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

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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 acitivity 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), NReady-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 °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) curcuminoids 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, ¹H-NMR and Mass spectral data.(Su et al., 1982) Hence, curcuminoids dissolved in 2% dimethylsufoxide (DMSO) and tested for
biological activity. Schematic representation 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; HCO₃⁻, 2.8; H₂PO₄⁻, 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).
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 separate 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 agreement 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 determined 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.

<table>
<thead>
<tr>
<th>Group (n=3)</th>
<th>Curcuminoid/ Concentration (μg/mL)</th>
<th>Force of contraction (N)(^d)</th>
<th>Heart rate (BPM)(^d)</th>
<th>Cardiac output (mL/min)(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)a</td>
<td>C</td>
<td>0.61 ± 0.20</td>
<td>116.03 ± 3.56</td>
<td>30.33 ± 3.60</td>
</tr>
<tr>
<td></td>
<td>DMC</td>
<td>0.67 ± 0.09</td>
<td>111.73 ± 8.58</td>
<td>30.17 ± 1.90</td>
</tr>
<tr>
<td></td>
<td>BDMC</td>
<td>0.92 ± 0.03</td>
<td>150.03 ± 3.24</td>
<td>31.50 ± 1.08</td>
</tr>
<tr>
<td>Group 2 (Vehicle control)b</td>
<td>C</td>
<td>0.61 ± 0.20</td>
<td>116.23 ± 3.56</td>
<td>31.00 ± 3.60</td>
</tr>
<tr>
<td></td>
<td>DMC</td>
<td>0.67 ± 0.09</td>
<td>111.50 ± 8.58</td>
<td>29.17 ± 1.90</td>
</tr>
<tr>
<td></td>
<td>BDMC</td>
<td>0.91 ± 0.03</td>
<td>150.07 ± 3.24</td>
<td>31.00 ± 1.08</td>
</tr>
<tr>
<td>Group 3 (Treatment)c</td>
<td>C (50)</td>
<td>0.83 ± 0.20</td>
<td>18.30 ± 3.5</td>
<td>24.17 ± 3.60</td>
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<td>C (100)</td>
<td>0.79 ± 0.20</td>
<td>21.80 ± 3.56</td>
<td>23.17 ± 3.60</td>
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<tr>
<td></td>
<td>C (150)</td>
<td>0.77 ± 0.20</td>
<td>20.73 ± 3.56</td>
<td>23.33 ± 3.60</td>
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<td>C (200)</td>
<td>0.80 ± 0.20</td>
<td>25.00 ± 3.56</td>
<td>21.33 ± 3.60</td>
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<tr>
<td></td>
<td>C (250)</td>
<td>0.77 ± 0.20</td>
<td>25.23 ± 3.56</td>
<td>21.50 ± 3.60</td>
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<tr>
<td></td>
<td>DMC (50)</td>
<td>0.62 ± 0.09</td>
<td>52.23 ± 8.58</td>
<td>15.33 ± 1.90</td>
</tr>
<tr>
<td></td>
<td>DMC (100)</td>
<td>0.66 ± 0.09</td>
<td>46.00 ± 8.58</td>
<td>13.00 ± 1.90</td>
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<td></td>
<td>DMC (150)</td>
<td>0.67 ± 0.09</td>
<td>60.40 ± 8.58</td>
<td>12.00 ± 1.90</td>
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<tr>
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<td>DMC (200)</td>
<td>0.64 ± 0.09</td>
<td>50.83 ± 8.58</td>
<td>11.17 ± 1.90</td>
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<td></td>
<td>DMC (250)</td>
<td>0.66 ± 0.09</td>
<td>51.43 ± 8.58</td>
<td>9.50 ± 1.90</td>
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<td>BDMC (50)</td>
<td>0.99 ± 0.03</td>
<td>21.13 ± 3.24</td>
<td>7.50 ± 1.08</td>
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<td></td>
<td>BDMC (100)</td>
<td>1.08 ± 0.03</td>
<td>16.60 ± 3.24</td>
<td>7.67 ± 1.08</td>
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<td></td>
<td>BDMC (150)</td>
<td>1.09 ± 0.03</td>
<td>21.13 ± 3.24</td>
<td>7.83 ± 1.08</td>
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<td>BDMC (200)</td>
<td>1.02 ± 0.03</td>
<td>21.63 ± 3.24</td>
<td>7.33 ± 1.08</td>
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<td></td>
<td>BDMC (250)</td>
<td>0.86 ± 0.03</td>
<td>21.40 ± 3.24</td>
<td>7.67 ± 1.08</td>
</tr>
</tbody>
</table>

aFrog ringer solution, b2% Dimethylsulfoxide (DMSO), cTreatment with curcuminoid, dAll 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 ionotrophic 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 50 μg/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 ≤ 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 ionotrophic 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.
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5. CONFLICT OF INTEREST
The authors declare that they have no conflict of interests to disclose.

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