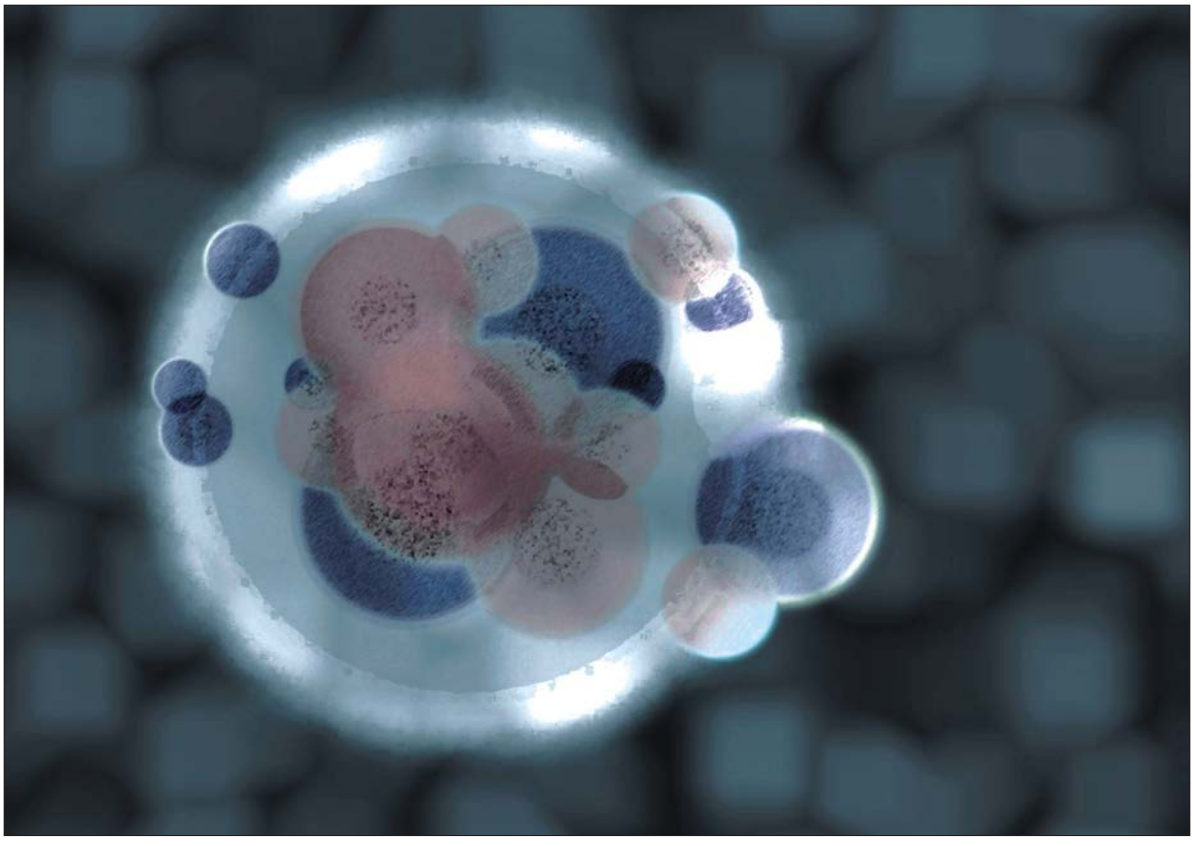

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Analgesic, Antioxidant and Anti-inflammatory Related Activities of 2^1-hydroxy-2,4^1-dimethoxychalcone and 4-hydroxychalcone in Mice

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Abstract: The present study was conducted to evaluate the analgesic, antioxidant and anti-inflammatory effects of two chalcones, 2^1-hydroxy-2,4^1-dimethoxychalcone [2-DMC] and 4-hydroxychalcone [4HC] synthesized in our laboratory. Antioxidant property of the two chalcones were compared with ascorbic acid and were evaluated in the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical assay. Results showed a potent analgesic effect of the two chalcones at 50, 100 and 200 mg kg^{-1} intraperitoneally. A significant inhibitions in acetic acid-induced abdominal contractions and significant percentage increase in pain threshold in hot-plate test were exhibited. However in flick test, highest analgesic activity was observed only with 4HC (200 mg kg^{-1}, i.p.) which exhibited 91.9% increase in pain threshold even more than the standard drug, acetylsalicylic acid that produced 85.0%. The % antioxidant activity (AA) of 2-DMC and 4HC ranges between 14.42- 48.99 and 17.09 -24.83%. AA of 2-DMC and 4HC were shown to be long and short acting respectively with the time period of 20 min. The anti-inflammatory effects of the two chalcones of both 2-DMC and 4HC produced% inhibitions between 27.0, 49.2, 78.9% and 30.2, 49.2, 77.8% respectively, while indomethacin gave 28.6% in oedema formation of mice right hind paw. In pulmonary oedema and leucocytes count from the mice pleurisy, only 200 mg kg^{-1} i.p of the chalcones produced significant anti-inflammatory effect. This study demonstrated that the inhibitory effects of 2-DMC and 4HC on various mediators responsible for pain and inflammation and indicated the effectiveness of the chalcones as potent analgesic, antioxidant and anti-inflammatory agents.

