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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and evaluation of novel indomethacin–antioxidant codrugs as gastrosparing NSAIDs

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Abstract Indomethacin has been conjugated with different antioxidants having antiulcerogenic activity with the objective of obtaining indomethacin–antioxidant codrugs as gastrosparing NSAIDs devoid of ulcerogenic side effects. Purified synthesized codrugs have been characterized by m.p., TLC, elemental analyses, FTIR, NMR, MS. The synthesized derivatives have been screened for their antiinflammatory, analgesic, and antiulcer activity. The codrugs showed retention of antiinflammatory activity with reduced ulcerogenic side effects. These results indicated that indomethacin–antioxidant codrugs have the potential to be developed as gastrosparing NSAIDs.

Keywords NSAIDs · Indomethacin · Antioxidant · Codrug · Ulcerogenicity

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are the most widely used drugs, with prescription as well as over the counter formulations being available in most countries. Since the introduction of NSAIDs in the market, enormous literature has been published regarding their side effects. Although these agents affect renal and cardiovascular systems, the most common, widely studied, reported, and reviewed side effects are related to gastrointestinal tract (GIT) (Gibson, 1988; Vane and Botting, 1998). The pharmacological activity of NSAIDs is related to their ability to inhibit the activity of the enzyme cyclooxygenases (COXs) involved in the biosynthesis of prostaglandin H_2 (PGH_2) (Vane, 1971; Hla and Neilson, 1992). It is now well known that COX exists in two isoforms, namely COX-I and COX-II, which are regulated differently (Xie *et al.*, 1991). COX-I is constitutively expressed in stomach to provide cytoprotection in the GIT. COX-II is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells. Since most of the NSAIDs used clinically inhibit both isoforms, long term use of these agents results in gastric ulcer and there is enough evidence that inhibition of COX-I rather than that of COX-II underlies gastric ulcer formation (McCarthy, 1989; Warner *et al.*, 1999; Wolfe *et al.*, 1999). As a result, a number of selective COX-II inhibitors, including Celecoxib and Rofecoxib have been introduced for clinical use with exceptional antiinflammatory properties with reduced gastric toxicity (Xie *et al.*, 1992; Hawkey, 1999). But initial enthusiasm for selective COX-II inhibitors as safer NSAIDs has faded due to emergence of serious cardiovascular side effects on long term use and need for design and development of safer agents still remain (Dogne *et al.*, 2005; Schnitzer, 2001).

Recently, it has been well known that local generation of various reactive oxygen species (ROS) plays a significant role in the formation of gastric ulceration associated with NSAID therapy (Bandyopadhyay *et al.*, 1999; Hassan *et al.*, 1998). These observations indicate that antioxidants may be used to prevent NSAIDs induced gastric ulcers. During the past few decades, a large number of naturally occurring compounds have been identified as antioxidants, which are viewed as promising therapeutic agents for treating free radical mediated diseases including NSAID induced peptic ulcers. Large number of herbs and spices are recognized as source of natural antioxidants and studies have confirmed their efficacy for the treatment of gastrointestinal ulcers (Nakatani, 2000). Based on these observations, it has been

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suggested that coadminstration of antioxidants and NSAIDS in formulated dosage form may possibly decrease the risk of NSAIDs induced gastrointestinal side effects (Jimenez and Alcaraz, 1988; Repetto and Llesuy, 2002). However, there are potential advantages in giving such coadministered drugs having complementary pharmacological activities in the form of a single chemical entity. Such agents are named as mutual prodrugs/codrugs which are designed with improved physicochemical properties and release the parent drug at the site of action (Singh and Sharma, 1994; Bhosle *et al.*, 2006; Leppanen *et al.*, 2002).

Indomethacin (**1**) is one of the most potent NSAIDs. However, its use is restricted due to high incidences of ulcerogenic side effects. In the present study, this potential NSAID has been selected. In literature various indomethacin-conjugates has been reported as ulcer protective agents (Sawraj *et al.*, 2010; Zhang *et al.*, 2005; Doulgkeris *et al.*, 2006). This work aims to synthesize indomethacin–antioxidant ester codrugs to get safer NSAIDs, devoid of ulcerogenic side effects while retaining the antiinflammatory and analgesic activity.

Result and discussion

Chemistry

For the preparation of indomethacin–antioxidant codrugs (**3a**–**g**), various natural antioxidants were identified for conjugation including, guaiacol (**2a**), eugenol (**2b**), thymol (**2c**), vanillin (**2d**), sesamol (**2e**), umbelliferone (**2f**), and menthol (**2g**) (Fig. 1). These agents have been an important part of human diet and therefore their safety profile is well known (Cotelle, 2001; Martin *et al.*, 1998). Sequence of steps involved in the synthesis of various indomethacin–antioxidant codrugs (**3a**–**g**) by conjugation of indomethacin (**1**) and various antioxidant (**2a**–**g**) are shown in Scheme 1. For this purpose, indomethacin (**1**) was dissolved in chloroform followed by the addition of DCC and stirred at room temperature for 1 h. To this solution, the corresponding antioxidant was added along with DMAP and reaction mixture was stirred at room temperature for 24 h. After the processing the reaction mixture, desired product was obtained. All the codrugs (**3a**–**g**) were prepared following this general procedure. These compounds were purified by recrystallization and obtained in reasonable yield (43–58%). Their structures were confirmed by the use of elemental analysis and spectral studies (Table 1).

