Phytoconstituents from *Curcuma longa* L. aqueous ethanol extract and its immunomodulatory effect on diabetic infected rats

Manal H. Shabana[a], Eman M. Shahy[b], Mona M. Taha[b], Gehan M. Mahdy[b], Maha H. Mahmoud[c]

Departments of [a]Phytochemistry and Plant Systematics, [b]Environmental and Occupational Medicine, [c]Department of Food Science & Nutrition, National Research Center, Dokki, Egypt

Correspondence to Manal H. Shabana, Department of Phytochemistry and Plant Systematics, National Research Centre, El-Tahrir Street, Dokki 12311, Egypt
Tel: +20 100 196 1517; fax: +20 333 70931; e-mail: shabanamhs61@hotmail.com

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Background and objectives

Curcuma longa L. (*C. longa*) has been shown to possess a wide variety of pharmacological properties against malignant proliferation, inflammation, and parasitosis. Further, *C. longa* has been reported to possess antioxidant and hypoglycemic activities.

The aim of this study was to isolate and elucidate the structure of the phytoconstituents of turmeric and test the hypoglycemic, antioxidant, antibacterial, and anti-inflammatory properties of the alcohol extract of *C. longa* on diabetic rats infected with *Staphylococcus aureus*.

Materials and methods

The extraction and isolation of the constituents of *C. longa* were performed according to the standard methods of column, paper, and thin-layer chromatography. The chemical structure of the isolated compounds was established by R_f, by characterization of acid hydrolysates, by ultraviolet spectral analysis, and by ^{1}H-NMR spectroscopy. Serum glucose, immunoglobulin E (IgE), nitric oxide, cytokine levels (IL-6, TNF-α, and IL-1β), and blood cell counts (total and differential) were determined.

Results and conclusion

Three compounds were isolated from the alcohol extract of *C. longa*. These compounds were identified as apigenin 7-*O*-rhamnoside 4′-*O*-glucoside, a new compound isolated for the first time from nature, and 7-methoxyapigenin-6-*C*-glucoside and *N*-(3-methoxyphenyl) acetamide, two known compounds isolated from *C. longa* for the first time. Also, the alcohol extract of *C. longa* ameliorated the serum glucose, IgE, nitric oxide, and cytokine levels in diabetic infected rats with *S. aureus*.

Keywords:
apigenin-7-O-rhamnoside- 4′-*O*-glucoside, *Curcuma longa*, phytoconstituents, hypoglycemic and immunomodulatory activities, diabetic rats, "*Staphylococcus aureus*" infection, cytokines, IgE, nitric oxide, blood counts

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Introduction

Curcuma longa L. (Zingiberaceae) is an aromatic perennial plant. Plants of this family are widely distributed throughout Asia, where its rhizome is extensively used in culinary and traditional medicinal practices [1]. Curcuminoids and the volatile oil are the principal active constituents contained in *C. longa* rhizomes [2]. Curcuminoids are curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcuminoids exhibit free radical scavenging property [3]. Traditional Indian medicine claims the use of its rhizome powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism, and sinusitis [4]. Anticancer [5], anti-inflammatory [6], antioxidant [6,7], and parasiticidal [8] activities of curcumin have been reported.

Staphylococcus aureus is a significant human pathogen that causes a wide range of diseases [9]. *S. aureus* is notorious for developing rapid resistance to antibiotics, which is caused primarily by antibiotic selection and the horizontal transfer of resistance genes [10,11]. The staphylococcal infection was shown to cause massive systemic cytokine release from T cells [interleukin-2 (IL-2), tumor necrosis factor-β (TNF-β), and IFN-γ] and macrophages (IL-1β and TNF-α) [9]. Antimicrobial resistance has emerged among pathogenic bacteria since the beginning of the antibiotic era as a consequence of the selective pressure generated by the therapeutic use, abuse, and sometimes misuse of antibiotics for humans and animals [12].