Key words: Chalcones, 2^1-hydroxy-2, 4^1-dimethoxychalcone, 4-hydroxychalcone, analgesic, antioxidant, anti-inflammatory activities

INTRODUCTION

Inflammation is the response to injury of cells and body tissues through different factors such as infections (malaria parasites, bacterial, viral and worms), chemicals (CCl_4, hormones, poisons, foods and drugs), thermal and mechanical injuries (Rang *et al*., 1998; Lian *et al*., 2004). These factors leading to inflammation has resulted into various diseases like asthma, rheumatoid arthritis, meningitis, malarial infection, diabetes, HIV/AIDS, cancers, Parkinson's disease and other neurodegenerative disorders which arose from neuroinflammation (Vadas *et al*., 1992; Cohn *et al*., 2001). Recently, it has been shown that generation of free radicals from body metabolic reactions could be a source of scavenging DNA and cell membrane and can cause disease conditions like cancer, inflammation and heart disease (Ames *et al*., 1993; Repetto and Llesuy, 2002).

In the recent years, flavonoids, chalcones and their derivatives have been detected from natural sources possessing anti-inflammatory property as found in active semi-synthetic derivative of hesperidin methylchalcones and O-rutosides (Alcaraz and Jimenez, 1988; Gabor, 1979). Chalcones are open analogues of flavonoids in which the two aromatic rings are joined by a three carbon, α,β-unsaturated carbonyl system. Fundamentally they can be considered to be derivatives of phenyl styryl ketone. Chalcones have been documented to possess quite a number of activities like: antimicrobial, antiviral, antioxidant, antiprotozoal, anticancer and gastroprotective effects, but the anti-inflammatory and analgesic properties are so scanty (Devia *et al*., 1998; Opletalová *et al*., 2000; Calliste *et al*., 2001; Liu *et al*., 2001; Machala *et al*., 2001). 2^1-hydroxy-2,4^1-dimethoxychalcone [2-DMC] and 4-hydroxychalcone [4-HC] were found to exhibit potent anti-trichomonal activity (Oyedapo *et al*., 2004) in our laboratory. As a continuation of our studies, we investigated the analgesic, antioxidant and anti-inflammatory properties of both 2-DMC and 4HC in various animal experimental models.

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MATERIALS AND METHODS

Animals: Swiss albino mice of either sex weighing 20-26 g were used. The animals were maintained under normal laboratory conditions of humidity, temperature (25+1 °C) and light (12 h day: 12 h night) and allowed free access to food and water *ad libitum* for at least 5 days, before the commencement of our experiments. The principle of laboratory animal care (NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised 1985).

Drugs: The following drugs were used: [Disprin® (acetylsalicyclic acid -ASA)]-(Reckiti Benckiser); Carrageenin (Sigma); and Indomethacin (KGN Pharmaceuticals). Synthesized Chalcones 2^1-hydroxy-2, 4^1-dimethoxychalcone [2-DMC], 4-hydroxychalcone [4-HC], Glacial acetic acid (Analar).

Synthesis of 1,3-diaryl-2-propen-1-ones: Using Claisen-Schmidt condensation method (Adewunmi *et al*., 1987), appropriate acetophenones were condensed with benzaldehydes to give 2^1-hydroxy-2,4^1-dimethoxychalcone [2-DMC], 4-hydroxychalcone [4-HC], in 65-81% yield.

Analgesic activity evaluation: Evaluation of the analgesic properties of 2^1-hydroxy-2,4^1-dimethoxychalcone [2-DMC] and 4-hydroxychalcone [4-HC],was carried out by using two different models of noxious stimuli; namely, chemical and thermal stimuli.

