WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 1, 121-140. Research Article ISSN 2277– 7105

REVISITING CARDIOPROTECTIVE ROLE OF CURCUMINOIDS: A COMPARISON OF CARDIAC OUTPUT, POSITIVE INOTROPIC, AND NEGATIVE CHRONOTROPIC EFFECTS IN ISOLATED PERFUSED FROG HEART PREPARATION

Vasudeva Rao Avupati[1]*, Nalini Govindasamy[2,3], Yuawa Rani[2], M. Mohamed Shabi[4], Ashwinder Singh[2] and Prasad Cheepurupalli[5]

[1]Department of Pharmaceutical Chemistry, School of Pharmacy, International Medical University, 18 Jalan Jalil Perkasa, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Asia Metropolitan University, Selangor Darul Ehsan, 43200, Malaysia.

[3]Faculty of Science, Technology and Engineering, La Trobe University, Bendigo, Australia.

[4]Department of Pharmaceutical Biology, Faculty of Pharmaceutical Sciences, UCSI University College, No. 1 Jalan Menara Gading, Taman Connaught, Cheras, 56000 Kuala Lumpur, Malaysia.

[5]Pharmaceutical Chemistry Division, Vignan Institute of Pharmaceutical Technology, Beside SEZ, Duvvada, Visakhapatnam, Andhra Pradesh, Pincode-5530049, India.

Article Received on
27 October 2018,

Revised on 17 Nov. 2018,
Accepted on 07 Dec. 2018

DOI: 10.20959/wjpr20191-13797

***Corresponding Author**
Dr. Vasudeva Rao Avupati
Department of Pharmaceutical Chemistry, School of Pharmacy, International Medical University, 18 Jalan Jalil Perkasa, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.

ABSTRACT

Curcuminoids, a curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) were isolated from *Curcuma longa* L. (*C. longa* L) by column chromatography and evaluated for relative cardioprotective potential in isolated frog heart preparation. Although DMC and BDMC are also principal curcuminoids but most of the studies reported on the cardioprotective role of *C. longa* L include C as effective and bioactive curcuminoid, thus DMC and BDMC were less explored. Based on our study, the results suggest that curcuminoids (C, DMC and BDMC) could exhibit cardioprotective activity as evidenced by improved hemodynamic variables such as cardiac output, positive inotropic and negative chronotropic effects. Our results demonstrated that the C has more significant effect on cardiac output, DMC exhibited enhanced negative chronotropic effect (increased heart rate),

and BDMC displayed intensified positive ionotropic effect (force of contraction) respectively, which were independently cardioprotective and revealed comparable potency with each other.

KEYWORDS: Curcumin, demethoxycurcumin, bisdemethoxycurcumin, positive inotropic, negative chronotropic, cardiac output, cardioprotection.

INTRODUCTION

Cardiovascular diseases have become a leading cause of death worldwide. World Health Organisation (WHO) estimated that it will reach up to 20 million in 2020.(Feigin *et al.*, 2017) Although substantial developments have been succeeded in treating vascular disorders such as thrombosis, (Marine *et al.*, 2017) atherosclerosis,(Dou *et al.*, 2017) cardiac arrhythmia, (Haron-Khun *et al.*, 2017) congenital heart disease (Larsen *et al.*, 2017) and heart failure, (Troughton *et al.*, 2000) the existing allopathic cardioprotective drugs have been reported massive side effects but are also very expensive.(Koleva *et al.*, 1988) In the recent past few decades, medicinal plants have investigated as potential alternative cardioprotective therapeutics due to their easy obtainability, relatively less side effects, and cost effective.(Calvo *et al.*, 2014).

Turmeric, the dried and powdered rhizomes of *Curcuma longa* L. (*C. longa* L), is a medicinal plant belonging to Zingiberaceae family, widely cultivated in tropical regions of Asia.(Nelson *et al.*, 2017) Curcuminoids, the natural polyphenolic secondary metabolites in turmeric, (Bahramsoltani *et al.*, 2017) mainly composed of curcumin and together with a small amount of demethoxycurcumin and bisdemethoxycurcumin responsible for the yellow color of the turmeric.(Yadav *et al.*, 2017) They have been used to treat a variety of diseases in traditional Indian Ayurvedic, Chinese and Indonesian medicinal practice.(Kocaadam *et al.*, 2017) The concept of using curcuminoids, that is, the mixture, rather than a single ingredient has accompanied in a new revolution in phytomedicine.(Ahmad *et al.*, 2017, Ahmad *et al.*, 2014 and Gaffey *et al.*, 2017) Recently, several studies have indicated that, compared to curcumin, demethoxycurcumin and bisdemethoxycurcumin have similar or higher biological activities in many cases,(Amalraj *et al.*, 2017) such as antimetastasis, (Yodkeeree *et al.*, 2009) anti-inflammatory, (Lukita-Atmadja *et al.*, 2002) antiprotozoal, (Rasmussen *et al.*, 2000) and protecting PC12 cells against 1-methyl-4-phenylpyridinium ion-induced apoptosis by bcl-2-mitochondria-ROS-iNOS pathway.(Kim *et al.*, 2001) Therefore, investigations of the total three major curcuminoids are more considerable. However, curcuminoids have gained

importance because of their minimal side effects, low cost, and abundance. Further, curcuminoids has also been demonstrated to exhibit biological activities such as antimicrobial, (De *et al.*, 2009) anticancer, (Aggarwal *et al.*, 2003) antiviral, (Zandi *et al.*, 2010) antimitotic, (John *et al.*, 2002) antitumor, (Ruby *et al.*, 1995) diuretic, (Elgazar *et al.*, 2013) antihypertensive,(Rachmawati *et al.*, 2016) antidiabetic, (Chuengsamarn *et al.*, 2012) hypoglycemic, (Nishiyama *et al.*, 2005) hepatoprotective, (García-Niño *et al.*, 2014) antioxidant, (Jitoe *et al.*, 1992) anti-inflammatory,(Chainani-Wu *et al.*, 2003) analgesic,(Zhao *et al.*, 2012) immunomodulatory, (Jagetia *et al.*, 2007) antiulcer,(Tuorkey *et al.*, 2009) gastroprotective, (Yadav *et al.*, 2013) antibacterial, (Rai *et al.*, 2008) antifungal,(Martins *et al.*, 2008) antidepressant, (Kulkarni *et al.*, 2008) anticonvulsant, (Bharal *et al.*, 2008) antiprotozoal, (Rasmussen *et al.*, 2008) anthelmintic, (Bazh *et al.*, 2013) antimalarial, (Reddy *et al.*, 2005) antibiotic, (Wang *et al.*, 2009) antiretroviral, (Riva *et al.*, 2008) antineoplastic, (Lin *et al.*, 2001) adrenergic agonist,(Dewar *et al.*, 2011) cholinergic agonist,(Cheng *et al.*, 2010) antiarrhythmic, (Dikshit *et al.*, 1995) antihyperlipidemic,(Babu *et al.*, 1997) anticoagulant,(Pan *et al.*, 2006) antiasthmatic,(Moon *et al.*, 2008) wound healing, (Krausz *et al.*, 2015) free radical scavenging, (Rao *et al.*, 1997) inhibitors of lipid peroxidation, (Rao *et al.*, 1994) nematocidal, (Kiuchi *et al.*, 1993) anti-alzheimer's, (Zhang *et al.*, 2006) cytotoxic, (Syu *et al.*, 1998) antimutagenic, (Anto *et al.*, 1996) antiarthritic, (Funk *et al.*, 2006) α-glucosidase inhibitor,(Du *et al.*, 2006) androgen receptor antagonist (Ohtsu *et al.*, 2002) and cardioprotective. (Ali *et al.*, 2009) The major problem associated with curcumin is its poor bioavailability due to its rapid metabolism in the liver and intestinal wall.(Purpura *et al.*, 2017) In an interesting study, it has been noted that DMC and BDMC are the natural stabilizers of C.(Zhongfa *et al.*, 2012) C has been reported to exhibit cardioprotective acitivity, (Wongcharoen *et al.*, 2012) but the other curcuminoids, DMC and BDMC have not been investigated whether they exhibit cardioprotective activity to the same extent as curcumin. However, there have been no reports regarding the relative cardioprotective role of each of the curcuminoids in the isolated frog heart preparation. Hence, in this context, we comparatively examined the cardoprotective effects of curcuminoids on isolated perfused functional frog heart preparation.