At present, bacteria like *S. aureus*, expressing phenotype MDR, are among the most important causes of infections in nosocomial and community settings, and new drugs are urgently needed [13–15]. Diabetes mellitus (DM) is a chronic metabolic disease. In recent decades, studies have shown that oxidative stress and inflammatory reactions have critical roles in the pathogenesis of DM [16]. DM has been associated with immune dysfunction (e.g. damage to the neutrophil function, depression of the antioxidant

system, and humoral immunity) [17]. Also, it has been associated with an increase in oxidative stress through enhanced formation of superoxides as well as a decrease in antioxidant capacity [18]. Experimental evidence indicates that hyperglycemia causes the autoxidation of glucose, glycation of proteins resulting in generation of reactive oxygen species (ROS) [19]. A number of studies have shown that dietary curcumin reduces inflammation. New improved methods of delivering curcumin are being developed, including nanoparticles and lipid/liposome formulations that increase absorption and bioavailability of curcumin. These technologies will enable cell-directed targeting of curcumin and improve the therapeutic outcome [20]. Curcumin can be considered a suitable supplementation for the prevention and treatment of diabetes through its antioxidant and anti-inflammatory activities [16].

The purpose of the present study was to examine the effect of *C. longa* extract supplementation in diabetic and *S. aureus*-infected diabetic rats and also its effects on immune response by the detection of TNF-α, IL-6, IL-1β, immunoglobulin E (IgE), and nitric oxide (NO) levels (marker of oxidative stress) along with blood index levels.

Experimental

Materials and methods

Phytochemical investigations

Plant material: Rhizomes of *C. longa* were purchased from a local herbal shop and were identified by Dr I. Elgarf, Taxonomy Department, Faculty of Sciences, Cairo University. A voucher specimen has been deposited in the Herbarium of the NRC (Cairo, Egypt).

Preparation of 80% ethanol extract: The crude extract was prepared by percolating the air-dried powdered rhizomes (1 kg) with 80% ethanol until exhaustion. The filtered percolate was evaporated to dryness under vacuum at 40˚C to give 45 g of dark residue, 20 g of which was kept for biological activities (Cl-EtOH).

Isolation of phytoconstituents: The ethanol extract of *C. longa* (25 g) was fractionated on a reversed-phase polyamide column, with gradient solvent system, starting from 100% water to 100% ethanol, obtaining four fractions (I–IV). Fraction II (20% EtOH) was further purified on preparative paper chromatography using Whatman No 3MM with (S1) *n*-buOH-HOAc-H_2O (4 : 1:5 upper phase) and (S2) 15% aqueous HOAc and subcolumns of Sephadex (LH-20) using methanol as eluent to yield compounds 1 (14 mg) and 2 (20 mg). Fraction III (30% EtOH) was chromatographed on silica gel column chromatography developed with benzene : ethyl acetate (9 : 1) to afford compound 3 (8 mg). It was further purified by TLC on silica gel F 254 precoated plates, developed with solvent system (S3) chloroform: methanol. The pure compounds were subjected to R_f determination, chemical analysis, ultraviolet (UV) spectral analysis, and ^{1}H-NMR.

Biological investigation

Animals: Thirty white male albino rats (Sprague–Dawley strain) with body weight ranging from 115 to 160 g were used. Each animal was housed individually in a separate polypropylene cage and maintained at 25˚C under a 12 h light/dark cycle. The animals were fed normal rodent chow (El-Nasr Pharmaceuticals, Chemical Industries, Egypt) and had free access to food and water. The study protocol was approved by scientific committees at Cairo University and National Research Center (NRC, Egypt). Animal experiments were conducted according to the guidelines of animal care and ethics committee of the NRC (Approval no: 09210). The animals were divided into five groups, each comprising six rats: Group I consisted of normal control rats fed normal rodent chow; group II consisted of diabetic control rats fed normal rodent chow; group III consisted of diabetic infected control rats fed normal rodent chow; group IV consisted of diabetic rats fed normal rodent chow and supplemented with Cl-EtOH at 50 mg/kg/day; and group V consisted of diabetic infected rats fed normal rodent chow and supplemented with Cl-EtOH extract.