Acetic acid-induced writhing method: Control group of mice (n = 5) received normal saline (0.3 mL kg^{-1} i.p.). Mice in the test groups received chalcones [2-DMC] and [4-HC], (50, 100 and 200 mg kg^{-1} i.p) or acetylsalicyclic acid (ASA, 100 mg kg^{-1} i.p.), respectively. ASA was used as the reference analgesic drug for comparison in this study. One hour following chalcones, ASA- or normal saline administration, 0.1 mL of a 3% acetic acid solution was injected to each of the test mice intraperitoneally (Koster *et al*., 1959). The number of abdominal contractions that occurred within the next 20 min following acetic acid administration was counted and recorded. A significant reduction in the number of acetic acid-induced abdominal contractions of the treated mice, compared to the contractions in the untreated control mice, was taken as an indication of analgesic activity.

Hot Plate test method: Control group of mice (n = 5) received normal saline (0.3 mL kg^{-1} i.p.) only. The control mean reaction time (in seconds) was determined and recorded. The test group mice (5 mice per group) were treated with different doses of chalcones [2-DMC] and [4-HC], (50, 100 and 200 mg kg^{-1} i.p) or ASA (100 mg kg^{-1} i.p.) respectively. One hour following the tested agent or ASA-administration, the mice were separately placed in an hot plate (Thermajust, Model 475, TechniLab Instruments, N.J, 07440) maintained at 55±1 °C. For both the control and test animals, the reaction time (in sec) was taken as the time when each of the mice jumped out of the beaker on the hot plate. The test mean reaction time (in sec) was also determined for each compound dose and ASA.

Tail immersion test method: Control group of mice (n = 5) received normal saline (0.3 mL kg^{-1} i.p.) only and the mean reaction time (in sec) was determined. Test groups of mice (5 mice per compound- or reference drug-dose) were treated with chalcones [2-DMC] and [4-HC], (50, 100 and 200 mg kg^{-1} i.p) or ASA (100 mg kg^{-1} i.p.), respectively. One hour following the drug-or reference drug (ASA) administration, the tail (up to 5 cm) of each mouse was immersed in hot water maintained at 50±1 °C (in a 1 L water bath). For both the control and test animals, the reaction time (in sec) was taken as the time when the animals withdrew their tails completely from the hot water in the bath (Parimaladevi *et al*., 2003). The test mean reaction time (in sec) was calculated for each dose, ASA and the control.

Anti-inflammatory activity determination:

Acute inflammation: Carrageenin-induced paw oedema in mice was used as a model of acute inflammation. 0.1 mL of a 1% carrageenin solution was injected into the plantar surface of the right hind paws of the mice. Control group mice (n = 5) received normal saline (0.3 mL kg^{-1} i.p) treatment only, while animals in the test groups were treated with either chalcones [2-DMC] and [4-HC], (50, 100 and 200 mg kg^{-1} i.p) or IND (10 mg kg^{-1} i.p.) 1 h before carrageenin injection. Two hours after carrageenin injection, the mice were anaesthetized by dropping them in a jar containing cotton wool soaked with chloroform and both the right and left hind limbs were cut identically at the ankle joint and weighed. The differences in weight gave the amount of oedema developed in the right hind limbs (Subramoniam *et al*., 2001).

Carrageenin-induced pleurisy in mice: The method used in these experiments was modified from that described in detail by (Vinegar *et al*., 1982). Five groups of five mice each, were treated with chalcones [2-DMC] and [4-HC], (50, 100 and 200 mg kg^{-1} i.p), IND (10 mg kg^{-1} i.p.) and normal saline [control] (0.3 mL kg^{-1} i.p) respectively. One hour after treatment, all the animals received an

intrapleural injection of 0.1 mL carrageenin on the right side of the thorax. Two hours later, the mice were anaesthetized with chloroform and the pleural cavity was washed with.0.1 mL of distilled water. The number of leucocytes in the pleural cavity was determined and recorded.