The IR spectrum of the derivative **3a** showed the absorption peaks at 3028.1 cm^{-1} characteristic of C–H stretching. The peaks at 1761.1, 1684.5 cm^{-1} showed presence of C=O (ester linkage) and C=O (indomethacin), respectively. The ^{1}H NMR spectrum of **3a** showed the

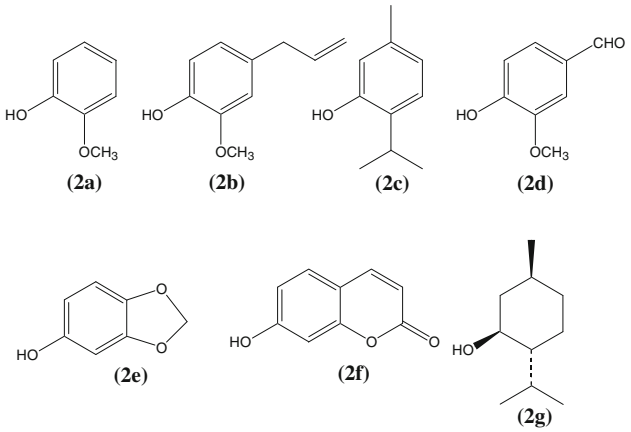
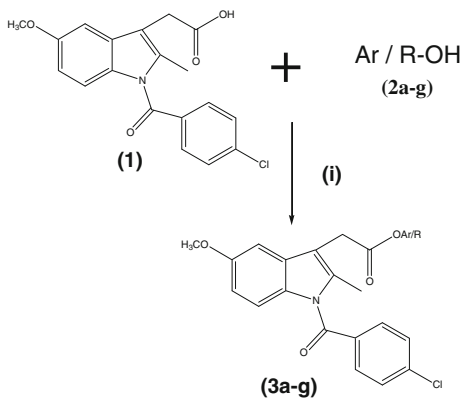


Fig. 1 Structures of naturally occurring antioxidants; guaiacol (**2a**), eugenol (**2b**), thymol (**2c**), vanillin (**2d**), sesamol (**2e**), umbelliferone (**2f**), and menthol (**2g**)



Scheme 1 Sequence of steps involved in the synthesis of indomethacin–antioxidant codrugs. Reagent and conditions: (i) CH_2Cl_2, DCC, DMAP, room temperature, 24 h

signals at δ 2.43 for CH_3 protons (s, indomethacin ring), δ 3.73 for OCH_3 protons (s, guaiacol), 3.83 for OCH_3 protons (s, indomethacin ring), δ 3.92 for CH_2 (s, indomethacin). Aromatic protons of indomethacin and guaiacol are overlapped and appeared between δ 6.89–7.25. Two distinct ABq were observed at 7.44–7.47 ($J = 9$ Hz) and 7.65–7.68 ($J = 9$ Hz), respectively, representing para coupling of the indomethacin molecule. In ^{13}C NMR, signals appeared at δ 168.39 and 169.02 for *C*OO (ester linkage) and *C*=O (indomethacin nucleus). The mass spectrum of the compound **3a** showed molecular ion peak at *m*/*z* 463.98 (M^+).

The IR spectrum of the derivative **3b** showed the absorption peaks at 3081 cm^{-1} characteristic of C–H stretching. The peaks at 1752.7, 1668.7 cm^{-1} showed presence of C=O (ester linkage) and C=O (indomethacin), respectively. The ^{1}H NMR spectrum of **3b** showed the signals at δ 2.46 for CH_3 protons (s, indomethacin ring), δ 3.74 for OCH_3 protons (s, eugenol), 3.86 for OCH_3 protons (s, indomethacin ring), δ 3.95 for CH_2 (s, indomethacin). The

Table 1 Physical properties

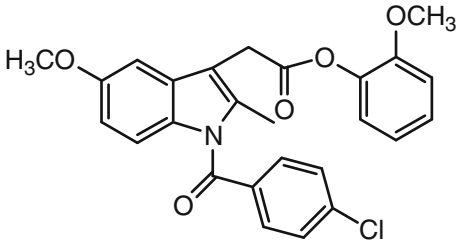
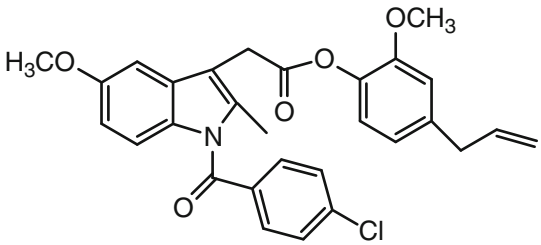
Name	Yield (%)	M.p. (°C)	Spectral and elemental data
 2-Methoxyphenyl[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (**3a**) $C_{26}H_{22}ClNO_5$	56	203	IR (KBr): 3028.1 (aromatic C–H st), 2928.3 (aliphatic C–H st), 2851.2 (C–H st of aromatic OCH_3), 1761.1 (C=O st, ester), 1684.5 (C=O st, indomethacin), 1500.6 (benzene ring C=C st), 1258.4 (asymm C–O–C st), 1138.5 (C–C(=O)–O st), 1042.2 (symm C–O–C st) cm^{-1} ^{1}H NMR ($CDCl_3$): δ 2.43 (s, 3H, C*H*$_3$, indomethacin), 3.73 (s, 3H, OC*H*$_3$, guaiacol), 3.83 (s, 3H, OC*H*$_3$, indomethacin), 3.92 (s, 2H, C*H*$_2$COO), 6.67–6.70 (dd, H, $J = 9.0$ Hz and 2.5 Hz Ar–*H*, indomethacin), 6.89–6.94 (m, 3H, Ar–*H*, indomethacin, guaiacol), 6.98–7.01 (dd, 1H, $J = 1.7$ Hz and 7.9 Hz, Ar–*H*, guaiacol), 7.08–7.09 (d, 1H, $J = 2.52$ Hz, Ar–*H*, indomethacin), 7.15–7.25 (m, 1H, Ar–*H*, guaiacol), 7.46–7.47 (Abq, 2H, $J = 9$ Hz, Ar–*H*, indomethacin), 7.65–7.68 (Abq, 2H, $J = 9$ Hz, Ar–*H*, indomethacin) ^{13}C NMR ($CDCl_3$): δ 13.49 (Ar–*C*H$_3$), 30.08 (Ar–*C*H$_2$COO), 55.77 (O*C*H$_3$), 101.61–133.99 (Ar-carbons), 136.22 (>N–*C*(CH$_3$)=C), 139.3 (CH$_2$COO*C*Ar), 139.90 (Ar*C*–Cl), 151.08 (*C*OCH$_3$, indomethacin), 156.11 (*C*OCH$_3$, guaiacol), 168.39 (CH$_2$*C*OO), 169.02 (>N*C*O–Ar) LC–MS: *m*/*z* 463.98 $[M]^+$ Calculated for $C_{26}H_{22}ClNO_5$: C, 67.31; H, 4.78; Cl, 7.64; N, 3.02. Found: C, 67.71; H, 4.39; N, 3.37%
 4-Allyl-2-methoxyphenyl[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (**3b**) $C_{29}H_{26}ClNO_5$	50	147.5	IR (KBr): 3081 (aromatic C–H st), 2933.4 (aliphatic C–H st), 2839.2 (C–H st of aromatic OCH_3), 1752.7 (C=O st, ester), 1668.7 (C=O st, indomethacin), 1506.5 (benzene ring C=C st), 1272.2 (asymm C–O–C st), 1149.2 (C–C(=O)–O st), 1034.3 (symm C–O–C st) cm^{-1} ^{1}H NMR ($CDCl_3$): δ 2.46 (s, 3H, C*H*$_3$, indomethacin), 3.38–3.39 (d, 2H, $J = 6.6$ Hz, –C*H*$_2$–, eugenol), 3.74 (s, 3H, OC*H*$_3$, eugenol), 3.86 (s, 3H, OC*H*$_3$, indomethacin), 3.95 (s, 2H, C*H*$_2$COO), 5.09–5.13 (m, 2H, =C*H*$_2$, eugenol), 5.93–6.00 (m, 1H, –C*H*=, eugenol), 6.70–6.79 (m, 3H, Ar–*H*, indomethacin, eugenol), 6.93–6.96 (dd, 2H, $J = 2.34$ Hz and 9.24 Hz, Ar–*H*, indomethacin, eugenol), 7.120–7.126 (d, 1H, $J = 2.2$ Hz, Ar–*H*, indomethacin), 7.48–7.50 (Abq, 2H, $J = 9$ Hz, Ar–*H*, indomethacin), 7.69–7.71 (Abq, 2H, $J = 9$ Hz, Ar–*H*, indomethacin) ^{13}C NMR ($CDCl_3$): δ 13.48 (Ar–*C*H$_3$), 30.07 (Ar–*C*H$_2$COO), 40.10 (*C*H$_2$–CH=CH$_2$), 55.74 (O*C*H$_3$), 114.93 (CH=*C*H$_2$), 101.58–138.12 (Ar-carbons), 134.2 (*C*H=CH$_2$), 136.16 (>N–*C*(CH$_3$)=C), 139.13 (CH$_2$COO*C*Ar), 139.26 (Ar*C*–Cl), 150.82 (*C*OCH$_3$, eugenol), 156.09 (*C*OCH$_3$, indomethacin), 168.35 (CH$_2$*C*OO), 169.12 (>N*C*O–Ar) LC–MS: *m*/*z* 503.93 $[M]^+$ Calculated for $C_{29}H_{26}ClNO_5$: C, 69.11; H, 5.20; N, 2.78. Found: C, 69.44; H, 5.15; N, 2.65%