Preparation of streptozotocin for induction of diabetes

Streptozotocin (STZ) was freshly prepared before induction by dissolving it in 0.1 mol/l citrate buffer (PH 4.5; Sigma-Aldrich Co., USA).

Preparation of bacteria for infection

The culture for isolation of *S. aureus* strain was kindly provided by the Department of bacteriology, Faculty of Medicine, Ain Shams University, Egypt. A urine sample, from a 42-year-old patient, allowed to culture and having a sensitivity for pus cells over hundred, was streaked into a nutrient agar plate and incubated at 37˚C for 2 days. After the incubation, bacteria were subjected to identification by Gram staining and a confirmatory test. The identified bacteria were shown to be *S. aureus*. Bacteria were harvested by centrifugation at 9000 *g* for 10 min. The bacteria were suspended in sterile saline solution and centrifuged again. Resuspension in sterile saline solution and centrifugation were repeated three times. Inoculum suspensions were prepared by resuspending the washed bacteria in 4 ml of sterile saline solution. The final suspension contained 10^8 *S. aureus* CFU.

Induction of diabetes

Groups II, III, IV, and V were induced with a single injection of freshly prepared STZ (50 mg/kg body weight) by the intraperitoneal route [21]. Four days after STZ administration, diabetes was confirmed by determination of fasting blood glucose concentration with the help of a glucometer. The animals with blood glucose more than 200 mg/dl were selected to compose the diabetic control, diabetic infected control, and supplemented groups (groups IV and V).

Infection with bacteria

The back of each diabetic rat to be infected was shaved with a razor blade and a wound measuring 1 cm in length was made using a surgical blade. One hundred microliters of the bacterial suspension (10^8 *S. aureus* cells) were slowly applied through a micropipette on the wounded area. One week later, bacterial infection was confirmed by the appearance of abscess [22].

Treatment with *Curcuma longa* ethanol extract

Groups IV and V were administered the antioxidant *C. longa* (Cl-EtOH extract) orally at a dose of 50 mg/kg for 6 weeks. Control animals (groups I, II, III) were also administered saline orally (50 mg/kg). At the end of 6 weeks of treatment, body weight was recorded.

Blood collection and biochemical analysis

At the end of the experimental period, blood was collected from the retro-orbital plexus using a glass capillary (after overnight fasting) and delivered into EDTA tubes and non-EDTA tubes. The obtained blood was used for the following analyses:

(1) The portion of blood in EDTA tubes from rats in each group was used to determine the total and differential leukocyte counts using cell dyne.
(2) The other portion of blood in non-EDTA tubes from rats in each group was left for 30 min at room temperature and centrifuged for 10 min at 3000 rpm. The separated serum was used for the following purposes:
   (a) Glucose estimation by glucose oxidase/peroxidase using a standard commercial kit supplied by Biodiagnostic Co. Cairo, Egypt: Glucose is oxidized by glucose oxidase to produce gluconate and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4-aminoantipyrene and phenol in the presence of peroxidase to yield a red quinoneimine dye that is measured at 505 nm. The absorbance nm is proportional to the concentration of glucose in the sample.
   (b) NO determination using a standard commercial kit supplied by Biodiagnostic Co.: It involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. Total nitrite is then identified as a colored azo-dye product of the Griess reaction that absorbs visible light at 540 nm [23].
   (c) Determination of cytokine (TNF-α, IL-6, and IL-1β) and total IgE levels by means of the sandwich enzyme-linked immunosorbent assay using a commercially available kit (Ray Biotech Inc. and Glory Sciences Co. Ltd, USA, www.raybiotech.com).

