Exploration of antinociceptive, antipyretic and anti-inflammatory activities of Curcumin in male rat

Fraid J. Al-Tahan
Department of physiology and pharmacology,College of veterinary medicine.,Baghdad,Iraq.
fared_altahan@yahoo.com.

Abstract

Curcumin, one of active ingredients of *Curcuma spp*. Roxb (Zingiberaceae) is referred to main medicinal part in this spice. In the present study, the analgesic, antipyretic and anti-inflammatory actions of Curcumin were investigated in male rats. Ninety Albino male rats (110-125g) were used in this study (six animals were used in each test for Curcumin, aspirin and Pethedine). The effects of curcumin on nociceptive response using writhing, tail flicking and formalin tests in rats were evaluated. The antipyretic activity in yeast-induced fever and anti-inflammatory activity in carrageenin-induced edema in rats were examined. Administration of curcumin significantly $P<0.05$ decreased the number of writhing and stretching induced by acetic acid, and suppressed the licking activity of the late phase in formalin test in rats. Curcumin had significant decrease $P<0.05$ effects on yeast-induced fever and carrageenin-induced edema in rats. Furthermore, the ED_{50} from Log dose response curve exhibited sequence order potency in formalin test, writhing reflex and tail flick as aspirin > curcumin. Curcumin showed synergistic effect centrally acting pethidine and peripheral analgesic aspirin. From these results it can be concluded that curcumin possesses analgesic effect via a different mechanism from that of aspirin and pethidine.

Keywords: Curcumin,Analgesia,an fiinflammation.

الكشف عن الفعالية المسكنة للالم والخافضة للحرارة والمضادة للالتهاب للكركمين (Curcumin) في ذكور الجرذان

فريد جميل عمر الطحان

قسم الفسلجة والادوية،كلية الطب البيطري،جامعة بغداد،بغداد، العراق.

الخلاصة

الكركمين، احد المكونات الفعالة لنبات الكركم والذي تعزى اليه اغلب الفعاليات الطبية لتلك النبتة. استهدف البحث دراسة الفعالية المسكنة للالم وخفض الحرارة والمضادة للالتهاب للكركمين في ذكور الجرذان. قيمت فعالية الكركمين المسكنة من خلال التحري عن قدرته على خفض عدد حركات التلوي (writhing) وخفق الذنب (flicking) وعتبة التحمل ضد التاثير المهيج للفورملين، كما تم التحري عن قدرته على خفض حرارة الجسم المحدثة باستخدام احدى الخمائر والفعل المضاد للالتهاب المحدث بالكاراغينان (carrageenan). ادت المعامله بالكركمين الى انخفاض معنوي في عدد مرات التلوي المحدث بفعل حمض الخليك وعدد مرات لعق القدم في المرحلة الاجله (late phase) من تاثير الفورمالين في الجرذان. كما ادت

المعامله الكركمين الى انحفاض معنوي $P<0.05$ لحجم الوذمة في اقدام الجرذان المعاملة بالكاراغينان. اظهرت الجرعة المؤثرة الوسطى المستخرجة من منحني تاثير لوغاريتمات الجرعات للفحوصات الانفة الذكر ان الكركمين يقع تاليا للاسبرين من حيث شدة الفعالية، كما اظهر الكركمين فعلا تأزريا لكل من المسكن المركزي (البثدين) والمحيطي (الاسبرين). يمكن الاستنتاج من هذه الدراسه ان الكركمين يحدث مفعوله المسكن عبر الية مختلفة عن تلك التي يعمل بموجبها كل من البثدين والاسبرين.