Pulmonary oedema: The lungs of the animals sacrificed in (b) above were dissected free from the trachea and weighed. Significant changes in the test 'wet-lung weight' compared to the distilled water-treated controls, was considered to reflect pulmonary oedema (Staub, 1974). Pulmonary oedema was calculated from the formula:

$$\text{Pulmonaryoedema} = \frac{\text{Lungs wet weight}}{\text{Body weight}} \times \frac{10\,000}{1}$$

Antioxidant assay:

Free radical scavenging capacity: The free radical scavenging activity of each compound was analysed by the DPPH assay (Sanchez-Moreno *et al*., 1998). The test compounds, at concentrations ranging from 10 to 100 μg mL^{-1}, were mixed with 3 mL of 0.1 mmol DPPH/l (in ethanol) in a cuvette. The time-course of the change in absorbance at 517 nm was monitored over 20 min. The antioxidant activities of the extracts/compounds were evaluated by measuring the value of the absorbance at 517 nm when the reaction plateau step was reached. A minimum of three different concentrations for each compound/extract in triplicate analyses was tested. The percentage of DPPH remaining was calculated according to the equation:

$$\%\ DPPH_{REM} = [DPPH]_{(t)}/[DPPH]_{(o)} \times 100$$

where $[DPPH]_{(o)}$ is its remaining concentration of the stable radical without the antioxidant and $[DPPH]_{(t)}$ is its remaining concentration at the reaction plateau step.

Therefore, %Antioxidant Activity [AA] = 100-% $DPPH_{REM}$

RESULTS

The results of the analgesic, antioxidant and anti-inflammatory activities of 2^1-hydroxy-2, 4^1-dimethoxychalcone [2-DMC] and 4-hydroxychalcone [4-HC] are shown in Table 1, 2 and Fig. 1a-c.

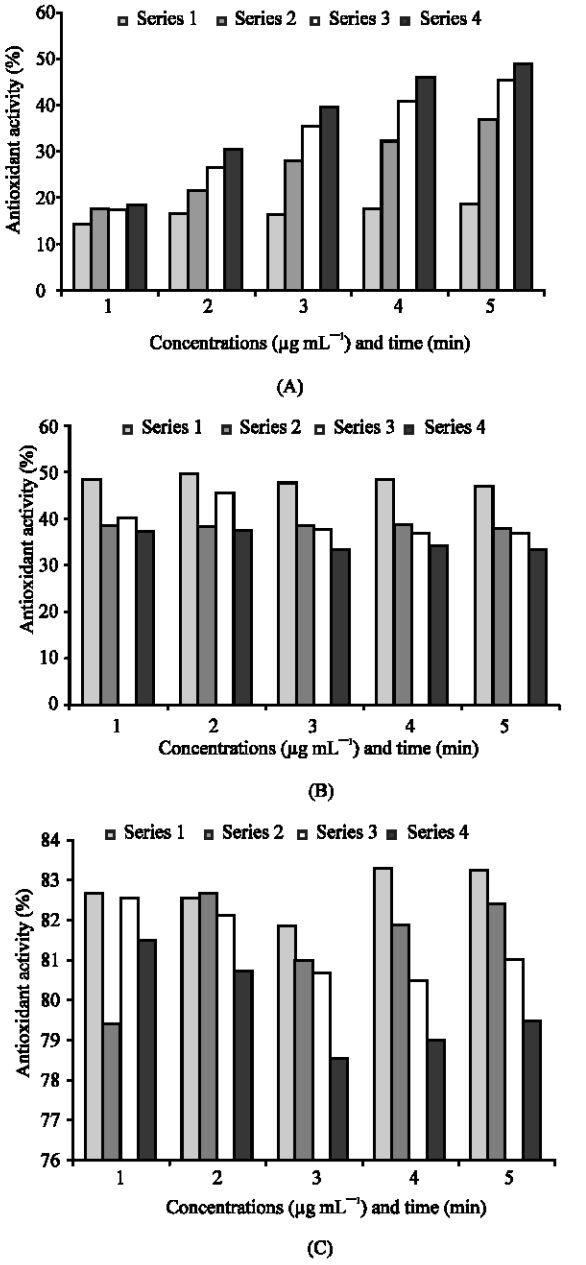

Fig. 1: The antioxidant effect of 2-DMC, 4-HC and ascorbic acid against concentration and time. Serials 1, 2, 3 and 4 represent 10, 25, 50 and 100 μg mL^{-1} of test agents. Numbers 1-5 represent timing T_0, T_1, T_5, T_{10} and T_{20} for 0, 1, 5, 10 and 20 min of agent incubation

Analgesic effect: Analgesic effects of the two chalcones were evident in all the three models used but in various degrees. In the flick test, 4-hydroxychalcone [4-HC] was significant at 200 mg kg^{-1}, while 2^1-hydroxy-2, 4^1-dimethoxychalcone [2-DMC] showed significant activity at 100 and 200 mg kg^{-1} (Table 1). In both hot plate and acetic-acid induced writhing models, the two chalcones exhibited reduction in pain significantly in an eqi-potent manner. The activities were however less when compared to acetylsalicylic acid as the standard drug.