1. MATERIALS AND METHODS

1.1. Reagents and instruments

All the chemicals and solvents used for the extraction were of AR-grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA), Malaysia. Separation of curcuminoids was

carried out on column chromatography using Silica gel (particle size 100-200 m), thin-layer chromatography (TLC) plates (20×20 cm, Merck-60 F254, 0.25 mm thick) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Malaysia. Evaporation under reduced pressure was carried out using Büchi® rotary evaporator Model R-200. Curcumin standard was purchased from HiMedia Laboratories Pvt.Ltd. (RM1449, C.I.No. 75300) containing C, DMC, and BDMC as reference. Spectra were recorded using Lambda 250UV/Vis double beam spectrophotometer, Fourier-Transform Infrared Spectrophotometer (FTIR, Shimadzu, IRTracer-100), NMReady-60PRO (Nanalysis Ready Pro60), Flexar™ SQ 300 MS Single-Quad LC/MS System PerkinElmer Inc. Bioassay was carried out using PowerLab® (Lab Tutor®, ADInstruments, Malaysia).

1.2. Plant material

The rhizomes of *C. longa* L were purchased from local market in January 2015 at Cheras, State of Selangor, Malaysia. Plant sample was identified botanically by Dr. Shamsul Khamis, Senior Science Officer, Biodiversity Unit, UPM (University Putra Malaysia). A voucher specimen (Ref: UPM/IBS/UB/H56-15) was deposited at the Herbarium of UPM, 43400 Serdang, Selangor, Malaysia.

1.3. Extraction, isolation and purification

The rhizomes of *C. Longa* L (0.7 kg) were dried at 35 ^{o}C and ground into a powder (0.5 kg), liquid-solid type solvent extraction (percolation) was carried out with acetone for every 24 hrs. (Pothitirat et al., 2004, Lee *et al.,* 2012) The extract filtered thorugh a Buchner funnel using Whatmann filter paper No. 1, concentrated using a rotary evaporator under reduced pressure, and subsequnetly the dark residue obtained was subjected to a silica-gel column (100-200 mesh, column size 20 cm), and eluted successively with different ratios of hexane and ethylacetate with increasing order of polarity index to isolate (elute) curucminoids as fractions and also to obtain pure single spots on TLC. (Paulucci *et al.,* 2013) The isolated fractions were then loaded on an analytical TLC plate (20×20 cm, Merck-60 F254, 0.25 mm thick) using dichloromethane–methanol (99.7:0.3, v/v) as the mobile phase solvent and visualised under UV chamber with curcumin standard from HiMedia Laboratories Pvt.Ltd. (RM1449, C.I.No. 75300) containing C, DMC, and BDMC as reference.(Li *et al.,* 2014) The purified individual curcuminoids (C, DMC, and BDMC) were then identified and characterized based on melting point, UV, IR, ^{1}H-NMR and Mass spectral data.(Su *et al.,* 1982) Hence, curcuminoids dissolved in 2% dimethylsufoxide (DMSO) and tested for

biological activity. Schematic represenation of the complete study protocol has shown in Fig.1.

1.4. Isolated frog heart preparation

The study protocol has been approved by the Asia Metropolitan University's Animal Ethics Committee (AEC) with proceeding number AMU/AEC/FOP/2014/43. Frogs (*Rana pipense*) of either sex weighing 100-120 g were pithed to spinal cord, to the level of third vertebra. The heart was quickly exposed, and inferior vena cava was cleaned and 'V' shaped cut was made in inferior vena cava near heart and Syme's cannula was inserted into it. The heart along with Syme's cannula was isolated from the body and fixed on a heart perfusion apparatus. The Syme's cannula was connected to the reservoir containing frog's Ringer solution (pH 7.4) which consisted the composition of frog ringer in mM was Na^{+}, 110.7; Cl^{-}, 114.2; K^{+}, 1.2; Ca^{++}, 1.10; HCO_3^{-}, 2.8; $H_2PO_4^{-}$, 0.1 and glucose, 11.1 respectively and continuously bubbled with air, at room temperature. The flow rate of frog's Ringer solution was kept at 2-5 mL/min by means of screw clip for about 15 min prior to administration of any dose. The heart was stabilized for fifteen minutes prior to the administration of curcuminoids (C, DMC and BDMC). Each of the curcuminoids (C, DMC and BDMC) were prepared in different doses such as 50, 100, 150, 200, 250 mg mL-1 respectively. The responses to curcuminoids (C, DMC and BDMC) were recorded on a PowerLab® (LabTutor®, ADInstruments, Malaysia) by attaching one end of thread to the apex of the heart by means of a pin clip and the other end of the thread to a force transducer (Model: MLT004/ST) to measure the PowerLab® unit's output includes amplitude of muscle contractions and action potentials. The study was conducted in three different groups of frog's hearts.(Zimmer, 2000) Control group hearts perfused with frog's Ringer (Group I), vehicle treated group perfused with 2% DMSO (Group II), frog's hearts perfused with C, DMC and BDMC at test concentrations 50, 100, 150, 200, 250 µg/mL (Group III). Similar conditions were maintained in all the experiments to relatively compare the activity of different doses of curcuminoids (C, DMC and BDMC) (Fig.1).