Introduction

Curcumin is one of the pungent active ingredients of turmeric [1,2,3]. It is commonly used as a beverage given to women in confinement to accelerate the lochia and decrease pain and inflammation of uterus [1,2]. It is considered to be depurative and used both internally and externally for treating exanthema as well as a poultice for virtually every curry dish made in the world. Besides being a culinary delight, several clinical trials have found curcumin to be a notable anti-inflammatory and analgesic compound [4,5] Moreover, *in vitro* studies have explored that curcumin, act as a chemopreventive agent, inhibits the expression and activity of COX-2 in several different gastrointestinal cell lines: colon, esophagus and small intestine [6,7] However, treatment of the cell with curcumin suppressed dose-dependent mediated induction of COX-2 protein, genetic COX-2 expression (as measured by mRNA), and the synthesis of prostaglandin E_2. Most impressive, however, was the discovery that curcumin directly inhibited the enzymatic activity of COX-2 [1,8] Although the Zhang study did not examine the action of curcumin on cells mediating chronic joint inflammation, it did offer a provocative suggestion that curcumin may modulate chronic inflammatory GI events such as Crohn's disease and ulcerative colitis. After exposing such cells to curcumin, the researchers found the compound not only inhibited cell growth but also reduced the expression of COX-2 mRNA in a time- and dose-dependent manner [9,10] Therefore, curcumin would appear to be a safe, natural COX-2 inhibitor in humans [11], giving its safety profiles and demonstrated anti-inflammatory activity. Therefore, the present study was undertaken to demonstrate the antinociceptive, antipyretic and anti-inflammatory activities of the curcumin in rats.

Materials and Methods

Ninety Albino male rats (110-125gm) were obtained from the animal house of Al-Nahrain University, College of Science. They were kept in the animal house at 25 ± 2 C^o and light and dark cycles of 10 and 14 hr. for 1 week before and during the experiments. The animals were provided with standard rodent pellet diet and water *ad libitum.*

Test agents and treatment considerations

Five different doses were used for performance of relative log dose response of curcumin (Riedel-de Haen Seelze-Hannover) (10, 20, 30, 40 and 50 mg/kg B.W) (Orally), a reference peripherally acting analgesic drug "aspirin" (Aspin, SDI Iraq) (50, 100, 200, 250 and 300 mg/kg B.W.) orally and centrally acting analgesic "Pethidine" (T&D Pharma GmbH, Germany) (15, 20, 25, 30, 35 mg/kg B.W.), and cosolvent vehicle was orally administered certain time of each test. Curcumin and Aspirin suspensions were prepared with the use of distilled water. Six animals were used for each dose of the above mentioned test agents in each segment of the present work. A two-week withdrawal period was left after each experiment to allow clearance of the used drug and reuse of the same animals. Pretreatment values were obtained prior to each respective test.

Curcumin synergistic maneuver

The ED_{50}s were calculated from regression of log dose response curve of curcumin and each drug; the pivot tables were drawn to determine type and degree of synergism.

A. Antinociceptive Activity

1. Writhing test

Writhing behavior was tested after each dose level of each of the used test agents in rats to which 0.6% acetic acid solution (10 ml/kg body weight) was injected intraperitoneally. The number of writhings and stretchings were calculated over a 20 min. period [4].

2. Tail flick latent period

The technique described previously [12,13] was adopted, using a techno analgesiometer

(DEP, Co. China). The rat was placed in a rat holder with its tail coming out through a slot in the lid. The tail was kept on the bridge (jacket) of the analgesiometer with an electrically heated nichrome wire underneath. The tail received radiant heat from the wire, heated by passing current by 6 mA. The time taken for the withdrawal of the tail after switching on the current was considered as a latent period (in seconds) of "tail flicking response and was considered as the index of nociception. The cut off time for determination of latent period was taken at 30 seconds to avoid injury to the skin [14]

3. Formalin test

Thirty minutes after administration of each of curcumin dose orally, aspirin orally and 15 min. after subcutaneous administration of Pethidine, 20 µl of 2.5% formalin (BDH. England) in saline were injected subcutaneously in a hind paw of the rats. The time spent before licking the injected paw was recorded and the data were expressed as total licking time in the early phase (0-5 min.) and the late phase (15-30 min.) after formalin injection [15,16].