Table 1 continued

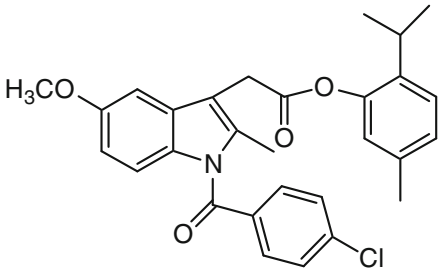
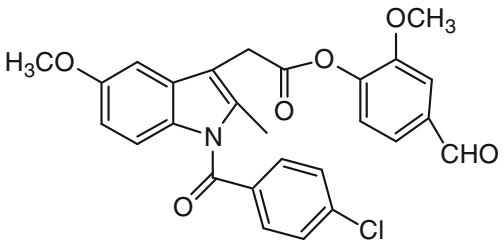
Name	Yield (%)	M.p. (°C)	Spectral and elemental data
 2-Isopropyl-5-methylphenyl[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (**3c**) $C_{29}H_{28}ClNO_4$	40.5	118.5	IR (KBr): 3042.2 (aromatic C–H st), 2927.6 (aliphatic C–H st), 1754.8 (C=O st, ester), 1680.3 (C=O st, indomethacin), 1504.7 (benzene ring C=C st), 1287.1 (asymm C–O–C st), 1134.7 (C–C(=O)–O st) cm^{-1}, 1031.5 (symm C–O–C st) cm^{-1}, 952.2 (C–H bend) ^{1}H NMR ($CDCl_3$): δ 1.01–1.02 (d, 6H, $J = 6.9$ Hz, $CH(CH_3)_2$), 2.27 (s, 3H, CH_3, indomethacin), 2.45 (s, 3H, Ar–CH_3, thymol), 2.73–2.77 (sept, 1H, $J = 6.9$, $CH(CH_3)_2$), 3.89 (s, 3H, OCH_3, indomethacin), 3.91 (s, 2H, CH_2COO), 6.67–6.70 (dd, 1H, $J = 2.5$ Hz and 9 Hz, Ar–*H* indomethacin), 6.7913–6.7936 (d, 1H, $J = 0.92$ Hz, Ar–*H*, thymol), 6.89–6.91 (d, 1H, $J = 9$ Hz, Ar–*H*, indomethacin), 6.96–6.98 (d, 1H, $J = 7.92$ Hz, Ar–*H*, thymol), 7.063–7.069 (d, 1H, $J = 2.48$ Hz, Ar–*H*, indomethacin), 7.11–7.13 (d, 1H, $J = 7.88$ Hz, Ar–*H*, thymol), 7.42–7.46 (Abq, 2H, $J = 9$ Hz, Ar–*H*, indomethacin), 7.62–7.66 (Abq, 2H, $J = 9$ Hz, Ar–*H*, indomethacin) ^{13}C NMR ($CDCl_3$): δ 13.38 (–NC(–CH_3))=C), 20.80 (Ar–CH_3, thymol), 22.85 ($CH(CH_3)_2$), 27.01 ($CH(CH_3)_2$), 30.55 (CH_2COO), 55.67 (OCH_3), 101.18–147.86 (Ar–carbons), 136.56 (>N–$C(CH_3)$=C), 139.6 (Ar*C*–Cl), 147.86 ($CH_2COOCAr$), 156.16 ($COCH_3$), 168.24 (CH_2COO), 169.50 (>N*C*O–Ar) LC–MS: *m*/*z* 489.06 $[M]^+$ Calculated for $C_{29}H_{28}ClNO_4$: C, 70.22; H, 6.91; N, 2.82. Found: C, 70.34; H, 6.91; N, 2.85%
 (4-Formyl-2-metho xyphenyl[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (**3d**) $C_{27}H_{22}ClNO_6$	42.5	128	IR (KBr): 3064.9 (aromatic C–H st), 2933.4 (aliphatic C–H st), 2843.5 (C–H st of aromatic OCH_3), 1752.9 (C=O st, ester), 1685.7 (C=O st aldehyde), 1683.6 (C=O st, indomethacin), 1503.5 (benzene ring C=C st), 1273.7 (asymm C–O–C st), 1138.8 (C–C(=O)–O st), 1032.9 (symm C–O–C st) cm^{-1}, 908.4 (C–H bend) cm^{-1} ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 3H, CH_3, indomethacin), 3.79 (s, 3H, OCH_3, vanillin), 3.83 (s, 3H, OCH_3, indomethacin), 3.95 (s, 2H, CH_2COO), 6.68–6.70 (dd, H, $J = 9.0$ Hz and 2.5 Hz, Ar–*H*), 6.88–6.90 (d, 1H, $J = 9.0$ Hz, Ar–*H*), 7.06–7.07 (d, 1H, $J = 2.4$ Hz, Ar–*H*), 7.17–7.19 (d, 1H, $J = 9.0$ Hz, Ar–*H*, vanillin), 7.432–7.436 (d, 2H, $J = 2.0$ Hz, Ar–*H*), 7.45–7.47 (Abq, 2H, $J = 9$ Hz, Ar–*H*, indomethacin), 7.65–7.69 (Abq, 2H, $J = 9$ Hz, Ar–*H*, indomethacin), 9.92 (s, 1H, C*H*O) ^{13}C NMR ($CDCl_3$): δ 13.40 (Ar–CH_3), 30.00 (Ar–CH_2COO), 55.75 (OCH_3), 55.97 (OCH_3), 37.99 (Ar–CH_2), 56.15 (OCH_3), 101.61–139.37 (Ar-carbons), 136.36 (>N–$C(CH_3)$=C), 139.37 (Ar*C*–Cl), 144.99 ($CH_2COOCAr$), 151.88 ($COCH_3$, indomethacin), 156.09 ($COCH_3$, vanillin), 168.32 (CH_2COO), 169.50 (>N*C*O–Ar), 191.00 (*C*HO) LC–MS: *m*/*z* 491.91 $[M]^+$ Calculated for $C_{27}H_{22}ClNO_6$: C, 65.92; H, 4.51; N, 2.85. Found: C, 65.04; H, 4.84; N, 2.92%