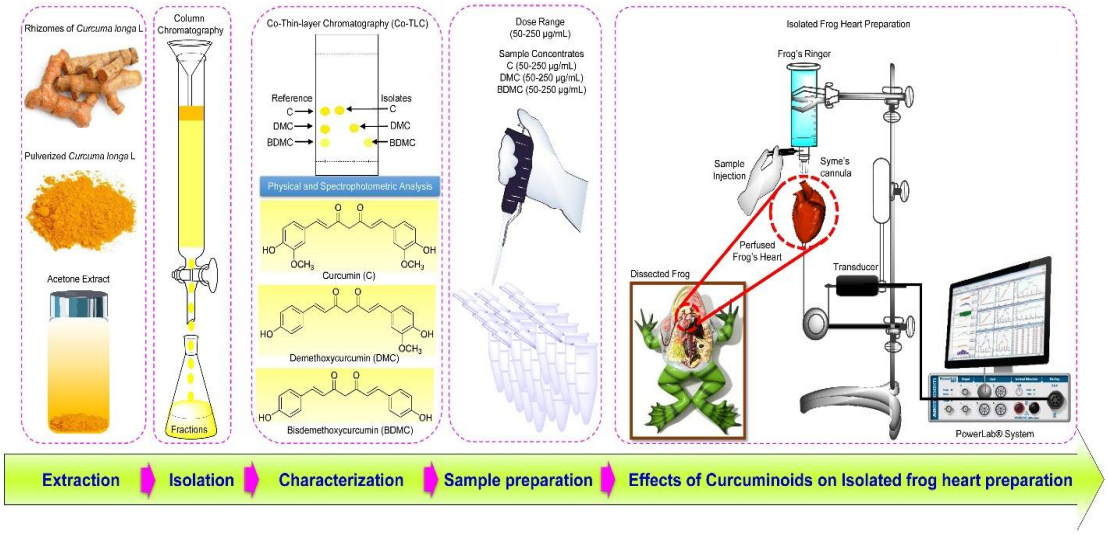

Fig. 1: Schematic representation of the comprehensive study protocol.

1.5. Statistical analysis

Statistical analysis has been performed using statistical software GraphPad Prism v 5.0. All experiments were performed in triplicates (n=3) and the numerical results were obtained as mean ± SEM. Group differences were determined using ANOVA (one-way analysis of variance); a statistical value of $P<0.05$ was taken as significant.

2. RESULTS AND DISCUSSION

2.1. Isolation and identification of curcuminoids (C, DMC and BDMC)

The rhizomes powder of *C. Longa* L was extracted sequentially with acetone, and subjected to normal phase gravity column chromatography using silica gel to seperate C, DMC and BDMC fractions, and they were characterized based on melting point, UV, IR, ^{1}H-NMR and Mass spectral data, eventually spectral data of all the isolates C, DMC and BDMC were keeping with the excepted chemical structures.(Rohman, 2012) The spectral data (data not shown) were in good agreeent with that of literature reports, the isolates were further confirmed as C, DMC and BDMC respectively. The melting points of C, DMC and BDMC were recorded as 183-184, 172-175 and 222-224 respectively. Purity of the isolated C, DMC and BDMC were confirmed by co-TLC using dichloromethane–methanol (99.7:0.3, v/v) as mobile phase, single spots with R_f values 0.7, 0.5, 0.1 were detected under UV inspection cabinet (Long wavelength), the UV absorption spectra were ddetermined in methanol, for C, DMC and BDMC respectively.

2.2. Effects of curcuminoids (C, DMC and BDMC) on isolated frog heart preparation.

From the results of curcuminoids treatment (Table 1), possible general statements were outlined; the order of positive ionotropic potential of curcuminoids resides as BDMC > C > DMC respectively. Similarly, the order of negative chronotropic potential of curcuminoids resides as DMC > C ≥ BDMC respectively. Further, the order of potential of curcuminoids on cardiac output of isolated frog heart resides as C > DMC > BDMC respectively.

Table 1: Effects of curcuminoids (C, DMC and BDMC) on isolated frog heart preparation.

Group (n=3)	Curcuminoid/ Concentration (µg/mL)	Force of contraction (N)[d]	Heart rate (BPM)[d]	Cardiac output (mL/min)[d]
Group 1 (Control)[a]	C	0.61 ± 0.20	116.03 ± 3.56	30.33 ± 3.60
	DMC	0.67 ± 0.09	111.73 ± 8.58	30.17 ± 1.90
	BDMC	0.92 ± 0.03	150.03 ± 3.24	31.50 ± 1.08
Group 2 (Vehicle control)[b]	C	0.61 ± 0.20	116.23 ± 3.56	31.00 ± 3.60
	DMC	0.67 ± 0.09	111.50 ± 8.58	29.17 ± 1.90
	BDMC	0.91 ± 0.03	150.07 ± 3.24	31.00 ± 1.08
Group 3 (Treatment)[c]	C (50)	0.83 ± 0.20	18.30 ± 3.56	24.17 ± 3.60
	C (100)	0.79 ± 0.20	21.80 ± 3.56	23.17 ± 3.60
	C (150)	0.77 ± 0.20	20.73 ± 3.56	23.33 ± 3.60
	C (200)	0.80 ± 0.20	25.00 ± 3.56	21.33 ± 3.60
	C (250)	0.77 ± 0.20	25.23 ± 3.56	21.50 ± 3.60
	DMC (50)	0.62 ± 0.09	52.23 ± 8.58	15.33 ± 1.90
	DMC (100)	0.66 ± 0.09	46.00 ± 8.58	13.00 ± 1.90
	DMC (150)	0.67 ± 0.09	60.40 ± 8.58	12.00 ± 1.90
	DMC (200)	0.64 ± 0.09	50.83 ± 8.58	11.17 ± 1.90
	DMC (250)	0.66 ± 0.09	51.43 ± 8.58	9.50 ± 1.90
	BDMC (50)	0.99 ± 0.03	21.13 ± 3.24	7.50 ± 1.08
	BDMC (100)	1.08 ± 0.03	16.60 ± 3.24	7.67 ± 1.08
	BDMC (150)	1.09 ± 0.03	21.13 ± 3.24	7.83 ± 1.08
	BDMC (200)	1.02 ± 0.03	21.63 ± 3.24	7.33 ± 1.08
	BDMC (250)	0.86 ± 0.03	21.40 ± 3.24	7.67 ± 1.08

[a]Frog ringer solution, [b]2% Dimethylsulfoxide (DMSO), [c]Treatment with curcuminoid, [d]All values are expressed as means ± standard error of the mean (mean ± SEM), C: Curcumin, DMC: Demethoxycurcumin, BDMC: Bisdemethoxycurcumin, N: Newton, BPM: Beats per minute, All values are significant at $P < 0.05$.

The comparison between positive ionotropic effect of C, DMC and BDMC on isolated frog heart gave intensified response in dose-dependent manner (Fig. 2a and 2b). With respect to C, the force of contraction at a dose of 50µg/mL was 0.83 N; and, 0.79 N, 0.77 N, 0.80 N and 0.77 N respectively for doses of 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL.