Table 1: Analgesic activity of 4–hydroxychalcone, [4-HC] 2^1-hydroxy-2,4^1-dimethoxychalcone [2-DMC] and Acetylsalicylic acid (ASA)

Treatments (mg kg^{-1})	Flick test (sec)	Increase in pain threshold %	Hot-plate test (sec)	Increase in pain threshold %	Acetic-acid induced writhings (No/20 mins)	Inhibition (%)
Control (0.3 mL normal saline)	5.4±0.7	-	7.6±0.7	-	62.0±1.6	-
4-hydroxychalcone, [4-HC]						
50	3.2±0.4	-	10.9±0.95*	30.3	13.6±2 1**	78.1
100	4.0±0.5	-	11.6±0.8**	34.2	10.0±1.8**	83.9
200	67.2±2.0**	91.9	16.7±0.9**	54.4	1.6±0.1**	97.4
2^1-hydroxy-2,4^1-dimethoxychalcone [2-DMC]						
50	6.8±0.8	20.6	10.96±0.7*	30.7	13.8±1.7**	77.7
100	9.2±0.7**	41.3	16.5±0.8**	53.9	11.4±1.0**	81.6
200	9.8±0.6**	45.0	21.9±1.1**	65.3	9.0±1.3**	85.5
Acetylsalicylic acid (ASA)						
100	36.0±1.9 **	85.0	15.9±1.2**	52.1	3.4±0.8**	94.5

Values are Means±±SEM of 5 mice. Significant-t-test level *p<0.05, **p<0.01

Table 2: Anti-inflammatory activity of 4-hydroxychalcone, [4-HC] 2^1-hydroxy-2,4^1-dimethoxychalcone [2-DMC] and Indomethacin (IND) on oedema formation leucocyte counts and pulmonary oedema

Treatments (mg kg^{-1})	Mice right hind paw (Oedema formation)	Inhibition (%)	Pulmonary oedema	Leucocyte counts
Control (0.3 mL normal saline)	0.063±0.013	-	79.3±2.1	92.6±10.1
4–hydroxychalcone, [4-HC				
50	0.044±0.004	30.2	129.5±3.9	121.4±9.4
100	0.032±0.003*	49.2	84.1±1.7	83.5±2.1
200	0.014±0.005**	77.8	48.4±1.8**	66.3±1.2*
2^1-hydroxy-2,4^1-dimethoxychalcone [2-DMC]				
50	0.046±0.008	27.0	105.6±2.1	226.2±13 8
100	0.032±0.005*	49.2	109.0±2.1	98.6±2 8
200	0.013±0.004**	78.9	64.3±1.7**	61.8±3.3**
Indomethacin				
10	0.045±0.001	28 6	73.1±3.5	39.8±3 8**

Values are Means±SEM of 5 mice. Significant-t-test level *p<0.05, **p<0.01

Antioxidant activity: Antioxidant property of the two chalcones were compared to that of ascorbic acid. From Fig. 1a and b; it is observed that 2-DMC is more potent than 4-HC. The % antioxidant activity of 2-DMC ranges between 14.42-48.99, while that of 4-HC were observed between 17.09-24.83. The antioxidant activity of 2-DMC increases with time, while that of 4-HC decreases with the period of 20 minutes. However, ascorbic acid gave very high activity that ranges between 78.98- 83.33 within the same time period (Fig. 1c).

Anti-inflammatory activity: Using three different models of determining the anti-inflammatory property of chalcones, the activity shown in both mice right hind paw and pulmonary oedema formation were better than the standard drug indomethacin (Table 2). Effects were also demonstrated against the leucocytes, but the activity was less compared to that of indomethacin. In oedema formation of mice right hind paw, both chalcones were eqi-potent, by demonstrating between 27.0-78.9% inhibition of oedema formation, while indomethacin gave 28.6%. In pulmonary oedema and leucocyte counts, only 200 mg kg^{-1} of the two chalcones exhibited significant effects where anti-inflammatory property was well demonstrated.

DISCUSSION

Great interest has been shown in natural products as anti-inflammatory agents by offering some advantages against classical or synthetic anti-inflammatory drugs. Non-steroidal anti-inflammatory drugs (NSAID) exhibit side effects such as ulcerogenicity, bleeding and other GIT disorders which could be eliminated by finding new agents or establishing the structural activity relationship as the basis to obtain compounds with greater activity.