Table 1 continued

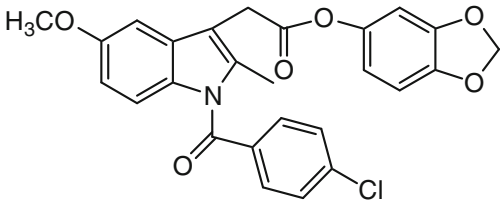
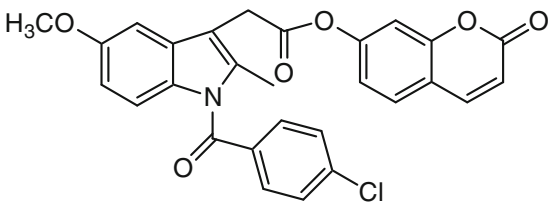
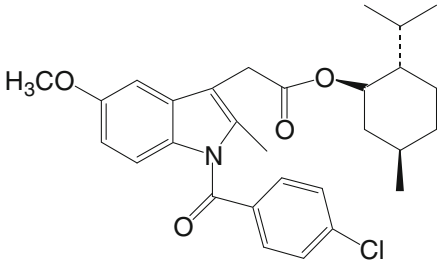
Name	Yield (%)	M.p. (°C)	Spectral and elemental data
 3,4-(Methylenedioxy)phenyl[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (**3e**) $C_{26}H_{20}ClNO_5$	48.4	77.5	IR (KBr): 3025.8 (aromatic C–H st), 2924.9 (aliphatic C–H st), 2852.9 (C–H st of aromatic OCH_3), 1759.8 (C=O st, ester), 1683.1 (C=O st, indomethacin), 1482.6 (benzene ring C=C st), 1258.4 (asymm C–O–C st), 1133.4 (C–C(=O)–O st), 1029.7 (symm C–O–C st), 927.6 (C–H bend) cm^{-1} ^{1}H NMR ($CDCl_3$): 2.43 (s, 3H, CH_3, indomethacin), 3.83 (s, 3H, OCH_3, indomethacin), 3.86 (s, 2H, CH_2COO), 5.95 (s, 2H, OCH_2), 6.47–6.49 (dd, 1H, J = 8.4 Hz and 2.4 Hz, Ar–*H*, sesamol), 6.562–6.568 (d, 1H, J = 2.28 Hz, Ar–*H*, sesamol), 6.67–6.70 (dd, 1H, J = 9.0 Hz and 2.5 Hz, Ar–*H*, indomethacin), 6.72–6.74 (d, 1H, J = 8.4 Hz, Ar–*H*, sesamol), 6.88–6.90 (d, 1H, J = 9.0 Hz, Ar–*H*, indomethacin), 7.03–7.04 (d, 1H, J = 2.4 Hz, Ar–*H*, indomethacin), 7.45–7.48 (Abq, 2H, J = 9 Hz, Ar–*H*, indomethacin), 7.66–7.68 (Abq, 2H, J = 9 Hz, Ar–*H*, indomethacin) ^{13}C NMR ($CDCl_3$): δ 13.49 (Ar–***C***H_3), 30.51 (Ar–*C*H_2COO), 55.77 (O*C*H_3), 101.20 (O*C*H_2O), 101.78–133.86 (Ar-carbons), 136.24 (>N–*C*(CH$_3$)=C), 139.39 (Ar*C*–Cl), 145.47 (CH$_2$COO*C*Ar), 148.02 (Ar*C*OCH$_2$O), 156.16 (*C*OCH$_3$), 168.35 (CH$_2$*C*OO), 169.68 (>N*C*O–Ar) LC–MS: m/z 477.92 $[M]^+$ Calculated for $C_{26}H_{20}ClNO_5$: C, 65.34; H, 4.22; N, 2.93. Found: C, 65.97; H, 4.31; N, 2.85%
 22-Oxo-2*H*-chromen-7-yl[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (**3f**) $C_{28}H_{20}ClNO_6$	42.5	224.5	IR (KBr): 3036.1 (aromatic C–H st), 2927.9 (aliphatic C–H st), 1776.7 (C=O st aldehyde), 1758.9 (C=O st, ester), 1687.6 (C=O st, indomethacin), 15037.5 (benzene ring C=C st), 1241.4 (asymm C–O–C st), 1186.1 (C–C(=O)–O st), 1049.6 (symm C–O–C st) cm^{-1}, 928.4 (C–H bend) cm^{-1} ^{1}H NMR ($CDCl_3$): δ 2.46 (s, 3H, CH_3, indomethacin), 3.83 (s, 3H, OCH_3, indomethacin), 3.94 (s, 2H, CH_2COO), 6.36–6.39 (d, 1H, J = 9.5 Hz, *H* of lactone ring, umbelliferone), 6.68–6.71 (dd, H, J = 9.0 Hz and 2.5 Hz, Ar–*H*, indomethacin), 6.86–6.89 (d, 1H, J = 9.0 Hz, Ar–*H*, indomethacin), 6.99–7.00 (d, 1H, J = 2.4 Hz, Ar–*H*, indomethacin), 7.02–7.03 (m, 1H, Ar–*H*, umbelliferone), 7.09–7.1 (d, 1H, J = 2.2 Hz, Ar–*H*, umbelliferone), 7.45–7.47 (m, 3H, Ar–*H*, indomethacin, umbelliferone), 7.65–7.69 (m, 3H, Ar–*H*, indomethacin, *H* of lactone ring, umbelliferone) ^{13}C NMR ($CDCl_3$): δ 13.47 (Ar–*C*H_3), 30.56 (Ar–*C*H_2COO), 55.79 (O*C*H_3), 101.19–133.73 (Ar-carbons), 110.32 (Ar–CH=*C*H–), 136.45 (>N–*C*(CH$_3$)=C), 139.50 (Ar*C*–Cl), 142.82 (Ar–*C*H=CH–), 153.14 (Ar*C*OCO), 154.66 (CH$_2$COO*C*Ar), 156.09 (*C*OCH$_3$), 160.27 (*C*=O, umbelliferone), 168.32 (CH$_2$*C*OO), 168.68 (>N*C*O–Ar) LC–MS: m/z 501.97 $[M]^+$ Calculated for $C_{28}H_{20}ClNO_6$: C, 67.00; H, 4.02; N, 2.79. Found: C, 67.09; H, 4.02; N, 2.93%