B. Antipyretic activity

Antipyretic activity of test agent was measured by slightly modifying the method described previously [17,18], Male rats were fasted overnight with water *ad libitum* before experiments. Pyrexia was induced by subcutaneously injecting 20% (w/v) brewer's yeast suspension (10 ml/kg) into the animals' dorsum region. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (Sato Keiryoki. Co., Ltd., Japan). Only rats that showing an increase in temperature of at least 0.7ºC were used for the experiments. Test agent or used for this purpose were curcumin and Aspirin. The temperature was measured at 1 and 4 hr. after drug administration.

C. Anti-inflammatory activity
Carrageenin-induced paw Edema

According to the method described by Woofle and MacDonald [19], the initial right hind paw volume of each rat was measured using a micrometer (Ugo Basile) and then 0.1 ml of 1% (w/v) carrageenin was subcutaneously injected into the subplantar region of the right hind paw. The volume of the paw was measured immediately prior to, and after 1 and 4 hr. post carrageenin injection, and the edema volume was determined. The data were expressed as paw volume, compared with the initial hind paw volume of each rat. Test agents (curcumin and Aspirin) were orally administered 30 min. before carrageenin injection.

Synergism and antagonism between agents:

The type of interaction between agents could be calculated according to [20], by the following formula:

$da/Da + db/Db < 1$ synergy effect
$da/Da + db/Db = 1$ Zero interaction
$da/Da + db/Db > 1$ antagonist effect

Where da and db were the doses of drugs A and B used in combination and Da and Db were their single doses which were effective with the combination (da + db) at any specified level of effect.

In case of presence of synergistic effect between agents the degree of synergism was determined according to Woofle and MacDonald [19], by using the following equation: $EA+B = EA + EB – (EA \times EB)$ when E can be expressed as a fraction of the maximum effect (1.0) using this equation and the data for experimentally obtained analgesic effect for drugs A and B, the theoretical curves for analgesic could be determined by using spreadsheet, while the experimental curve exported the combination of drugs had a positive synergy.

Statistical Analysis

Data are expressed as mean±SEM and were analyzed statistically by two way ANOVA procedures, followed by LSD test. A difference was considered significant at $p<0.05$.

Results

Effects of curcumuin on nociceptive responses "Writhing test"

Administration of curcumin (10, 20, 30, 40 and 50 mg/kg B.W.) dose dependently reduced the number of writhing induced by an intraperitoneal injection of 0.6% acetic acid **(Table-1A)**. Aspirin dose (50, 100, 200, 250 and 300 mg/kg), standard analgesic drug, also reduced the number of writhing in rats **(Table-1B)**. Pethidine (15, 20, 25, 30 and 35 mg/kg B.W.) (A centrally acting analgesic) however, significantly and dose dependently reduced the number of writhing even after low doses **(Table-1C)**. In respect to analgesia, curcumin showed an obvious synergistic effect both with Aspirin and Pethidine **(Table-1D)**. The degree of

synergism with pethidine was about 2/3 of that show after combination of aspirin and pethidine **(Table-1E)**.

Tail flick test

The mean latency of nociceptive responses to thermal stimuli is shown in **(Table-2).** Curcumin (A), Aspirin (B) and Pethidine (C) significantly exerted protective effects from heat-induced pain in Rats. In spite of being centrally acting analgesic drug, Pethidine showed markedly highly significant synergism in the pain latency period with curcumin.

Formalin test

(Table-3) shows effect of curcumin (A), Aspirin (B) and Pethidine (C) licking times of hind paw practiced by rats both at early and late phase due to subcutaneously injected formalin. Curcumin caused dose-dependent reduction of licking time only at late phase, while a high reduction results due to Aspirin and still higher due to pethidine at both phases.