Meanwhile, for the same dose pattern, the force of contraction produced by DMC was 0.62 N, 0.66 N, 0.67 N, 0.64 N, 0.66 N respectively. On the other hand, for the similar parameter (force of contraction), BDMC, produced 0.99 N, 1.08 N, 1.09 N, 1.02 N and 0.86 N respectively for the selected doses. Likewise, comparison between negative chronotropic effect of C, DMC and BDMC on isolated frog heart has also followed a dose-dependent order (Fig. 2c and 2d). As per C, the heart rates were recorded as 18.30 bpm, 21.80 bpm, 20.73 bpm, 25.00 bpm and 25.23 bpm correspondingly for the doses of 50µg/mL, 100µg/mL, 150µg/mL, 200µg/mL and 250 µg/mL. Besides that, the heart rates recorded for DMC for the similar dose pattern were 52.23 bpm, 46.00 bpm, 60.40 bpm, 50.83 bpm and 51.43 bpm respectively. Meanwhile, the heart rates depicted by BDMC were 21.13 bpm, 16.60 bpm, 21.13 bpm, 21.63bpm and 21.40 bpm, respectively for the doses of 50µg/mL, 100µg/mL, 150µg/mL, 200 µg/mL and 250 µg/mL. Subsequently, comparison between effect of C, DMC and BDMC on cardiac output of isolated frog heart has showed a dose-dependent response too (Fig. 2e and 2f). In the case of C, the measured cardiac output readings were 24.17mL, 23.17 mL, 23.33 mL, 21.33 mL and 21.50 mL respectively for the doses of 50µg/mL, 100µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL. Next, in the scenario of DMC, the cardiac outputs were depicted as 15.33 mL, 13.00 mL, 12.00 mL, 11.17 mL and 9.50 mL respectively for the similar above-mentioned dose pattern. In the interim, the cardiac output readings for BDMC were observed as 7.50 mL, 7.67 mL, 7.83 mL, 7.33 mL and 7.67 mL respectively for the doses of 50µg/mL, 100µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL. The above-mentioned values for all three parameters; force of contraction, heart rate and cardiac output were proven to be statistically significant with $P \leq 0.05$.

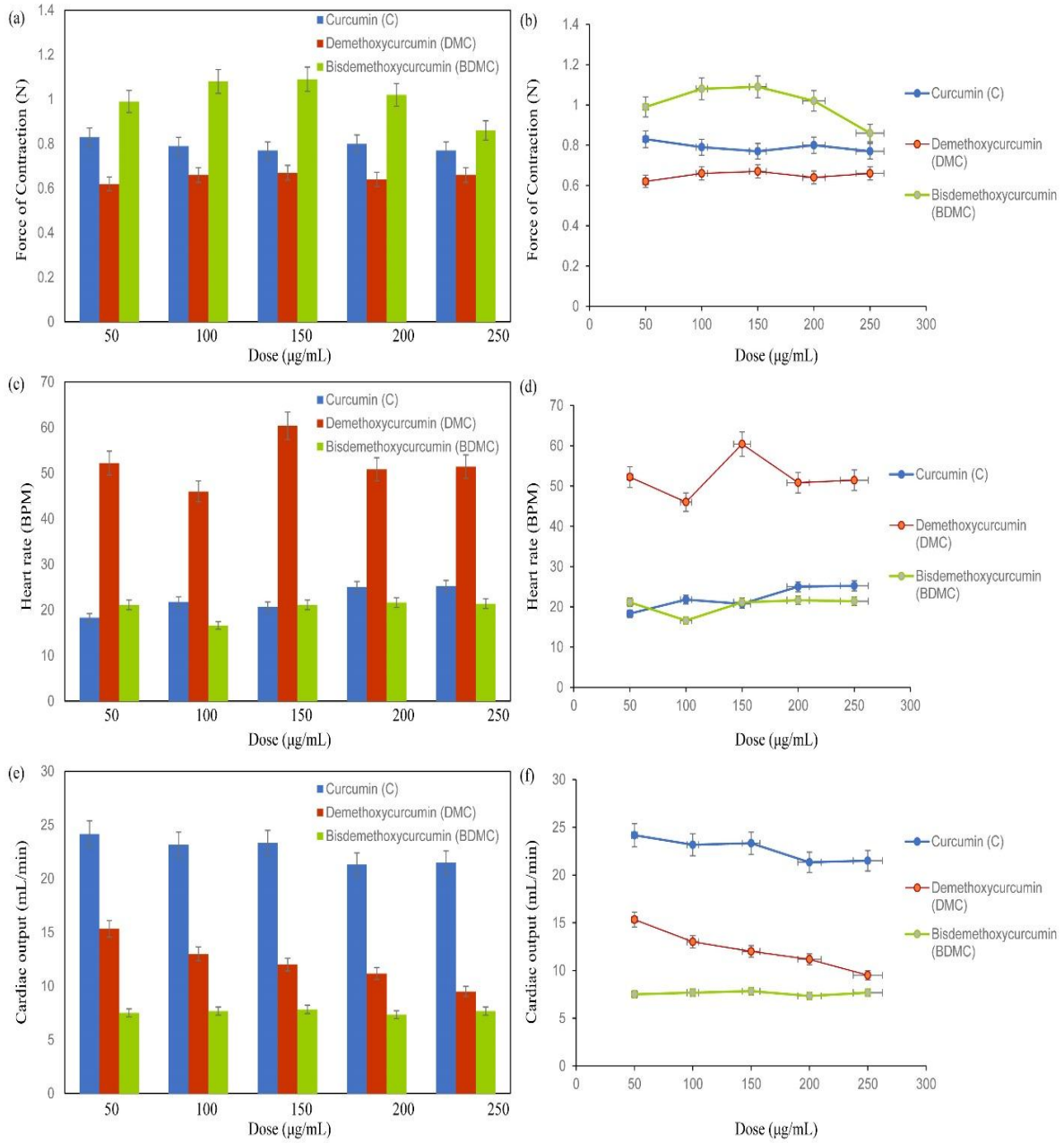

Fig. 2. (a). Relative effects of curcuminoids C, DMC and BDMC on the Force of Contraction (N) of the isolated frog heart preparation. (b) Dose-response curve of curcuminoids C, DMC and BDMC on the Force of Contraction (N) of the isolated frog heart preparation. (c). Relative effects of curcuminoids C, DMC and BDMC on the Force of Contraction (N) of the isolated frog heart preparation. (d) Dose-response curve of curcuminoids C, DMC and BDMC on the Heart Rate (BPM) of the isolated frog heart preparation. (e). Relative effects of curcuminoids C, DMC and BDMC on the Cardiac Output (mL) of the isolated frog heart preparation. (f) Dose-response curve of curcuminoids C, DMC and BDMC on the Cardiac Output (mL) of the isolated frog heart preparation.