Statistical analysis

Analysis of variance and a post-hoc test of least-significant differences were used to test for statistically significant differences between the different groups other than control with the control groups.

Results

Three compounds were isolated and identified from the hydroalcoholic extract of *C. longa*: Apigenin-7-O-α-L-rhamnoside-4′-O-β-glucoside (**1**), in the form of pale yellow powder (14 mg). R_f PC in (BAW) 0.18, (15% Ac.) 0.70. UV (MeOH) λ_{max} nm 269, 330; (+NaOMe) 293, 378 (decrease in intensity); (+$AlCl_3$) 278, 298, 338, 380; (+$AlCl_3$/HCl) 279, 298, 334, 379; (+NaOAc) 270, 330; (+NaOAc/H_3BO_3) 270, 335. ^{1}H-NMR(300 MHz, DMSO,d_6) δ 7.90 (2H,d, *J* = 8.5 Hz, H-2′,6′); 6.85 (2H,d, *J* = 8.5 Hz, H-3′,5′); 6.79 (1H,d, *J* = 2 Hz, H-8); 6.76 (1H,s,H-3); 6.41 (1H,d, *J* = 2 Hz, H-6); 5.39 (1H,br s,H-1″Rha.); 5.04 (1H,d, *J* = 7.5 Hz, H-1‴Glc.); 3.20–3.60 (m, sugars protons hidden by OH groups); 1.1 (3H,d, *J* = 6 Hz, Rha CH_3).

7-methoxyapigenin-6-C-β-glucoside (**2**), in the form of yellowish white powder (20 mg). R_f PC in (BAW) 0.50, (15% Ac.) 0.46. UV (MeOH) λ_{max} nm 268, 336; (+NaOMe) 268, 380; (+$AlCl_3$) 278, 300, 340, 380; (+$AlCl_3$/HCl) 279, 300, 339, 379; (+NaOAc) 270, 335; (+NaOAc/H_3BO_3) 269, 333. ^{1}H-NMR(300 MHz, DMSO,d_6) δ 7.80 (2H,d, *J* = 8.5 Hz,H-2′,6′); 6.90 (2H,d, *J* = 8.5 Hz, H-3′,5′); 6.60 (1H,s,H-8); 6.50 (1H,s,H-3); 4.60 (1H,d, *J* = 8.0 Hz,H-1″glucose.); 3.88 (3H,s,OCH_3 at C-7.); 3.50–4.00 (m, sugar protons hidden by OH groups).

N-(3-methoxyphenyl) acetamide (3), in the form of brown amorphous powder (8 mg). R_f PC in ($CHCl_3$: MeOH, 9: 1) 0.60. UV (MeOH) λ_{max}: 245 nm. EI-MS: *m/z* 165 for molecular formula $C_9H_{11}NO_2$. ^{1}H-NMR(300 MHz, DMSO,d_6) δ 9.18 (1H,br s

N-H); 8.85 (1H,dd, J = 8.5, 2.0 Hz, H-6); 8.75 (1H,dd, J = 8.5, 2.0 Hz, H-4); 8.30 (1H,d, J = 2 Hz, H-2); 8.10 (1H,m,H-5); 3.78 (3H,s, OCH_3 at C-3); 1.95 (3H,s, acetyl).

In both STZ-induced diabetic rats and infected diabetic rats there was significant increase in serum glucose (P < 0.001) with reduction in body weight (P < 0.001) compared with normal control rats. In addition, the level of NO revealed significant increase in both groups (P < 0.05 and P < 0.001, respectively) compared with normal control. Treatment with Cl-EtOH extract for 6 weeks demonstrated significant decrease in blood glucose (P < 0.001) in diabetic and diabetic infected groups compared with both normal and diabetic controls, whereas body weight gain showed significant decrease in both groups compared with normal controls after supplementation. The level of NO revealed a significant decrease in the diabetic infected group after supplementation (P > 0.001) compared with the diabetic control group. In contrast, supplementation with Cl-EtOH extract showed a nonsignificant change in serum glucose, body weight, and NO levels (Table 1).