Yeast-induced fever in rats

Both curcumin and Aspirin had significant reduction effects on pyrexia induced by yeast in rats for all given times **(Table -4).**

Effect of Curcumin on carrageenin-induced paw edema in rats

Significant suppression on carrageenan-induced paw edema was observed after curcumin treatment, while Aspirin (200 mg/kg) highly significantly reduced the carrageenin-induced paw edema in rats **(Table-5).**

Discussion

The ED_{50} results demonstrate that the curcumin and peripheral acting analgesic agents attenuated and modified nociceptive responses to chemical stimuli significantly ($P<0.05$) (acetic acid-induced writhing response in rats) which were ordered according to potency as follows: curcumin<Aspirin<Pethidine, where the synergistic maneuver of curcumin ED_{50} associated with ED_{50} of both centrally acting Pethidine and peripherally acting Aspirin displayed different pattern in their potency curcumin<Pethidine<curcumin-Aspirin. Only curcumin and aspirin and curcumin - aspirine concealed the licking activity in the late phase of formalin test. Whereas had significant effects on yeast-induced fever or paw edema induced by carrageenin in rats. Curcumin and the aspirin exerted protective action against nociception in the writhing test similarly to the reference.

(Table-1) Effect of Curcumin (A), Aspirin (B) and Pethidine (C) on acetic-induced writhing and their synergisms in male rats.

	Pretreatment	Number of writhing	% of inhibition
	0	26.81±4.01 e	----------
1A	**Curcumine Dose (mg/kg BW)**		
	10	18.30±3.14 a	26.76 a
	20	14.86±2.53 b	32.14 b
	30	12.69±2.85 b	51.25 c
	40	9.27±1.16 c	62.00 d
	50	6.23±1.41 d	79.32 e
1B	**Aspirin Dose (mg/kg BW)**		
	50	14.75±380 a	32.55 a
	100	10.69±1.40 b	60.84 b
	200	7.25±1.72 b	76.21 c
	250	6.61±0.70 cb	81.34 d
	300	4.14±0.92 c	90.80
1C	**Pethidine Dose (mg/kg BW)**		
	15	7.30±0.14 a	76.48 a
	20	3.25±0.95 b	97.04 b
	25	1.10±0.20 c	99.08 c
	30	0.00 d	100.00 d
	35	0.00 d	100.00 d

Different letters significant ($P<0.05$) between doses.,N=6

Type (1D) and degree (1E) of synergism (analgesic effect) of curcumine with Aspirin and Pethidine in male rats.

1.D **1.E**

	Aspirin	Pethidine		Aspirin	Pethidine
Curcumin	11.01±2.50	25.0±1.98	Curcumin	1.81	3.05 a
Aspirin	------	36.40±7.11	Aspirin	------	4.19 b

Different letters significant (P<0.05) between doses.,N=6

(Table-2) Effect of curcumine (A) Aspirin (B) and Pethidine (C) on tail flick test and their synergisms in male rats.

2A

Pretreatment	**Initial**	**After 30 seconds**
0	5.98±1.17 d	6.50±1.18 d
Curcumin Dose (mg/kg BW)		
10	9.05±1.02 a	10.01±1.30 a
20	9.68±1.54 a	10.92±1.01 a
30	11.87±1.35 a	14.25±1.92 b
40	13.80±1.41 b	15.90±1.48 b
50	16.70±1.03 c	11.94±1.25 c

2B

Aspirin Dose (mg/kg BW)		
50	6.78±0.93 a	7.92±1.61 a
100	9.05±1.69 b	11.5±1.18 b
200	9.98±0.54 b	11.2±0.73 b
250	11.07±1.42 b	14.5±0.78 c
300	15.88±2.12 c	14.38±1.139 c

2C

Pethidine Dose (mg/kg. BW.)	**Initial**	**After 30 seconds**
15	10.65±0.98 a	18.50±1.47 a
20	10.97±1.54 a	20.25±1.38 b
25	12.96±1.23 b	25.50±1.15 c
30	13.40±1.09 b	28.00±1.12 d
35	13.88±4.01 b	29.14±1.11 e

Different letters significant (P<0.05) between doses.,N=6

Synergism type **Synergism fold**

2.D **2.E**

	Aspirin	Pethidine
Curcumin	15.75±1.71	≤30.81±4.59a
Aspirin	------	28.14±3.80a

	Aspirin	Pethidine
Curcumin	1.79	2.80 a
Aspirin	------	1.64

(Table-3) Antinociceptive effect of Curcumin (A), Aspirin (B) and Pethidine (C) in formalin test and their synergisms in male rats.(sec.)