Chalcones can be obtained from natural plant sources and synthesized in the laboratory. Chalcones have been reported to possess a variety of biological properties, including anti-inflammatory, analgesic, antioxidant, antibacterial, antifungal and antiprotozoal activities (Haraguchi *et al*., 1998; Hsieh *et al*., 1998). They are also reported to be gastric protectant, antimutagenic and antitumorogenic (Makita *et al*., 1996). Various 2'-substituted chalcones have been shown to possess anti-inflammatory and antioxidant properties (Yu *et al*., 1995). For example, 2',5'-dihydroxychalcone prevents platelet aggregation (Lin *et al*., 1997) and 2',3-dihydroxychalcone and 2',5'-dihydroxychalcone inhibit polymixin B-induced hind-paw oedema (Hsieh *et al*., 1998). Butein (3,4,2',4'-tetrahydroxychalcone) prevents antiglomerular basement membrane antibody-associated glomerulonephritis in rats (Hayashi *et al*., 1996). 2'-Substituted chalcones have also been shown to inhibit production of IL-1from monocytes stimulated with LPS and also prevent LPS-induced septic shock in mice) (Batt *et al*., 1993). LPS-induced septic shock involves excessive infiltration of neutrophils into the liver because of uncontrolled up-regulation of ICAM-1 expression in the liver (Xu *et al*., 1994). As a result, ICAM-1 deficient mice are protected against septic shock (Xu *et al*., 1994) and inhibitors of ICAM-1 prevent

lethality induced by septic shock in mice. Recently, 2'-hydroxychalcone has been shown to be a potent antioxidant by inhibiting lipid peroxidation and is antitumorogenic (Anto *et al*., 1995). Having the hydroxyl group at the *ortho*-position on the benzene ring of chalcone increases its antioxidant property compared with other substituted chalcones (Anto *et al*., 1995). The antioxidant activity of 4-HC was earlier reported by (Calliste *et al*., 2001). To our knowledge, the analgesic and anti-inflammatory related activities of these compounds was not reported. Because 2'-hydroxychalcone has been found to be pharmacologically important, we are therefore interested in the action of 2^1-hydroxy-$2,4^1$-dimethoxychalcone [2-DMC] and 4-hydroxychalcone [4-HC] on pain, oxygen radical production and inflammatory reactions.

2-DMC and 4-HC demonstrated potent and significant analgesic, antioxidant and Anti-inflammatory effects in this work. In the acetic acid-induced writhing, 2-DMC and 4-HC produced 77.7, 81.6, 85.5% and 78.1, 83.9, 97.4% inhibitions respectively. Using the hot-plate model, 2-DMC and 4-HC produced percentage increase in pain threshold as 30.7, 53.9, 65.3% and 30.3, 34.2, 54.4% respectively. In flick test, the same trend of analgesic activity was observed. However administration of 4-HC (200 mg kg^{-1}, i.p.) exhibited 97.4% inhibition of abdominal contraction, 54.4, 91.9% increase in pain threshold even more than the standard drug acetylsalicylic acid that produced 94.5% inhibition of abdominal contraction, 52.1, 85.0% increase in pain threshold, in acetic- acid contraction, hot-plate and flick tests respectively. There is tendency to suggest here that these two chalcones can suppress acute pains which could be mediated peripherally and centrally. Antioxidant property of the two chalcones were compared with ascorbic acid and were evaluated in the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical assay. The % antioxidant activity (AA) of 2-DMC and 4-HC ranges between 14.42- 48.99 and 17.09-24.83 respectively. The % AA of 2-DMC increases with time while that of 4-HC decreases with the time period of 20 min (Fig.1a and b). The anti-inflammatory effects of the two chalcones were carried out using three different models. In oedema formation of mice right hind paw, both 2-DMC and 4-HC produced 27.0, 49.2, 78.9% and 30.2, 49.2, 77.8% inhibitions, respectively, while indomethacin gave 28.6%. In pulmonary oedema and leucocytes count from the mice pleurisy, only 200 mg kg^{-1} i.p of 2-DMC and 4-HC produced significant anti-inflammatory effect.

