistical analysis: All the data were expressed as mean ± S.E. Statistical significance of the difference between control and treated group were assessed by the method of student's t-test. $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The anti-inflammatory effects of all the five compounds are presented in Table 1 as the difference in paw volume and % inhibition. The ligand showed only 33 % activity. The Co(II) and Cu(II) complexes showed 94 % and 92 % inhibition with $p < 0.001$ respectively at the end hour of experiment as standard drug, diclofenac (94 %, $p < 0.001$) for the dose level taken. The drug action *i.e.* reduction of paw volume is observed from 2nd h for standard drug (100 mg/kg, po). But for Co(II) and Cu(II) complexes (200 mg/kg, po), it is observed even from 1st h. The Zn(II) complex showed 73 % inhibition with $p < 0.01$.

The tail withdrawal reflux times for untreated and treated groups are given in Table 2. The standard drug showed peak activity at 2nd h, and at the 4th h its activity was reduced to half the extent. The ligand and Ni(II) complex showed less activity. The Co(II) and Cu(II) complexes showed peak activity at 3rd h, and Zn(II) complex at 4th h with the significance of $p < 0.01$.

The results of effect of ligand and complexes on yeast induced pyrexia in albino rats were depicted in Table 3. The Co(II), Cu(II) and Zn(II) complexes showed 1.05, 0.85, 0.78°C

TABLE 1: ANTI-INFLAMMATORY ACTIVITY OF TEST SAMPLES AGAINST CARRAGEENAN INDUCED HIND PAW EDEMA

Groups	Dose p.o (mg/kg)	Paw volume (mm)					% inhibition
		0 h	1 h	2 h	3 h	4 h	
control	--	0.37±0.08	0.60±0.09	0.74±0.08	1.01±0.09	1.5±0.13	--
Diclofenac	100	0.39±0.04	0.81±0.04	0.53±0.05	0.29±0.07**	0.09±0.03*	94
BA	200	0.69±0.18	1.10±0.19	1.12±0.19	1.12±0.19	1.01±0.17	33
Co	200	0.62±0.04	0.51±0.04	0.33±0.03#	0.18±0.03*	0.09±0.01*	94
Ni	200	0.62±0.09	0.64±0.07	0.59±0.09	0.58±0.08	0.86±0.28	43
Cu	200	0.62±0.03	0.55±0.02	0.40±0.02#	0.24±0.02*	0.12±0.02*	92
Zn	200	0.78±0.11	1.32±0.09	1.07±0.09	0.54±0.18	0.41±0.14**	73

Values are expressed as mean±SEM (standard error of the mean) *p < 0.001, **p = 0.001, #p < 0.01 compared to control

fall in temperature at 4th h from peak temperature at 0 h, whereas paracetamol showed 1.1°C. But the ligand and Ni(II) complex showed only 0.4°C and 0.45°C fall in temperature.

Carrageenan induced rat paw edema method is a primary screen for cyclooxygenase-2 (COX-2) inhibitors. The inflammatory response to carrageenan consists of three phases[19]. The primary phase is mediated by histamine and 5-hydroxytryptamine, secondary phase is mediated kinin notably the endogenous nonapeptide bradykinin produced by kallikrein. The final phase is attributed to local production of prostaglandins (PG) of E series. Non-steroidal anti-inflammatory drugs exert their activity through blocking the synthesis of prostaglandins by inhibiting cyclooxygenase enzyme[3,5,6]. The N, N' -propylenebis(salicylamide) is a salicylic acid derivative and it will also belong to non-steroidal anti-inflammatory drug. It showed only mild activity (33 %). Activities of all the complexes are higher than ligand particularly Co(II), Cu(II) and Zn(II) complexes. It is well known that Co(II), Cu(II)[13,20,21] and Zn (II)[12,22] complexes of many non-steroidal anti-inflammatory drugs exhibited enhanced anti-inflammatory action. Even, Co(II)[23,24] and Cu(II)[25] complexes of many non-anti-inflammatory agents exhibited anti-inflammatory action, where the ligand may simply acts as a carrier that brings metal to the therapeutic target. During inflammation higher concentration of zinc ions were found in inflamed tissues. This opens the view of the anti-inflammatory properties of zinc[26]. The same activity was also reported for copper[21,27,28]. There is an increased demand for Cu(II) during inflammatory conditions[29,30]. So, with the added effect of metal ions, all the complexes showed enhanced activity, especially for Co(II), Cu(II) and Zn(II) complexes.