Curcuminoids, are major bioactive components of turmeric,(Priyadarsini, 2014) are extracted and isolated from the powdered rhizomes of *Curcuma longa Linn* (Zingiberaceae) and which have been used since ancient times as herbal medicine.(Prasad, 2014, Anand *et al.,* 2008) To investigate the relative cardioprotection role of the curcuminoids,(Aruna *et al.,* 2014) the crude extract of *C. longa* was located into the column and the curcuminoids were eluted and eluates were collected in portions. Then each elate was evaporated under reduced pressure to obtain desired curcuminoid. Therefore, for further activity, the curcuminoids used were C, DMC and BDMC respectively. We presented that curcuminoids have a cardioprotective role in isolated frog heart preparation. The observed cardioprotective activity of curcuminoids might be attributed to the methoxyl and hydroxyl groups present in phenyl ring substituted with β-diketone moiety, for modifying hemodynamic properties in isolated frog heart.(Jiang *et al.,* 2012) Curcuminoids are shown to improve the hemodynamic parameters such as positive ionotropic effect, negative chronotropic effect and cardiac output.(Tilak-Jain *et al.,* 2006).

Literature on cardioprotective potential of curcuminoids highlights the protective role against, sodium fluoride-induced oxidative stress in rat heart,(Nabavi *et al.,* 2011) lead-induced cardiotoxicity in rats,(Mahjoub *et al.,* 2011) myocardial oxidative damage induced by isoproterenol in rats,(Mohanty *et al.,* 2008) doxorubicin-induced cardiotoxicity in rats,(El-Sayed *et al.,* 2011) and acute myocardial infarction after coronary artery bypass grafting respectively.(Wongcharoen *et al.,* 2012) Recent studies have revealed that curcuminoids reduce the levels of proinflammatory cytokines throughout the cardiopulmonary bypass surgery and inhibit the incidence of cardiomyocytic apoptosis next to cardiac ischemia-reperfusion injury in different animal models. There are number of studies that had dealt with the molecular basis of the cardioprotective role exhibited by curcumin.(Nawaz *et al.,* 2011) Curcumin exerts cardioprotective protective property through multiple mechanisms which include the activation of the JAK2/STAT3 signaling pathway in myocardial ischemia and reperfusion, (Duan *et al.,* 2012) by attenuation of oxidant stress and mitochondrial dysfunction in cardiac reperfusion damage, (González-Salazar *et al.,* 2011) protects against regional myocardial ischemia/reperfusion injury through activation of RISK/GSK-3β (Jeong *et al.,* 2012) and inhibition of p38 MAPK and JNK, by inhibition of cardiac oxidative and endoplasmic reticulum stress-mediated apoptosis, (Mito *et al.,* 2011) mediated by toll-like receptor 2 in cardiomyocytes, (Kim *et al.,* 2012) protects rat myocardium against isoproterenol-induced ischemic injury by attenuation of ventricular dysfunction through

increased expression of hsp27 with strengthened antioxidant defense system.(Tanwar *et al.*, 2010) According to this study's data, the curcuminoids suppressed the myocardial contractility in a dose-dependent manner without decreasing the heart rate.

The fact that the curcuminoids dose-dependently reduced the cardiac contractility of isolated frog heart muscle indicates the positive inotropic mechanism of curcuminoids.(Kapakos *et al.*, 2012) During the study, it was also being demonstrated that the curcuminoids of *C. longa* dose-dependently reduced the negative chronotropic effect.(Imbaby *et al.*, 2014) It was revealed that the negative ionotropic effect was due to the potency of DMC. Furthermore, relative comparison of the curcuminoids C, DMC and BDMC appeared to an observation which would lead to believe that the activity order as C > DMC > BDMC. Our study has tried to show that all three major curcuminoids of *C. longa* have cardioprotective nature which can be isolated by column chromatography and that the isolated functional frog heart can be used to monitor the relative potency of curcuminoids. Apparently, the observed activity order is further supported by the dose-response curve elicited by the curcuminoids and the three curcuminoids (C, DMC and BDMC), which were isolated from the crude extract. Based on the enhancement of the potency and quantitative similarity between the cardioprotective effects elicited by the curcuminoids, it would be logical to conclude that the all three curcuminoids plays a key role in the cardioprotective effect.(Ahuja *et al.*, 2011) The need to refine this further to accomplish the complete mechanism of cardioprotection *in vivo* is indicated.

3. CONCLUSION

The present investigation concludes that the curcuminoids; even after two centuries of extensive research remains to be an interesting phytoconstituents for vascular drug discovery owing to their cardioprotection. This study also unfolds novel views on the efficacy and potential of using all three major curcuminoids (C, DMC and BDMC) in polyherbal formulations to attain maximum protection against cardiovascular diseases. The cardioprotective nature exhibited by these compounds validates the use of turmeric in the traditional treatment of cardiovascular related diseases. Further studies on *in vivo* testing, combination with other herbal or synthetic drugs of these compounds and evaluating them against specific drug targets are required.

4. ACKNOWLEDGEMENTS

One of the authors (Nalini A/P Govindasamy) is thankful to the Dean, Faculty of Pharmacy and to the Vice-chancellor, La Trobe University, Australia and Asia Metropolitan University (AMU), Malaysia for providing Bachelor of Pharmacy (Hons) Graduate Research Grant, instrument facilities to carry out the research work.

5. CONFLICT OF INTEREST

The authors declare that they have no conflict of interests to disclose.

6. REFERENCES

1. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res*., 2003; 23: 363-398.
2. Ahmad N, Ahmad R, Naqvi AA, Alam MA, Ashafaq M, Iqbal Z, Ahmad FJ. Isolation, characterization, and quantification of curcuminoids and their comparative effects in cerebral ischemia. *J Liq Chromatogr Relat Technol*., 2017; 40: 133-146.
3. Ahmed T, Gilani AH. Therapeutic potential of turmeric in Alzheimer's disease: curcumin or curcuminoids?. *Phytother Res*., 2014; 28: 517-525.
4. Ahuja S, Kohli S, Krishnan S, Dogra D, Sharma D, & Rani V. Curcumin: a potential therapeutic polyphenol, prevents noradrenaline-induced hypertrophy in rat cardiac myocytes. *J Pharm Pharmacol*, 2011; 63(12): 1604-1612.
5. Ali M, Mudagal MP, Goli D. Cardioprotective effect of tetrahydrocurcumin and rutin on lipid peroxides and antioxidants in experimentally induced myocardial infarction in rats. *Pharmazie*, 2009; 64: 132-136.
6. Amalraj A, Pius A, Gopi S, Gopi S. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives–A review. *J Tradit Complement Med*, 2017; 7: 205-233.
7. Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, Tharakan ST, Misra K, Priyadarsini IK, Rajasekharan KN, Aggarwal BB. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem Pharmacol*, 2008; 76: 1590-1611.
8. Anto RJ, George J, Babu KD, Rajasekharan KN, Kuttan R. Antimutagenic and anticarcinogenic activity of natural and synthetic curcuminoids. *Mutat Res*., 1996; 370: 127-131.