The main pathological feature of chronic inflammatory diseases including asthma, rheumatoid arthritis is the infiltration of leucocytes to the affected area. These leucocytes release cell adhesion molecules (CAM) like intracellular CAM-1 and vascular CAM-1 that are induced by various inflammatory cytokines (TNF-α, IL-1, LPS) (Madan *et al*., 2000). Inhibition of leucocytes migration and ICAM-1 and VCAM-1 expression has been shown to produce therapeutic efficacy in various animal models of inflammation (Oguchi *et al*., 2000). Some natural products have been reported as potent inhibitors of leucocytes, ICAM-1 and VCAM-1. A common structural features of these natural products (e.g., chalcones) is an α, β-unsaturated carbonyl with substitutions in various positions on ring A and B. It could therefore be inferred that the two chalcones could produced their anti-inflammatory effects by inhibiting leucocytes migration and prevent further inflammatory reactions as observed in this study. It is also worthwhile to note that the anti-inflammatory action of the chalcones used in this study are more or greater on oedema formation in mice right hind paw and lungs than that observed with the standard drug indomethacin. However, the reverse is the case with the leucocyte count model. It could then be postulated that apart from inhibiting leucocytes migration, there are other mechanisms. Mechanism of anti-inflammatory activity of these agents using these models might not be the same. There is a need of further study on the multi-facial mechanism of action of these two chalcones.

REFERENCES

Adewunmi, C.O., F.O. Ogungbamila and J.O. Oluwadiya, 1987. Molluscicidal activities of some synthetic chalcones, Planta Med., 53: 110-112.

Alcaraz, M.J. and M.J. Jimenez, 1988. Flavonoids as anti-inflammatory agents. Fitoterapia LIX: 25-38.

Ames, B.N., M.K. Shigenaga and T.M. Hagen, 1993. Oxidants, antioxidants and the degenerative diseases of aging Proceedings of Nat. Acad. Sci., 90: 7915-7922.

Anto, R.J., K. Sukumaran, G. Kuttan, M.N.A. Rao, V. Subbaraju and R. Kuttan, 1995 Anticancer and antioxidant activity of synthetic chalcones and related compounds. Cancer Lett., 97: 33-37.

Batt, D.G., R. Goodman, D.G. Jones, J.S. Kerr, L.R. Mantegna, C. Macallister, R.C. Newton, S. Nurnberg, P.K .Welch and M.B. Covington, 1993. 2'-Substituted chalcone derivatives inhibitor of Interleukin-1 biosynthesis. J. Med. Chem., 36: 1434-1442.

Calliste, C.A, J.C. Le Bail, P. Trouillas, C. Pouget, G. Habrioux, A.J. Chulia and J.L. Duroux, 2001. Chalcones: structural requirements for antioxidant, estrogenic and antiproliferative activities. Anticancer Res., 21: 3949-3956.

Cohn, M.A., S.S. Frankel, S. Rugpao, M.A. Young and G. Willet *et al*., 2001. Chronic inflammation with increased Human Immunodeficiency Virus (HIV) RNA expression in the vaginal epithelium of HIV-infected Thai women. J. Infect. Dis., 184: 410-417.

Devia, C.M., N.B. Pappano and N.B. Debattista, 1998. Structure-Biological Acitivity Relationship of synthetic trihydroxilated Chalcones. Rev. Microbiolo., 29: 307-310.

Gabor, M., 1979. In: Handbook of Experimental Pharmacology: Anti-inflammatory Drugs. Vane J.R. and S.H. Ferreira (Eds.), Springer, New York, pp: 698-710.

Haraguchi, H., H. Ishikawa, K. Mizutani, Y. Tamura and T. Kinoshita, 1998. Antioxidative and superoxide scavenging activities of retrochalcones in *Glycyrrhiza inflata*. Bioorg. Med. Chem., 6: 339-347.

Hayashi, K., T. Nagamatsu, S. Honda and Y. Suzuki, 1996. Butein (3,4,2',4'-tetrahydroxychalcone) ameliorates experimental anti-glomerular basement membrane antibody-associated glomerulonephritis. Eur. J. Pharmacol., 316: 297-306.

Hsieh, H.K, T.H. Lee, J.P. Wang, J.I. Wang and C.N. Lin, 1998. Synthesis and anti-inflammatory effect of chalcones and related compounds. Pharm. Res., 15: 39-46.

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

Lian, X., C. Yan, L. Yang, Y. Xu and H. Du, 2004. Lysosomal acid lipase deficiency causes respiratory inflammation and destruction in the lung. Am. J. Physiol. Lung Cell. Mol. Physiol., 286: L801-L807.