The anti-inflammatory activity of test samples concludes that few compounds are efficient cyclooxygenase inhibitor. Usually cyclooxygenase inhibitors have analgesic activity. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non-steroidal anti-inflammatory drugs inhibit only peripheral pain[31,32]. But some of the NSAIDs are reported to have both[33,34]. Tail immersion method was used to evaluate analgesic activity involving centrally mediated mechanism. Pain is centrally modulated *via* a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems[35-37]. The painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. Analgesic effect against thermal noxious stimuli may be elicited through opoid receptors or through modulation of several neurotransmitters involved in relevant phenomena[38]. The tail withdrawal reflux time for test group was found to be higher than control group. This suggests that the test samples have central mechanism of analgesic action. But, the extent of activity showed by the test samples is less than that of the standard drug pentazocine. The ligand and Ni(II) complex showed poor activity compared to standard drug and other complexes. The Co(II), Cu(II) and Zn(II) complexes showed appreciable activity but the results are only the half extend compared to standard drug.

TABLE 2: ANALGESIC ACTIVITY OF TEST SAMPLES IN TAIL IMMERSION METHOD

Groups	Dose (mg/kg)	Basal reaction time (S)	Basal reaction time (S) after drug administration			
			1 h	2 h	3 h	4 h
control	--	1.25±0.22	1.25±0.22	1.25±0.22	1.25±0.22	1.5±0.25
Pentazocine	4 i.p	1.25±0.22	6.75±0.54*	8.25±0.41*	8.0±0.35*	4.5±0.25*
BA	200 p.o	1.5±0.25	2.0±0.35	2.25±0.22	1.75±0.22	1.5±0.25
Co	200 p.o	2.0±0.35	3.0±0.35	3.75±0.41**	4.25±0.41**	4.25±0.41**
Ni	200 p.o	1.5±0.25	2.5±0.25	3.25±0.41	3.25±0.22	3±0.5
Cu	200 p.o	1.75±0.22	3.25±0.22**	4.0±0.35**	4.25±0.41**	4.25±0.22*
Zn	200 p.o	1.75±0.22	2.5±0.25	3.0±0.35	3.75±0.22*	4.0±0.35**

Values are expressed as mean±SEM (standard error of the mean) *p < 0.001, **p < 0.01 compared to control

TABLE 3: ANTIPYRETIC ACTIVITY OF TEST SAMPLES AGAINST BREWER'S YEAST-INDUCED PYREXIA

Groups	Dose (mg/kg)	Normal Temperature (°C)	Rectal temperature (°C) after drug administration				
			0 h	1 h	2 h	3 h	4 h
Control	--	37.33±0.35	38.15±0.28	38.15±0.29	38.15±0.26	38.15±0.28	38.17±0.26
Paracetamol	100 p.o	36.78±0.28	38.1±0.11	37.85±0.08	37.6±0.07	37.25±0.16	37.03±0.22*
BA	200 p.o	37.3±0.08	38.67±0.11	38.53±0.10	38.43±0.12	38.4±0.12	38.3±0.12
Co	200 p.o	36.8±0.25	38.33±0.04	37.95±0.06	37.73±0.04	37.58±0.04	37.28±0.07*
Ni	200 p.o	37.08±0.10	38.33±0.16	38.25±0.13	38.13±0.16	38.0±0.15	37.88±0.14
Cu	200 p.o	37.0±0.14	38.2±0.11	37.88±0.09	37.58±0.09	37.38±0.10	37.35±0.08*
Zn	200 p.o	37.35±0.06	38.63±0.05	38.23±0.04	37.93±0.05	37.9±0.06	37.85±0.04

Values are expressed as mean±SEM (standard error of the mean) *p < 0.05 compared to control

Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect[39, 40]. The experimental rats showed a mean increase of about 1.2-1.5°C in rectal temperature 24 h after the yeast injection. Yeast induced fever is a pathogenic fever. Its etiology comprises of production of prostaglandins[41]; particularly PGE2 appears to be a final pathway responsible for fever induced by several pyrogens[42]. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action[43] as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. The Co(II) and Cu(II) showed considerable inhibition. The fall in elevated body temperature of experimental animals showed that for Co(II) complex there was a gradual and maximum reduction in temperature upto 4 h. But for Cu(II) and Zn(II) complexes, efficient reduction in temperature of 0.83°C and 0.7°C were observed within 3 h and 2 h respectively; compared to Co(II) complex at same time period (3 h, 0.75°C and 2 h, 0.6°C). Less useful inhibition was observed for ligand (0.4°C) and Ni(II) complex (0.45°C).