9. Aruna R, Sathiyarajeswaran P, Gopakumar K, Ramaswamy RS. Cardioprotective effects of kitchen culinaries mentioned in Siddha literature. *J Pharmacogn Phytochem*, 2014; 3: 71-79.
10. Babu PS, Srinivasan K. Hypolipidemic action of curcumin, the active principle of turmeric (Curcuma longa) in streptozotocin induced diabetic rats. *Mol Cell Biochem*, 1997; 166: 169-175.
11. Bahramsoltani R, Rahimi R, Farzaei MH. Pharmacokinetic interactions of curcuminoids with conventional drugs: A review. *J Ethnopharmacol*, 2017; 209: 1-2.
12. Bazh EK, El-Bahy NM. In vitro and in vivo screening of anthelmintic activity of ginger and curcumin on Ascaridia galli. *Parasitol Res*., 2013; 112: 3679-3686.
13. Bharal N, Sahaya K, Jain S, Mediratta PK, Sharma KK. Curcumin has anticonvulsant activity on increasing current electroshock seizures in mice. *Phytother Res*., 2008; 22: 1660-1664.
14. Calvo MI, Cavero RY. Medicinal plants used for cardiovascular diseases in Navarra and their validation from official sources. *J Ethnopharmacol*, 2014; 157: 268-273.
15. Chainani-Wu N. Safety and anti-inflammatory activity of curcumin: a component of tumeric (Curcuma longa). *J Altern Complement Med*, 2003; 9: 161-168.
16. Cheng TC, Lu CC, Chung HH, Hsu CC, Kakizawa N, Yamada S, Cheng JT. Activation of muscarinic M-1 cholinoceptors by curcumin to increase contractility in urinary bladder isolated from Wistar rats. *Neurosci Lett*., 2010; 473: 107-109.
17. Chuengsamarn S, Rattanamongkolgul S, Luechapudiporn R, Phisalaphong C, Jirawatnotai S. Curcumin extract for prevention of type 2 diabetes. *Diabetes care*., 2012; 35: 2121-2127.
18. De R, Kundu P, Swarnakar S, Ramamurthy T, Chowdhury A, Nair GB, Mukhopadhyay AK. Antimicrobial activity of curcumin against Helicobacter pylori isolates from India and during infections in mice. *Antimicrob Agents Chemother*, 2009; 53: 1592-1597.
19. Dewar AM, Clark RA, Singer AJ, Frame MD. Curcumin mediates both dilation and constriction of peripheral arterioles via adrenergic receptors. *J Invest Dermatol*, 2011; 131: 1754-1760.
20. Dikshit M, Rastogi L, Shukla R, Srimal RC. Prevention of ischaemia-induced biochemical changes by curcumin & quinidine in the cat heart. *Indian J Med Res*., 1995; 101: 31-35.

21. Dou Y, Chen Y, Zhang X, Xu X, Chen Y, Guo J, Zhang D, Wang R, Li X, Zhang J. Non-proinflammatory and responsive nanoplatforms for targeted treatment of atherosclerosis. *Biomaterials*, 2017; 143: 93-108.
22. Du ZY, Liu RR, Shao WY, Mao XP, Ma L, Gu LQ, Huang ZS, Chan AS. α-Glucosidase inhibition of natural curcuminoids and curcumin analogs. *Eur J Med Chem*., 2006; 41: 213-238.
23. Duan W, Yang Y, Yan J, Yu S, Liu J, Zhou J, Zhang J, Jin Z, Yi D. The effects of curcumin post-treatment against myocardial ischemia and reperfusion by activation of the JAK2/STAT3 signaling pathway. *Basic Res Cardiol*, 2012; 107: 1-2.
24. Elgazar AF, AboRaya AO. Nephroprotective and Diuretic Effects of Three Medicinal Herbs Against Gentamicin-Induced Nephrotoxicity in Male Rats. *Pakistan J Nutr*., 2013; 12: 715-722.
25. El-Sayed EM, El-azeem AS, Afify AA, Shabana MH, Ahmed HH. Cardioprotective effects of Curcuma longa L. extracts against doxorubicin-induced cardiotoxicity in rats. *J Med Plants Res*., 2011; 5: 4049-4058.
26. Feigin VL, Norrving B, Mensah GA. Global burden of stroke. *Circ Res*., 2017; 120: 439-448.
27. Funk JL, Oyarzo JN, Frye JB, Chen G, Lantz RC, Jolad SD, Sólyom AM, Timmermann BN. Turmeric Extracts Containing Curcuminoids Prevent Experimental Rheumatoid Arthritis. *J Nat Prod*, 2006; 69: 351-355.
28. Gaffey A, Slater H, Porritt K, Campbell JM. The effects of curcuminoids on musculoskeletal pain: a systematic review. *JBI Database System Rev Implement Rep*., 2017; 15: 486-516.
29. García-Niño WR, Pedraza-Chaverrí J. Protective effect of curcumin against heavy metals-induced liver damage. *Food Chem Toxicol*, 2014; 69: 182-201.
30. González-Salazar A, Molina-Jijón E, Correa F, Zarco-Márquez G, Calderón-Oliver M, Tapia E, Zazueta C, Pedraza-Chaverri J. Curcumin protects from cardiac reperfusion damage by attenuation of oxidant stress and mitochondrial dysfunction. *Cardiovasc Toxicol*, 2011; 11: 357-364.
31. Haron-Khun S, Weisbrod D, Bueno H, Yadin D, Behar J, Peretz A, Binah O, Hochhauser E, Eldar M, Yaniv Y, Arad M. SK4 K+ channels are therapeutic targets for the treatment of cardiac arrhythmias. *EMBO Mol Med*, 2017; 9: 415-429.