Table 1 continued

Name	Yield (%)	M.p. (°C)	Spectral and elemental data
 2-Isopropyl-5-methylcyclohexyl[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (**3g**) $C_{29}H_{34}ClNO_4$	42.5	82	IR (KBr): 2957.9 (aliphatic C–H st), 1730.1 (C=O st, ester), 1683.6 (C=O st, indomethacin), 1602.5 (Ar C=C st); 1226.0 (asymm C–O–C st), 1171.6 (C–C(=O)–O st) cm^{-1}, 1038.6 (symm C–O–C st) cm^{-1}, 926.7 (C–H bend) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.67–0.69 (d, 3H, $J = 7.0$ Hz, CH_3, menthol), 081–0.89 (d, 3H, CH_3), 0.91–0.96 (d, 4H, CH_3, CH), 0.99–1.05 (m, 2H, CH), 1.33–1.35 (m, 1H, CH), 1.46–1.48 (m, 1H, CH), 1.63–1.74 (m, 3H, CH), 1.99–2.02 (dd, 1H, CH), 2.38 (s, 3H, CH_3, indomethacin), 3.65 (s, 2H, CH_2COO), 3.83 (s, 3H, OCH_3, indomethacin), 4.46–4.79 (m, 1H, CH), 6.66–6.69 (dd, H, $J = 9.0$ Hz and 2.5 Hz, Ar–H), 6.88–6.91 (d, 1H, $J = 9.0$ Hz, Ar–H), 6.97–6.98 (d, 1H, $J = 2.4$ Hz, Ar–H), 7.45–7.47 (Abq, 2H, $J = 9$ Hz, Ar–H, indomethacin), 7.64–7.66 (Abq, 2H, $J = 9$ Hz, Ar–H, indomethacin) ^{13}C NMR ($CDCl_3$): 13.45 (Ar–CH_3), 30.46 (Ar–CH_2COO), 55.89 (OCH_3), 101.19–133.35 (Ar-carbons), 110.46 (Ar–CH=CH–), 136.51 (>N–$C(CH_3)$=C), 139.25 (ArC–Cl), 142.72 (Ar–CH=CH–), 153.47 (Ar$COCO$), 154.22 ($CH_2COOCAr$), 157.07 ($COCH_3$), 168.32 (CH_2COO), 168.68 (>NCO–Ar) LC–MS: m/z 496.0 $[M]^+$ Calculated for $C_{29}H_{34}ClNO_4$: C, 70.22; H, 6.91; N, 2.82. Found: C, 70.34; H, 6.91; N, 2.82

Spectral and elemental data of indomethacin–antioxidant codrugs (**3a–g**)

signal appeared at δ 3.38–3.39 for methylene (CH_2–CH=CH_2) proton as doublet, at δ 5.93–6.0 for methine (CH_2–CH=CH_2) proton as multiplet and at 5.09–5.13 for terminal methylene (CH_2–CH=CH_2) protons as multiplet of eugenol moiety. The signals for the aromatic protons appeared in the range of δ 6.70–7.12, overlapped with Ar–H signals of indomethacin moiety as multiplets. Two distinct ABq were observed at 7.48–7.50 ($J = 9$ Hz) and 7.69–7.71 ($J = 9$ Hz), respectively, representing para coupling of the indomethacin molecule. In ^{13}C NMR, signals appeared at δ 168.35 and 169.12 for COO (ester linkage) and C=O (indomethacin nucleus). The mass spectrum of the compound **3b** showed molecular ion peak at m/z 503.93 (M^+).