3A

Pretreatment	**Early phase**	**Late phase**
0	66.36±11.20 f	88.8±5.25 f
Curcumine Dose (mg/kg BW)		
10	60.37±4.27 a	53.99±11.08 a
20	71.16±5.60 b	20.82±3.82 b
30	87.65±4.14 c	11.81±1.10 c
40	76.38±7.92 d	3.75±0.61 d
50	69.11±12.72	1.13±0.00

3B

Aspirin Dose (mg/kg BW)		
50	29.36±3.9 a	11.20±1.45 e
100	26.11±4.12 b	9.96±2.30 b
200	19.64±1.40 c	7.64±4.03 c
250	11.20±2.85d	4.00±1.11d

	300	6.70±0.93e	1.13±0.10e
3C	**Pethidine Dose (mg/kg BW)**		
	15	1.02±0.36 a	0.20±0.05 a
	20	0.33±0.01 b	0.00 b
	25	0.00 c	0.00 b
	30	0.00 c	0.00 b
	35	0.00 c	0.00 b

Different letters significant (P<0.05) between doses.,N=6

Synergism type **Synergism fold**

3.D **3.E**

	Aspirin	Pethidine
Curcumin	6.82±1.18	20.10±3.79 a
Aspirin	------	16.43±4.10 b

	Aspirin	Pethidine
Curcumin	6.82±1.18	20.10±3.79 a
Aspirin	------	16.43±4.10 b

(Table-4) Effect of curcumin (A) and Aspirin (B) on yeast-induced fever and their synergisms in male rats.

	Pre-induced fever	**Body temperature C°**	**1 hour**	**4 hours**
4A	0	36.02±1.23	36.29±0.19 b	35.60±.15 b
	Curcumine Dose (mg/kg BW)			
	10	38.65±0.23	37.69±2.15	37.00±0.13
	20	37.92±0.38	37.00±0.23	36.5±0.20
	30	37.56±0.21	36.95±0.86	35.99±0.10 b
	40	38.00±0.42	36.36±0.10 a	35.99±0.10 b
	50	38.85±1.14	36.30±0.25 ba	35.99±0.16

	Aspirin Dose (mg/kg BW)	**1 hour**	**4 hours**
4B	50	36.90±0.20	35.90±0.26
	100	36.60±0.25	35.60±0.23
	200	36.05±0.10	35.30±0.10 a
	250	36.00±0.23	35.30±0.10 a
	300	35.70±0.10 a	35.30±0.10 a

Different letters significant (P<0.05) between doses.,N=6

Synergism type's **Synergism fold**

4.D **4.E**

	Curcumin	Aspirin
Curcumin	------	3.58±0.30
Aspirin	------	-------

	Curcumin	Aspirin
Curcumin	------	2.85±0.22
Aspirin	------	-----

(Table-5)Anti-inflammatory Effect of curcumin (A) and Aspirin (B) on carragninan-induced paw edema and their synergisms in male rats.(volume of paw mm.)

	Curcumine Dose (mg/kg BW)	**0 hour**	**1 hour**	**4 hours**
5A	10	3.48±0.29	5.24±0.13 a	6.46±0.17 a
	20	3.58±0.45	4.87±0.29 b	6.33±0.33 c
	30	3.87±0.13	5.32±0.29 a	6.72±0.10 a
	40	4.93±0.20	5.66±0.21 a	6.67±0.33 a
	50	4.13±0.28	6.01±0.15 a	6.99±0.28 a
5B	**Aspirin Dose (mg/kg BW)**		**1 hour**	**4 hours**
	50		5.03±0.26 a	5.95±0.14 a
	100		5.02±0.28 a	5.14±0.12 a
	200		4.67±0.25 a	4.88±0.26 a
	250		4.43±0.14 a	4.84±0.24 a
	300		3.73±0.14	3.58±0.19