32. Imbaby S, Ewais M, Essawy S, Farag N. Cardioprotective effects of curcumin and nebivolol against doxorubicin-induced cardiac toxicity in rats. *Hum Exp Toxicol*, 2014; 33: 800-813.
33. Jagetia GC, Aggarwal BB. "Spicing up" of the immune system by curcumin. *J Clin Immunol*, 2007; 27: 19-35.
34. Jeong CW, Yoo KY, Lee SH, Jeong HJ, Lee CS, Kim SJ. Curcumin protects against regional myocardial ischemia/reperfusion injury through activation of RISK/GSK-3β and inhibition of p38 MAPK and JNK. *J Cardiovasc Pharmacol Ther*, 2012; 17: 387-394.
35. Jiang JL, Jin XL, Zhang H, Su X, Qiao B, Yuan YJ. Identification of antitumor constituents in curcuminoids from Curcuma longa L. based on the composition–activity relationship. *J Pharm Biomed Anal*., 2012; 70: 664-670.
36. Jitoe A, Masuda T, Tengah IG, Suprapta DN, Gara IW, Nakatani N. Antioxidant activity of tropical ginger extracts and analysis of the contained curcuminoids. *J Agric Food Chem*, 1992; 40: 1337-1340.
37. John VD, Kuttan G, Krishnankutty K. Anti-tumour studies of metal chelates of synthetic curcuminoids. *J Exp Clin Cancer Res*., 2002; 21: 219-224.
38. Kapakos G, Youreva V, Srivastava AK. Cardiovascular protection by curcumin: molecular aspects. *Indian J Biochem Biophys*, 2012; 49: 306-315.
39. Kim DS, Park SY, Kim JY. Curcuminoids from Curcuma longa L.(Zingiberaceae) that protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial cells from βA (1–42) insult. *Neurosci Lett*., 2001; 303: 57-61.
40. Kim YS, Kwon JS, Cho YK, Jeong MH, Cho JG, Park JC, Kang JC, Ahn Y. Curcumin reduces the cardiac ischemia–reperfusion injury: involvement of the toll-like receptor 2 in cardiomyocytes. *J Nutr Biochem*, 2012; 23: 1514-1523.
41. Kiuchi F, Goto Y, Sugimoto N, AKAO N, KONDO K, TSUDA Y. Nematocidal activity of turmeric: synergistic action of curcuminoids. *Chem Pharm Bull*, 1993; 41: 1640-1643.
42. Kocaadam B, Şanlier N. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. *Crit Rev Food Sci Nutr*, 2017; 57: 2889-2895.
43. Koleva T, Madzharova IU, Baleva V, Dragoĭcheva TS, Stoĭkova K. Side effects of cardiovascular drugs. *Vutr Boles*., 1988; 28: 24-30.
44. Krausz AE, Adler BL, Cabral V, Navati M, Doerner J, Charafeddine RA, Chandra D, Liang H, Gunther L, Clendaniel A, Harper S. Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent. *Nanomedicine*, 2015; 11: 195-206.

45. Kulkarni SK, Bhutani MK, Bishnoi M. Antidepressant activity of curcumin: involvement of serotonin and dopamine system. *Psychopharmacology*, 2008; 201: 435-442.
46. Larsen SH, Olsen M, Emmertsen K, Hjortdal VE. Interventional Treatment of Patients With Congenital Heart Disease: Nationwide Danish Experience Over 39 Years. *J Am Coll Cardiol*, 2017; 69: 2725-2732.
47. Lee KJ, Yang HJ, Jeong SW, Ma JY. Solid-phase extraction of curcuminoid from turmeric using physical process method. Korean J Pharmacogn, 2012; 43: 250–256.
48. Li M, Ngadi MO, Ma Y. Optimisation of pulsed ultrasonic and microwave-assisted extraction for curcuminoids by response surface methodology and kinetic study. Food Chem, 2014; 165: 29-34.
49. Lin JK, Lin-Shiau SY. Mechanisms of cancer chemoprevention by curcumin. *Proc Natl Sci Counc Repub China B*., 2001; 25: 59-66.
50. Lukita-Atmadja W, Ito Y, Baker GL, McCuskey RS. Effect of curcuminoids as anti-inflammatory agents on the hepatic microvascular response to endotoxin. *Shock*, 2002; 17: 399-403.
51. Mahjoub S, Moghaddam AH. The Role of Exercising and Curcumin on the Treatment of lead-induced Cardiotoxicity in Rats. *Iran J Health Phys Act*., 2011; 2: 1-5.
52. Marine L, Urbina J, Bergoeing M, Valdes F, Mertens R, Kramer A. Mechanical and pharmacomechanical trombolysis in deep venous thrombosis with no clinical response to conventional treatment. *Rev Med Chil*, 2017; 145: 63-71.
53. Martins CV, Da Silva DL, Neres AT, Magalhaes TF, Watanabe GA, Modolo LV, Sabino AA, De Fátima A, De Resende MA. Curcumin as a promising antifungal of clinical interest. *J Antimicrob Chemother*, 2008; 63: 337-339.
54. Mito S, Thandavarayan RA, Ma M, Lakshmanan A, Suzuki K, Kodama M, Watanabe K. Inhibition of cardiac oxidative and endoplasmic reticulum stress-mediated apoptosis by curcumin treatment contributes to protection against acute myocarditis. *Free Radic Res*., 2011; 45: 1223-1231.
55. Mohanty IR, Arya DS, Gupta SK. Dietary Curcuma longa protects myocardium against isoproterenol induced hemodynamic, biochemical and histopathological alternations in rats. *Int. J Appl Res. Nat Prod*, 2008; 1: 19-28.
56. Moon DO, Kim MO, Lee HJ, Choi YH, Park YM, Heo MS, Kim GY. Curcumin attenuates ovalbumin-induced airway inflammation by regulating nitric oxide. *Biochem Biophys Res Commun*, 2008; 375: 275-279.

57. Nabavi SF, Nabavi SM, Ebrahimzadeh MA, Eslami S, Jafari N, Moghaddam HA. The protective effect of curcumin against sodium fluoride-induced oxidative stress in rat heart. *Arch Biol Sci*., 2011; 63: 563-569.
58. Nawaz A, Khan GM, Hussain A, Ahmad A, Khan A, Safdar M. Curcumin: a natural product of biological importance. *Gomal Univ J Res*., 2011; 27: 07-14.
59. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The Essential Medicinal Chemistry of Curcumin: Miniperspective. *J Med Chem*, 2017; 60: 1620-1637.
60. Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M, Sashida Y, Takahashi K, Kawada T, Nakagawa K, Kitahara M. Curcuminoids and sesquiterpenoids in turmeric (Curcuma longa L.) suppress an increase in blood glucose level in type 2 diabetic KK-Ay mice. *J Agric Food Chem*, 2005; 53: 959-963.
61. Ohtsu H, Xiao Z, Ishida J, Nagai M, Wang HK, Itokawa H, Su CY, Shih C, Chiang T, Chang E, Lee Y. Curcumin analogues as novel androgen receptor antagonists with potential as anti-prostate cancer agents. *J Med Chem*, 2002; 45: 5037-5042.
62. Pan CJ, Tang JJ, Weng YJ, Wang J, Huang N. Preparation, characterization and anticoagulation of curcumin-eluting controlled biodegradable coating stents. *J Control Release*, 2006; 116: 42-49.
63. Paulucci VP, Couto RO, Teixeira CC, Freitas LA. Optimization of the extraction of curcumin from Curcuma longa rhizomes. Braz J Pharmacogn, 2013; 23: 94-100.
64. Pothitirat W, Gritsanapan W. Extraction method for high curcuminoid content from Curcuma longa. Mahidol Univ J Pharm Sci., 2004; 31: 44-47.
65. Prasad S, Gupta SC, Tyagi AK, Aggarwal BB. Curcumin, a component of golden spice: from bedside to bench and back. *Biotechnol Adv*., 2014; 32: 1053-1064.
66. Priyadarsini KI. The chemistry of curcumin: from extraction to therapeutic agent. *Molecules*, 2014; 19: 20091-20112.
67. Purpura M, Lowery RP, Wilson JM, Mannan H, Münch G, Razmovski-Naumovski V. Analysis of different innovative formulations of curcumin for improved relative oral bioavailability in human subjects. *Eur J Clin Nutr*, 2017; 16: 1376-1379.
68. Rachmawati H, Soraya IS, Kurniati NF, Rahma A. In vitro study on antihypertensive and antihypercholesterolemic effects of a curcumin nanoemulsion. *Sci Pharm*, 2016; 84: 131-140.
69. Rai D, Singh JK, Roy N, Panda D. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. *Biochem J*., 2008; 410: 147-155.