The IR spectrum of the derivative **3c** showed the absorption peaks at 3042.2 cm^{-1} characteristic of C–H stretching. The peaks at 1754.8, 1680.3 cm^{-1} showed presence of C=O (ester linkage) and C=O (indomethacin), respectively. The ^1H NMR spectrum of **3c** showed the signals at δ 2.27 for CH_3 protons (s, indomethacin ring), 2.45 for CH_3 protons (s, thymol), 3.89 for OCH_3 protons (s, indomethacin ring), δ 3.91 for CH_2 (s, indomethacin). The signal for gem dimethyl group of thymol appeared at δ 1.01–1.02 as doublet ($J = 6.9$ Hz). Methine (–CH) proton signal appeared as septet at δ 2.73–2.77 ($J = 6.9$ Hz) due to splitting by gem dimethyl group. Aromatic protons appeared in the range of δ 6.79–7.13 as multiplet. Two distinct ABq were observed at 7.42–7.46 ($J = 9$ Hz) and 7.62–7.66 ($J = 9$ Hz), respectively, representing para coupling of the indomethacin molecule. In ^{13}C NMR, signals appeared at δ 168.24 and 169.50 for COO (ester linkage) and C=O (indomethacin nucleus). The mass spectrum of the compound **3c** showed molecular ion peak at m/z 489.06 (M^+).

The IR spectrum of the derivative **3d** showed the absorption peaks at 3064.9 cm^{-1} characteristic of C–H stretching. The peaks at 1752.9, 1685.7, and 1683.6 cm^{-1} showed presence of C=O (ester linkage), (C=O st aldehyde), and C=O (indomethacin), respectively. The ^1H NMR spectrum of **3d** showed the signals at δ 2.45 for CH_3 protons (s, indomethacin ring), 3.79 (s, 3H, OCH_3, vanillin), 3.83 for OCH_3 protons (s, indomethacin ring), δ 3.95 for CH_2 (s, indomethacin). Aromatic protons appeared in the range of δ 6.68–7.43 as multiplet. Two distinct ABq were observed at 7.45–7.47 ($J = 9$ Hz) and 7.65–7.69 ($J = 9$ Hz), respectively, representing para coupling of the indomethacin molecule. The signal for –CHO proton appeared at δ 9.92 as singlet. In ^{13}C NMR, signals appeared at δ 168.32, 169.50, and 191 for COO (ester linkage), C=O (indomethacin nucleus) and CHO (aldehyde, vanillin). The mass spectrum of the compound **3d** showed molecular ion peak at m/z 491.91 (M^+).

The IR spectrum of the derivative **3e** showed the absorption peaks at 3025.8 cm^{-1} characteristic of C–H stretching. The peaks at 1759.8 and 1683.1 cm^{-1} showed presence of C=O (ester linkage) and C=O (indomethacin), respectively. The ^1H NMR spectrum of **3e** showed the signals at δ 2.43 for CH_3 protons (s, indomethacin ring), 3.83 for OCH_3 protons (s, indomethacin ring), 3.86 for CH_2 (s, indomethacin), and 5.95 for methylenedioxy (–OCH_2O) of

sesamol as singlet. Aromatic protons appeared in the range of δ 6.47–7.04 as multiplets. Two distinct ABq were observed at 7.45–7.48 ($J = 9$ Hz) and 7.66–7.68 ($J = 9$ Hz), respectively, representing para coupling of the indomethacin molecule. In ^{13}C NMR, signals appeared at δ 168.35 and 169.68 for *C*OO (ester linkage) and *C*=O (indomethacin nucleus). The mass spectrum of the compound **3e** showed molecular ion peak at *m*/*z* 477.92 (M^+).

The IR spectrum of the derivative **3f** showed the absorption peaks at 3036.1 cm^{-1} characteristic of C–H stretching. The peaks at 1776.7, 1758.9, and 1687.6 cm^{-1} showed presence of C=O st (aldehyde), C=O (ester linkage), and C=O (indomethacin), respectively. The ^{1}H NMR spectrum of **3f** showed the signals at δ 2.46 for CH_3 protons (s, indomethacin ring), 3.83 for OCH_3 protons (s, indomethacin ring), 3.94 for CH_2 (s, indomethacin). Lactone ring protons of umbelliferone showed doublet at δ 6.36 and 7.65 ($J = 9.6$ Hz), respectively, indicating the *cis* configuration. Aromatic protons appeared in the range of δ 6.68–7.47 as multiplets. In ^{13}C NMR, signals appeared at δ 160.27, 168.32, and 168.68 for *C*=O (umbelliferone), *C*OO (ester linkage), and *C*=O (indomethacin nucleus). The mass spectrum of the compound **3f** showed molecular ion peak at *m*/*z* 501.97 (M^+).

The IR spectrum of the derivative **3g** showed the absorption peaks at 2957.9 cm^{-1} characteristic of C–H stretching. The peaks at 1730.1 and 1683.6 cm^{-1} showed presence of C=O (ester linkage) and C=O (indomethacin), respectively. The ^{1}H NMR spectrum of **3g** showed the signals at δ 2.38 for CH_3 protons (s, indomethacin ring), 3.83 for OCH_3 protons (s, indomethacin ring), 3.65 for CH_2 (s, indomethacin). The signals for methyl group of menthol appears at δ 0.67–0.69 as doublet with $J = 7.0$ Hz. The methine proton appears as multiplet along with gem dimethyl protons at δ 0.81–0.96 as multiplet. The cyclic methylene and methine protons appeared as multiplets in the range of δ 0.99–2.02. Aromatic protons appeared in the range of δ 6.66–6.98 as multiplets. Two distinct ABq were observed at 7.45–7.47 ($J = 9$ Hz) and 7.64–7.66 ($J = 9$ Hz), respectively, representing para coupling of the indomethacin molecule. In ^{13}C NMR, signals appeared at δ 168.32 and 168.68 for *C*OO (ester linkage) and *C*=O (indomethacin nucleus). The mass spectrum of the compound **3g** showed molecular ion peak at *m*/*z* 496 (M^+).