Lin, C.N., T.H. Lee, M.F. Hsu, J.P. Wang, F.N. Ko and C.M. Teng, 1997. 2',5'-dihydroxychalcone as a potent chemical mediator and cyclooxygenase inhibitor. J. Pharm. Pharmacol., 49: 530-536.

Liu, M., P. Wilairat and M.L. Go, 2001. Antimalarial alkoxylated and hydroxylated chalcones [corrected]: Structure-activity relationship analysis. J. Med. Chem., 44: 4443-4452.

Machala, M., R. Kubinova, P. Horavova and V. Suchy, 2001. Chemoprotective potentials of homoisoflavonoids and chalcones of *Dracaena cinnabari*: Modulations of drug-metabolizing enzymes and antioxidant activity. Phytother. Res., 15: 114-118.

Madan, B., S. Batra and B. Ghosh, 2000. 2'-Hydroxychalcone inhibits nuclear factor-KB and blocks tumor necrosis factor-α- and lipopolysaccharide-induced adhesion of neutrophils to human umbilical vein endothelial cells. Mol. Pharmacol., 58: 526-534.

Makita, H., T. Tanaka, H. Fujitsuka, N. Tatematsu, K. Satoh, A. Hara and H. Mori, 1996. Chemoprevention of 4-nitroquinoline 1-oxide-induced rat oral carcinogenesis by the dietary flavonoids chalcone, 2-hydroxychalcone and quercetin. Cancer Res., 56: 4904-4909.

Oguchi, S., P. Dimayuga, J. Zhu, K-Y. Chyu, J. Yano, P.K. Shah, J. Nilsson and B. Cercek, 2000. Monoclonal antibody against vascular cell adhesion molecule-1 inhibits neointimal formation after periadventitial carotid artery injury in genetically hypercholesterolemic Mice Arterioscler. Thromb. Vasc. Biol., 20: 1729-1736.

Opletalová, V., P. Øièièáøová, D. Šedivý, D. Meltrová and J. Køiváková, 2000. Chalcones and their Heterocyclic Analogues as Potential Medicaments Folia Pharmaceutica Universitatis Carolinae., 25: 21-33.

Oyedapo, O.A., C.O. Adewunmi, V.O. Makanju, T.K. Adenowo and E.O. Iwalewa, 2004. Anti-trichomonal activities of synthetic 1,3-diaryl-2-propen-1-ones on *Trichomonas gallinae*. Afr. J. Trad. Compl. Alter. Med., 1: 55-62.

Parimaladevi, B., R. Boominathan and S.C. Mandal, 2003. Studies on analgesic activity of *Cleome viscosa* in mice. Fitoterapia, 74: 262-266.3.

Rang, H.P., M.M. Dale and J.M. Ritter, 1998. Pharmacology 3rd Edn., Churchill Livingstone London, pp: 214-245.

Repetto, M.G. and S.F. Llesuy, 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz. J. Med. Biol. Res., 35: 523-534.

Sanchez-Moreno, C., J.A. Larrauri and F. Saura-Calixto, 1998. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric., 76: 270-276.

Staub, N.C., 1974. Pulmonary oedema. Physiol. Rev., 54: 698-811.

Subramoniam, A., D.A. Evans, S. Rajasekharan and G. Sreekandan-Nair, 2001. Effect of *Hemigraphis colorata* (Blume) leaf on wound healing and inflammation in mice. Ind. J. Pharmacol., 33: 283-285.

Vadas. P., J. Keystone, E. Stefanski, K. Scott and W. Pruzanski, 1992. Induction of circulating group II phospholipase. A2 expression in adults with malaria Infect. Immunol., 60: 3928-3931.

Vinegar, R., J.F. Truax, J.L. Selph and F.A. Voelker, 1982. Pathway of onset, development and decay of carrageenan pleurisy in the Rat. Fed. Pro., 41: 2588-2595.

Xu, H., J.A. Gonzalo, Y.S. Pierre, I.R. Williams T.S. Kupper, R.S. Cotran, T.A. Springer, J. Carlos and G. Ramos, 1994 Leukocytosis and resistance to septic shock in intercellular adhesion molecule1-defecient mice. J. Exp. Med., 180: 95-101.

Yu, S.M., Z.J. Cheng and S.C. Kuo, 1995. Endothelium-dependent relaxation of rat aorta by butein, a novel cyclic AMP-specific phosphodiesterase inhibitor. Eur. J. Pharmacol., 280: 69-77.