70. Rao MN. Curcuminoids as potent inhibitors of lipid peroxidation. *J Pharm Pharmacol*, 1994; 46: 1013-1016.
71. Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol, 1997; 49: 105-107.
72. Rasmussen HB, Christensen SB, Kvist LP, Karazmi A. A simple and efficient separation of the curcumins, the antiprotozoal constituents of Curcuma longa. *Planta Med*, 2000; 66: 396-398.
73. Rasmussen HB, Christensen SB, Kvist LP, Karazmi A. A simple and efficient separation of the curcumins, the antiprotozoal constituents of Curcuma longa. *Planta Med*, 2000; 66: 396-398.
74. Reddy RC, Vatsala PG, Keshamouni VG, Padmanaban G, Rangarajan PN. Curcumin for malaria therapy. *Biochem Biophys Res Commun*, 2005; 326: 472-474.
75. Riva DA, Fernandez-Larrosa PN, Dolcini GL, Martinez-Peralta LA, Coulombie FC, Mersich SE. Two immunomodulators, curcumin and sulfasalazine, enhance IDV antiretroviral activity in HIV-1 persistently infected cells. *Arch Virol*, 2008; 153: 561-565.
76. Rohman A. Analysis of curcuminoids in food and pharmaceutical products. *Int Food Res J.*, 2012; 19: 19-27.
77. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer lett.*, 1995; 94: 79-83.
78. Su HC, Horvat R, Jilani G. Isolation, purification, and characterization of insect repellents from Curcuma longa L. J Agric Food Chem, 1982; 30: 290-292.
79. Syu WJ, Shen CC, Don MJ, Ou JC, Lee GH, Sun CM. Cytotoxicity of curcuminoids and some novel compounds from Curcuma zedoaria. *J Nat Prod.*, 1998; 61: 1531-1534.
80. Tanwar V, Sachdeva J, Golechha M, Kumari S, Arya DS. Curcumin protects rat myocardium against isoproterenol-induced ischemic injury: attenuation of ventricular dysfunction through increased expression of hsp27 alongwith strengthening antioxidant defense system. *J Cardiovasc Pharmacol*, 2010; 55: 377-384.
81. Tilak-Jain JA, Devasagayam TP. Cardioprotective and other beneficial effects of some Indian medicinal plants. *J Clin Biochem Nutr*, 2006; 38: 9-18.
82. Troughton RW, Frampton CM, Yandle TG, Espine EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet*, 2000; 355: 1126-1130.

83. Tuorkey M, Karolin K. Anti-ulcer activity of curcumin on experimental gastric ulcer in rats and its effect on oxidative stress/antioxidant, IL-6 and enzyme activities. *Biomed Environ Sci*., 2009; 22: 488-495.
84. Wang Y, Lu Z, Wu H, Lv F. Study on the antibiotic activity of microcapsule curcumin against foodborne pathogens. *Int J Food Microbiol*, 2009; 136: 71-74.
85. Wongcharoen W, Jai-Aue S, Phrommintikul A, Nawarawong W, Woragidpoonpol S, Tepsuwan T, Sukonthasarn A, Apaijai N, Chattipakorn N. Effects of curcuminoids on frequency of acute myocardial infarction after coronary artery bypass grafting. *Am J Cardiol*, 2012; 110: 40-44.
86. Wongcharoen W, Jai-Aue S, Phrommintikul A, Nawarawong W, Woragidpoonpol S, Tepsuwan T, Sukonthasarn A, Apaijai N, Chattipakorn N. Effects of curcuminoids on frequency of acute myocardial infarction after coronary artery bypass grafting. *Am J Cardiol*, 2012; 110: 40-44.
87. Yadav DK, Sharma K, Dutta A, Kundu A, Awasthi A, Goon A, Banerjee K, Saha S. Purity Evaluation of Curcuminoids in the Turmeric Extract Obtained by Accelerated Solvent Extraction. *J AOAC Int*., 2017; 100: 586-591.
88. Yadav SK, Sah AK, Jha RK, Sah P, Shah DK. Turmeric (curcumin) remedies gastroprotective action. *Pharmacogn Rev*., 2013; 7: 42-46.
89. Yodkeeree S, Chaiwangyen W, Garbisa S, Limtrakul P. Curcumin, demethoxycurcumin and bisdemethoxycurcumin differentially inhibit cancer cell invasion through the down-regulation of MMPs and uPA. *J Nutr Biochem*, 2009; 20: 87-95.
90. Zandi K, Ramedani E, Mohammadi K, Tajbakhsh S, Deilami I, Rastian Z, Fouladvand M, Yousefi F, Farshadpour F. Evaluation of antiviral activities of curcumin derivatives against HSV-1 in Vero cell line. *Nat Prod Commun*, 2010; 5: 1935-1938.
91. Zhang L, Fiala M, Cashman J, Sayre J, Espinosa A, Mahanian M, Zaghi J, Badmaev V, Graves MC, Bernard G, Rosenthal M. Curcuminoids enhance amyloid-β uptake by macrophages of Alzheimer's disease patients. *J Alzheimers Dis*., 2006; 10: 1-7.
92. Zhao X, Xu Y, Zhao Q, Chen CR, Liu AM, Huang ZL. Curcumin exerts antinociceptive effects in a mouse model of neuropathic pain: descending monoamine system and opioid receptors are differentially involved. *Neuropharmacology*, 2012; 62: 843-854.
93. Zhongfa L, Chiu M, Wang J, Chen W, Yen W, Fan-Havard P, Yee LD, Chan KK. Enhancement of curcumin oral absorption and pharmacokinetics of curcuminoids and curcumin metabolites in mice. *Cancer Chemother. Pharmacol*, 2012; 69: 679-689.

94. Zimmer HG. Modifications of the isolated frog heart preparation in Carl Ludwig's Leipzig Physiological Institute: relevance for cardiovascular research. Can J Cardiol, 2000; 16: 61-69.