Pharmacological evaluation

The parent drug, indomethacin has been used as reference substance.

Antiinflammatory activity

Antiinflammatory activity was determined by using carrageenan induced rat paw edema model (Winter *et al.*, 1962). Carrageenan (1% w/v) was used to produce paw edema. Edema is presented as percentage increase in right hind paw, in comparison to the uninjected left hind paw. Percentage change in paw volume was calculated and expressed as the amount of inflammation. For antiinflammatory activity, the test compounds (**3a–g**) were administered orally at molar equivalent doses of indomethacin (12 mg/kg, p.o.). All codrugs at molar equivalent doses showed significantly increased antiinflammatory activity as compared to that produced by indomethacin (Table 2). This increased activity may be due to the combined effect of improved physicochemical properties of conjugates and contribution by their corresponding promoieties (indomethacin and antioxidants). Furthermore, equimolar physical mixtures of indomethacin and promoieties in equimolar proportion were also studied for the antiinflammatory activity. These physical mixtures showed comparable results to the parent indomethacin, but lower than their corresponding conjugates (Table 2).

Analgesic activity

For the analgesic activity, abdominal writhing assay was performed (Koster *et al.*, 1959). Writhing was induced by intraperitoneal (i.p.) injection of freshly prepared acetic acid solution (1%, 10 ml/kg, i.p.) in mice. The number of writhes (constriction of abdomen, turning of trunk, and extension of hind limbs) due to acetic acid was expressed as a nociceptive response. Vehicle treated control mice were given 1% acetic acid and writhing response was noted for 20 min. Indomethacin (10 mg/kg, p.o.) as well as synthesized conjugates at equimolar doses significantly reduced the writhing response (Table 2). The results showed that these derivatives (**3a–g**) possess analgesic activity comparable to the parent drug (Table 2).

Antiulcer activity

The codrugs (**3a–g**) were screened for their ulcerogenicity in rats, using parent drug induced acute gastric ulcerations (Cioli *et al.*, 1979). The animals were fasted for 24 h, divided into different groups containing six animals in each group. Control group was treated with an equal volume of 0.5% carboxy methyl cellulose (CMC) vehicle. Animals were killed 8 h after the treatment. The stomach was removed, opened along the greater curvature, washed with saline, and observed for ulcers. For the acute gastric damage evaluation, the parent drug indomethacin was used to produce gastric ulcers. For this purpose, indomethacin (48 mg/kg, p.o.) was administered which produced a significant increase in ulcer index (5.54 ± 0.09) as compared to the control group (0.2 ± 0.06). All conjugates (**3a–g**) showed significantly reduced gastric damage (Table 2). The reduction in ulcer

Table 2 Antiinflammatory, analgesic, and antiulcer activity of indomethacin, indomethacin–antioxidant codrugs, and indomethacin + antioxidant physical mixtures

Compound	Antiinflammatory activity			Analgesic activity		Antiulcer activity	
	Dose (mg/kg, p.o.)	% Increase in paw volume mean ± SEM		Dose (mg/kg, p.o.)	% Inhibition mean ± SEM	Dose (mg/kg, p.o.)	Ulcer index mean ± SEM
		2 h	4 h				
Control	0.5% CMC	64.73 ± 0.72	81.58 ± 0.63	0.5% CMC	–	0.5% CMC	0.23 ± 0.09
Indomethacin (**1**)	12.0	48.35 ± 0.56*	57.39 ± 0.60*	10.0	74.87 ± 0.88	48	5.54 ± 0.84
3a	15.6	46.89 ± 0.65*,#	48.21 ± 0.88*,#	12.9	78.66 ± 0.6#	62.2	1.15 ± 0.58*,#
1 + 2a	12 + 4.2	48.1 ± 0.76*	54.61 ± 0.57*	–	–	48 + 16.6	4.17 ± 0.19*,#
3b	16.9	44.33 ± 0.97*,#	45.39 ± 0.83*,#	14.08	84.75 ± 0.83#	67.6	0.93 ± 0.28#
1 + 2b	12 + 5.5	47.68 ± 0.67*	52.71 ± 1.06*,#	–	–	48 + 22	4.08 ± 0.34*,#
3c	16.4	50.25 ± 0.65*,#	54.61 ± 0.95*	13.69	73.19 ± 0.91#	65.7	1.37 ± 0.63*,#
1 + 2c	12 + 5.1	50.99 ± 0.17*	59.13 ± 1.01*,#	–	–	48 + 20	4.77 ± 0.46*
3d	16.5	47.13 ± 0.96*,#	49.87 ± 0.77*,#	13.75	81.46 ± 1.49#	66	1.23 ± 0.36*,#
1 + 2d	12 + 5.1	49.27 ± 0.78*	57.51 ± 0.62*	–	–	48 + 20	4.26 ± 0.76*,#
3e	16.0	43.17 ± 0.81*,#	44.52 ± 0.59*,#	13.96	85.98 ± 0.79#	67.1	0.79 ± 0.47#
1 + 2e	12 + 4.7	47.01 ± 0.49*	51.36 ± 0.69*,#	–	–	48 + 19	3.92 ± 0.14*,#
3f	16.8	48.06 ± 0.61*,#	50.27 ± 1.39*,#	13.35	75.23 ± 0.54#	64.1	1.28 ± 0.44*,#
1 + 2f	12 + 5.5	49.66 ± 0.98*	56.92 ± 1.07*,#	–	–	48 + 22	4.35 ± 0.49*,#
3g	16.6	52.01 ± 0.89*,#	55.54 ± 1.47*,#	13.9	73.94 ± 1.78#	66.5	1.41 ± 0.19*,#
1 + 2g	12 + 5.3	52.25 ± 1.27*	58.85 ± 0.79*,#	–	–	48 + 21	4.96 ± 0.51*

* $P < 0.05$ as compared to control, # $P < 0.05$ as compared to indomethacin

index by the physical mixture of indomethacin and antioxidant was negligible as compare to their conjugates (Table 2). This may be due to the polar nature of the antioxidants resulting in their poor bioavailability, whereas reduction in ulcer index by the conjugates was significant which may be due to the improved physicochemical properties and contribution by the antioxidant promoiety after the cleavage of the codrug.