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REFERENCES

[1] Purnima A, Koti BC, Thippeswamy AHM, Jaji MS, Vishwanatha Swami AHM, Kurhe YV *et al*. Antiinflammatory, Analgesic, and Antipyretic activites of *Mimusops elengi* Linn. Indian J Pharm Sci 2010;72(4):480-485.

[2] Weder JE, Dillon CT, Hambley TW, Kennedy BJ, Lay PA, Ray Biffin J *et al*. Copper complexes of non-steroidal anti-inflammatory drugs: an opportunity yet to be realized. Coord Chem Rev 2002;232: 95-126.

[3] Vane JR, Ferreira SH (Eds.). Inflammation. vol. 1 & 2. Berlin: Springer-Verlag; 1978.
[4] Vaday GG, Lider O. Extracellular matrix moieties, cytokines, and enzymes: dynamic effects on immune cell behavior and inflammation. J Leukoc Biol 2000;67:149-159.
[5] Vane SJ. Differential Inhibition of Cyclooxygenase Isoforms: An explanation of the action of NSAIDs. J Clin Rheumatol 1998;4(5):3-10.
[6] Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY *et al*. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents.. Nature (London) 1996;384:644-648.
[7] Malemud CJ, Goldberg VM. Directions for future research and treatment of osteoarthritis. Front Biosci. 1999;4:762-771.
[8] Krrogsgaard-Larsen P, Liljefors T (Eds.). A Textbook of Drug Design and Development, Amsterdam: Harwood Academic Publishers; 1996.
[9] Way E, Leong, Takemori AE, Smith GE Jr, Anderson HH, Brodie DC. The toxicity and analgesic activity of some congeners of salicylamide. J Pharmacol & Exper Therap. 1953;108: 450-460.
[10] Kubanova P, Buchta V, Perina M, Waisser K, Pour M. Evaluation of *in vitro* antifungal activity of N-benzylsalicylamide derivatives. Fol microbial. 2003;48(3):346-50.
[11] Daidone G, Raffa D, Plescia S, Matera M, Caruso A, Leone V, Amico-Roxas M. Synthesis and evaluation of the analgesic and antiinflammatory activities of N-substituted salicylamides, Farmaco. 1989;44(5):465-73.
[12] Singla AK, Wadhwa H. Zinc–aspirin complex: synthesis, physicochemical and biological evaluation. Int J Pharm. 1994;108:173-185.
[13] Singla, AK, Mediratta DK, Pathak K. Bioavailability of indomethacin from zinc-indomethacin complex. Int J Pharm. 1990;60:27–33.
[14] Abou-Mohamed G, El-Kashef H, Salem HA, Elmazar MM. Effect of zinc on the anti-inflammatory and ulcerogenic activities of indomethacin and diclofenac. Pharmacology. 1995;50: 266-272.
[15] Dash SC, Brahma GS, Das R, Das NN, Mohanty P. Synthesis, characterisation and substitution reaction of trans-(diaqua)(N,N'-ethylene-bis-salicylamide)chromium(III) ion. Ind J Chem. 2006;45A:2406-2411.
[16] Winter CA, Risley EA, Nuss GW. Carrageenin induced edema in hind paws of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962; 111: 544-547.
[17] Ali Khalaj, Mohammad Abdollahi, Abbas Kebriaeezadeh, Neda Adibpour, Zahra Pandi, Sara Rasoulamini. The antinociceptive and antiinflammatory activities and lack of ulcerogenicity of a benzodioxin-4-one and its analog benzoxazine as cyclic acetal-like derivatives of salicylic acid and salicylamide in mice and rats. Ind J Pharmacol. 2002;34:184-188.
[18] Soldatos CR, Kales A, Bixler EO, Scharf MB, Kales JD. Hypnotic effectiveness of sodium salicylamide with short-term use: sleep laboratory studies. Pharmacology. 1978;16(4):193-8.
[19] Paul G. Winyard, Derek A. Willoughby. Methods in molecular biology: Inflammation protocols. Vol. 225. Totowa, NJ: Humana press; 2003.
[20] Kovala-Demertzi D. Transition metal complexes of diclofenac with potentially interesting anti-inflammatory activity. J Inorg Biochem. 2000;79:153-157.
[21] Kostandinidou M, Kourounakis A, Yangou M, Hadjipetrou L, Kovala-Demertzi D, Hadjikakou S, Demertzis M. Anti- inflammatory properties of diclofenac transition metalloelement complexes. J Inorg Biochem. 1998;70:63-69.
[22] Singla AK, Wadhwa H. Zinc-indomethacin complex: synthesis, physicochemical and biological evaluation in the rat. Int J Pharm. 1995;120:145-155.
[23] Hunoor RS, Patil BR, Badiger DS, Vadavi RS, Gudasi KB, Magannavar CV, Muchandi IS. A study of anti-inflammatory and analgesic activity of new 2,3-disubstituted 1,2-dihydroquinazolin-4(3h)-one derivative and its transition metal complexes. Chem Pharm Bull. 2010;58:712-716.
[24] Konstantinovic SS, Cakic VS. Pharmacological characteristics of some 3-salicylidenehydrazono-2-indolinone coordination compounds. Med Chem Res. 2010;19;771-781.
[25] Sorenson JRJ. Copper complexes offer a physiological approach to treatment of chronic disease. Prog Med Chem. 1989;26:437-586.
[26] Simkin PA. Oral zinc sulfate in rheumatoid arthritis. Lancet. 1976;308(7985):539-542.
[27] Marrella M, Moretti U, Concari E, Velo GP, Milanino R. Changes of Cu and Zn in carrageenan pleurisy and paw-oedema in rat. Pharm Res Commun. 1988;20:629-630.
[28] Howell JMC, Gawthorne JM. Copper in Animals and Man. vol. 2. 1st edn. Florida: CRC Press, Boca Raton; 1987.
[29] Milanino R, Rainsford KD, Velo GP (Eds.). Copper and Zinc in Inflammation. Dordrecht: Kluwer Academic Publishers; 1989.