Different letters significant (P<0.05) between doses.,N=6

Synergism type's 5.D

	Curcumin	Aspirin
Curcumin	------	3.57±0.79
Aspirin	------	------

Synergism fold 5.E

	Curcumin	Aspirin
Curcumin	-------	1.75±0.03
Aspirin	-------	---------

Discussion

Peripheral analgesic compound, aspirin

This test is generally used for screening of antinociceptive effect [1,21] Tail flick painful stimuli are known to be selective to centrally, but not peripherally, acting analgesic drugs [22]. In the present study, Pethidine produced an inhibitory effect on the nociceptive response in writhing test, while Aspirin did that in a lesser extent. Curcumin, however, was medium between Pethidine and Aspirin. These findings, therefore, suggest that the apparent antinociceptive action of the active compound (s) in curcumin is mediated through peripheral and central mechanism (s). Formalin test is another pain model, which assesses the way an animal responds to moderate, continuous pain generated by injured tissue [19]. The effects of drugs on the licking responses in the early and late phases reportedly represent antinociceptive action on sensory receptor and anti-inflammatory action, respectively [20,16].

Curcumin dose-related results of anti-inflammatory and antinociceptive activity in the challenge tests were in agreement with [23,24 and 25]. In addition [12, 19] also pointed anti-inflammatory activity on carrageenin-induced hind paw edema in rats. Since the curcumin suppressed writhing and licking activity of the late phase in mice but had no significant effects on yeast-induced pyrexia or paw edema induced by carrageenin in rats, it also had no inhibitory activity on COX assay *in vitro*, as compared to the reference drug Aspirin, (a nonsteroidal anti-inflammatory drug possessing analgesic, antipyretic and anti-inflammatory activities by inhibition of prostaglandin synthesis via cyclooxygenase pathway) [6]. As nociceptors are exposed to noxious stimuli, some chemical pain mediators such as bradykinins, prostaglandins, histamine, serotonin and substance P are released from damaged tissues [4, 20 and 26]. Thus, the antinociceptive activity of curcumin may act on or involve some of those pain mediators-that is, a different mechanism from that of aspirin. So curcumin act centrally may be trans blood brain barrier comparable with pethedine. Orderly the synergistic profile between curcumine and centrally and peripherally action drugs through major act on brain centers and nociceptors peripherally that displays with pethedine more effective on than aspirin. Based on these results, we conclude that curcumin possesses analgesic effect, and that its action on nociception may be different from that of aspirin. In addition, it had remarkable synergistic action when given in combination with either of used references drugs.

References

[1]Mukhopadhyay, A., Basu, N., Ghatak, N., Gujral, P.K. **1982**. Anti-inflammatory and irritant activities of curcumin analogues in rats. Agents Actions. **1982** Oct;12(4):508-15.

[2]Claeson, P., Panthong, A., Tuchinda, P., Reutrakul, V., Kanjanapothi, D.,Taylor, W.C. and Santisuk, T. **1993**. Three non-phenolic diarylheptanoids with anti-inflammatory activity from Curcuma xanthorrhiza. Planta Med., 59: 451-454.

[3]Otake, T., Mori, H., Morimoto, M. and Ueba, N. **1995**. Screening of Indonesian plant extracts for antihuman immunodeficiency virus-type 1 (HIV-1) activity. Phytother. Res., 9: 6-10.

[4]Babcock, T.A., Helton, W.S., Anwar, K.N., Zhao, Y.Y., Espat N.J. **2004**. Synergistic anti-inflammatory activity of omega-3 lipid and rofecoxib pretreatment on macrophage proinflammatory cytokine production occurs via divergent NF-kappaB activation. JPEN J.Parenter. Enteral. Nutr. 28 (4): 232-9; discussion 239-40.

[5]Perry, L.M. **1980**. Medicinal plant of East and Southeast Asia: attributed properties and uses, The MIT Press, Massachusetts, USA, p. 439.

[6]Goel A, **1999** Inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. Proceedings of the American Association for Cancer Research Annual Meeting; 40:528-9.