The results listed in Table 2 showed that these indomethacin–antioxidant codrugs (**3a–g**) lack gastrointestinal ulcerogenic side effects with retention of antiinflammatory and analgesic activity.

Conclusion

In our attempt to combine antiinflammatory and antioxidant activities, it has been possible to synthesize indomethacin–antioxidant codrugs as safer NSAIDs using different naturally occurring phytophenols as antioxidant promoieties. Further, these agents were found to possess encouraging results with retention of antiinflammatory and analgesic activity with significant reduction in ulcerogenic side effects of the parent NSAID. The indomethacin–guaiacol (**3a**), indomethacin–eugenol (**3b**), indomethacin–vanillin (**3d**), indomethacin–sesamol (**3e**), conjugates showed maximum antiulcer activity. The absence of gastric damage in all these cases may be attributed to the combined effect of antioxidant activity of the compounds as well as improved physicochemical properties of the codrugs. Furthermore, indomethacin with antioxidants physical mixture did not effectively reduce the risk of GI side effects in comparison to their corresponding conjugates. These results suggest that there is a potential advantage in giving such drugs having complementary pharmacological activities, in the form of single chemical entity, i.e., codrugs which are designed with improved physicochemical properties.

Experimental protocols

Chemistry

Melting points (mp) were determined on a Veego melting point apparatus and are uncorrected. For TLC, glass plates coated with silica gel (E. Merck) were used. The TLC plates were activated at 110°C for 30 min and visualized by exposure to iodine vapors. Glass columns of appropriate sizes were used. Silica gel (60–120 mesh, BDH) was used as adsorbent. IR spectra were recorded on Perkin Elmer 882 spectrometer using potassium bromide pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker AC 300 F, 400 MHz spectrometer using $CDCl_3$ or DMSO-d_6

as solvents and tetramethylsilane as internal standard. Mass spectra were obtained with Vg-11-250J 70s mass spectrometer at 70 eV using electron ionization (EI) sources. The synthetic reactions were monitored by TLC. The structures of all new compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR data, elemental analysis, and mass spectrometer; homogeneity was confirmed by TLC. Solutions were routinely dried over anhydrous sodium sulfate prior to evaporation. Guaicaol, eugenol, vanillin, menthol, thymol, sesamol, and umbelliferone were purchased from Sigma-Aldrich. All other reagents and solvents were of AR grade.

General procedure for the synthesis of indomethacin–antioxidant codrugs (**3a**–**g**)

Indomethacin (3.57 g, 0.01 M) was dissolved in 25 ml of chloroform followed by the addition of DCC (2.06 g, 0.01 M). The reaction mixture was stirred at room temperature for 1 h. To this solution, antioxidant (0.01 M) and DMAP (40 mg) were added and stirred for 24 h at room temperature. The precipitated dicyclohexyl urea was filtered off and the solvent of the filtrate was removed under reduced pressure. To the residue obtained, cold ether (20 ml) was added. The ethereal solution was filtered and the filtrate was washed with acetic acid (1%, 3 × 50 cml), HCl (5%, 3 × 50 cml), $NaHCO_3$ (5%, 3 × 50 ml), and finally with water (3 × 50 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and solvent was removed under reduced pressure to obtain crude product, which was recrystallized from petroleum ether and ethyl acetate to obtain pure compounds. This general procedure was used starting with different antioxidants (**2a**–**g**) to prepare various indomethacin–antioxidant codrugs (**3a**–**g**). The final products were obtained as solids and recrystallized from petroleum ether and ethyl acetate (Table 1).

Pharmacology

Animals

Sprague-Dawley (sd) rats (weighing 150–200 g) of both sex and LACCA mice (male, 25.35 g) procured from central animal house, Panjab University, Chandigarh, India were used. The animals were housed in plastic cages (five rats/cage) under standard laboratory conditions and maintained on rat chow and water.

Unless otherwise stated, the following conditions were employed in all experiments. The test compounds were suspended in 0.5% carboxymethylcellulose (CMC) and administered per orally (p.o.). Control animals were given the corresponding amount of vehicle (0.5%, CMC). The test drugs were administered on molar equivalent basis of indomethacin.

Antiinflammatory activity

Antiinflammatory activity was determined by using carrageenan induced rat paw edema model. Rats were divided into different groups and the indomethacin–antioxidant codrugs were administered to each group. Acute edema was induced in left hind paw of rats by injecting freshly prepared solution of carrageenan (Type IV, 0.1 ml, 1%) under plantar region of left hind paw. In the right paw, saline (1 ml, 0.9%) was injected, which served as control for comparison. The increase in paw volume was measured by using plethysmometer (water displacement, UGO BASILE, Italy) at 2 and 4 h after carrageenan challenge. Percentage change in paw volume was calculated and expressed as the amount of inflammation.

Analgesic activity

Analgesic activity was determined by using abdominal writhing assay. Mice were divided into different groups containing six animals in each group. Writhing response was elicited by intraperitoneal (i.p.) injection of freshly prepared acetic acid solution (1%, 10 ml/kg, i.p.). The number of writhes due to acetic acid was expressed as antinociceptive response. The number of writhes per animal was counted during a 20 min period. Writhings were counted 3 min after the injection of acetic acid solution.

$$\%\,\text{Inhibition} = (1 - N_t/N_c) \times 100$$

where N_c number of writhes in control group and N_t number of writhes in drug treated group

Antiulcer activity

The fasted animals (rats) were divided into different groups containing six animals in each group. Animals were treated with indomethacin (48 mg/kg, p.o.), equimolar doses of indomethacin–antioxidant codrugs and their physical mixture. Animals were killed 8 h after the treatment. The stomach was removed, opened along greater curvature, washed with saline, and observed for the ulcers. The ulcers were scored as: 0 normal colored stomach, 0.5 red coloration, 1.0 spot ulcers, 1.5 hemorrhagic streaks, 2.0 ulcers >3 but <5, 3.0 ulcers >5.

Statistical analysis

Statistical analysis was carried out on in vivo studies data. The ulcer index data was subjected to student *t* test (unpaired), analysis of variance (ANOVA) test, followed

by Dunnett's test for determining the levels of significance in antioxidant studies. P values <0.05 were considered statistically significant.

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