[30] Milanino R, Frigo A, Bambara LM, Marrella M, Moretti U, Pasquialiccio M *et al*. Clin. Copper and zinc status in rheumatoid arthritis: studies of plasma, erythrocytes, and urine, and their relationship to disease activity markers and pharmacological treatment. Clin Exp Rheumatol. 1993;11:271-281.
[31] Ferreira SH. Peripheral analgesic sites of action of anti-inflammatory drugs. Int J Clin Pract Suppl. 2002;128:2-10.
[32] Romsing J, Moiniche S, Ostergaard D, Dahl JB. Local infiltration with NSAIDs for postoperative analgesia: evidence for a peripheral analgesic action. Acta Anaesthesiol Scand. 2000;44(6):672-83.
[33] Nakamura H, Shimoda A, Ishii K, Kadokawa T. Central and peripheral analgesic action of non-acidic non-steroidal anti-inflammatory drugs in mice and rats. Arch Int Pharmacodyn Ther. 1986;282(1):16-25.
[34] Cashman JN. The mechanisms of action of NSAIDs in analgesia. Drugs. 1996;52:13-23.
[35] Bensreti MM, Sewell RDE. Selective effects of dopaminergic modifiers on antinociception produced by different opioid receptor agonists. Pro Br Pharmacol Soc. 1983; 6th-8th July:70.
[36] Headley PM, Shaughnessy CT. Evidence for opiate and dopamine interaction in striatum. Br J Pharmacol 1985;86:700.
[37] Wigdor S, Wilcox GL. Central and systemic morphine-induced antinociception in mice: Contribution of descending serotonergic and noradrenergic pathways. J Pharmacol Exp Ther 1987;242: 90-95.
[38] Uddin ME, Islam AMT, Chowdhury MAU, Rahman MK, Islam MS, Islam MR. Sedative and analgesic activities of Ludwigia repens. Phytopharmacology 2012;2(2):202-211.
[39] Devi BP, Boominathan R, Mandal SC. Evaluation of antipyretic potential of Cleome viscosa Linn. (Capparidaceae) extract in rats. J Ethnopharmacol. 2003;87(1):11-13.
[40] Moltz H. Fever: causes and consequences. Neurosci Biobehav Rev. 1993;17(3):237-269.
[41] Tomazetti J, Avila DS, Ferreira AP, Martins JS, Souza FR, Royer C. Baker yeast-induced fever in young rats: Characterization and validation of an animal model for antipyretic screening. J Neurosci Methods 2005;147:29-35.
[42] Santhi V, Sivakumar V, Thangathirupathi A, Thilaga RD. Analgesic, Anti-pyretic and Anti-inflammatory activities of chloroform extract of prosobranch mollusk *purpura presica*. Int J Pharmacol Bio Sci. 2011;5(2):9-15.
[43] Sattayasai J, Bantadkit J, Aromdee C, Lattmann E and Airarat W. Antipyretic, analgesic and anti-oxidative activities of *Aquilaria crassna* leaves extract in rodents. J Ayurveda Integr Med. 2012; 3(4): 175–179.