[7]Tjolsen, A., Berge, O-G., Hunskaar, S., Rosland, J.H. and Hole, K. **1992**. The formalin test: an evaluation of the method. Pain, 51: 5-17.

[8]Pongbunrod, S. **1979**. Mai - Tet - Murng - Thai: Medicinal characteristic of foreign and

Thai traditional medicines, 1st ed., Khrungthon Press, Bangkok, Thailand, p. 493.
[9]Hunskaar, S., Fasmer, O.B. and Hole, K. **1985**. Formalin test in mice, a useful technique for evaluating mild analgesics. J. Neurosci. Meth., 14: 69-76.
[10]Sirat, H.M., Jamil, S. and Hussain, J. 1998b. Essential oil of Curcuma aeruginosa Roxb. From Malaysia. J. Essential Oil Res., 10: 453-458.
[11]Sirat, H.M., Jamil, S. and Rahman, A.A. **1998** a Sesquiterpenes from Curcuma aeruginosa. Planta Med., 64: 584-585
[12]Bruce, R.D. **1985**. An up- and down procedure for acute toxicity testing. Fundam. Appl. Toxicol., 5: 151-157
[13]Chau, T. **1989**. Pharmacology methods in the control of inflammation. In: Modern Methods in Pharmacology, Vol. V, Alan. R. Liss., Inc., New York, pp. 195-212.
[14]Gebhart, G.F. and McCormack, K.J. **1994**. Neuronal plasticity. Implication for pain therapy. Drugs, 47 (5): 1-47.
[15]Dubuisson, D. and Dennis, S.G. **1977**. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem oils. Phytother Res., 14: 240-244.
[16]Vane, J. **1987**. The evolution of non-steroidal anti-inflammatory drugs and their mechanisms of action. Drugs, 33 Suppl 1: 18-27.
[17]Inmaculada P., Mariarosaria B., Fiorentina R., Antonietta R, Luca P., Lidia S. and Giuseppe C. **2004**. Carrageenan-induced mouse paw edema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression Br. J. Pharmacol. 142(2): 331–338.
[18]Zhang F, **1999** Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated human gastrointestinal epithel al cells. Carcinogenesis; 20(3):445-51
[19]Manchikanti, L., Fellows, B., Ailinani, H., Pampati, V. **2010** Therapeutic use, abuse, and nonmedical use of opioids: a ten-year perspective Pain Physician. 2010; 13(5):401-35.
[20]Woolfe, G. and MacDonald, A.D. **1944**. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J. Pharmacol. Exp. Ther., 80: 300-307.
[21]Bin Jantan, I., Ahmad, A.S., Ali, N.A.M., Ahmad, A.R. and Ibrahim, H. **1999**. Chemical composition of the rhizome oils of four Curcuma species from Malaysia. J. Essential Oil Res., 11: 719-723.
[22]Jantan, I., Rafi, I.A.A. and Jalil, J**. 2005**. Plateletactivating factor (PAF) receptor-binding antagonist activity of Malaysian medicinal plants. Phytomedicine, 12: 88-92.
[23]Hunskaar, S. and Hole, K. **1987**. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain, 30: 103-114.
[24]Jirovetz, L., Buchbauer, G., Puschmann, C., Shafi, M.P. and Nambiar, M.K.G. **2000**. Essentia l oil analysis of Curcuma aeruginosa Roxb. Leaves from South India. J. Essential Oil Res., 12: 47- 49
[25]Nandal, S., Dhir, A., Kuhad, A., Sharma. S., Chopra, K. **2009**. Curcumin potentiates the anti-inflammatory activity of cyclooxygenase inhibitors in the cotton pellet granuloma pouch model. Methods Find Exp. Clin. Pharmacol.31(2):89-93
[26]Xuan Dung, N., Thi Bich, N. and Leclercq, P.A. **1995**. Characterization of the leaf oil of Curcuma aeruginosa Roxb. from Vietnam. J. Essential Oil Res., 7: 657-659.