The levels of different cytokines (IL-6, IL-1β, and TNF-α) and total IgE demonstrated a significant increase in diabetic and infected diabetic rat groups compared with normal controls. Table 2 shows a slight nonsignificant increase in the diabetic infected group compared with the diabetic one, whereas supplementation with Cl-EtOH extract for 6 weeks revealed a decrease in different cytokines and total IgE levels compared with diabetic and diabetic infected control groups. It should be noted that after supplementation the levels tend to return to the values of normal controls.

The total leukocyte count (TLC) showed a significant increase in diabetic, diabetic infected, and supplemented rats (P < 0.001) compared with controls.

Table 1 Effect of Cl-EtOH extract on body weight, serum glucose, and nitric oxide levels

Groups	Body weight gain (g)	Serum glucose (mg/dl)	Nitric oxide (μmol/l)
Normal control	111.67 ± 11.59	82.83 ± 9.13	19.90 ± 1.44
Diabetic infected control	−13.0 ± 11.15[a]	285.50 ± 19.53[a]	30.57 ± 8.59[a]
Diabetic control	47.03 ± 15.03[a]	274.67 ± 27.22[a]	27.57 ± 6.02[a]
Diabetic infected + Cl-EtOH extract	91.67 ± 19.26[a]	187.5 ± 3.62[a]	24.97 ± 1.33
Diabetic + Cl-EtOH extract	63.17 ± 21.96[a]	179.5 ± 7.79[a]	22.47 ± 2.18
F-ratio	33.64	110.92	4.22
P value	<0.001	<0.001	<0.01

Data are expressed as mean ± SD (n = 6); [a]P < 0.001 versus normal control.

These increases in diabetic and diabetic infected groups started to reduce nonsignificantly on supplementation with Cl-EtOH extract (Table 3). This increase in TLC appeared along with significant increase in differential lymphocyte percentage and nonsignificant decrease in the segmented neutrophil count in diabetic and infected diabetic rats compared with the normal control group. The lymphocyte percentage decreased significantly, whereas the neutrophil percentage increased upon supplementation with Cl-EtOH compared with the diabetic infected or diabetic group, and tended to return to normal control levels.

Discussion

Recently, we reported the isolation of flavonoids and curcuminoids from *C. longa* [24]. In the course of our continuing studies on the chemical constituents of *C. longa*, three compounds, two known and one new compound, were isolated and identified from the hydroalcoholic extract of *C. longa* [Figure 1]. The known compounds, 7-methoxyapigenin-6-C-β-glucopyranoside (**2**) and *N*-(3-methoxyphenyl) acetamide (**3**), were identified by comparison with authentic samples and spectroscopic data [25–27].

Compound **1** was isolated as a yellow powder. It showed chromatographic properties similar to those reported for flavonoid diglycosides [25]. Acid hydrolysis using 2N HCl for 5 h at 100°C afforded D-glucose and L-rhamnose as the sugar moieties after Co-PC with authentic specimens. The aglycone part was found to be apigenin (UV, MS, ^{1}H-NMR). The UV spectral data with diagnostic shift reagents indicated an apigenin structure [25] and the hydroxyl groups at positions 7- and 4′ were substituted. The ^{1}H-NMR spectrum confirmed that compound **1** is a diglycoside of apigenin on the basis of the signals of the two anomeric protons for glucose and rhamnose at δ 5.04 and 5.39. These chemical shifts confirmed that glucose and rhamnose are directly attached to aglycone. The doublet at δ 5.04 was assigned to anomeric proton H-1‴ of glucose with a coupling constant value equal to 7 Hz, indicating a β-configuration. The doublet at δ 5.39 was assigned to anomeric proton H-1″ of rhamnose. The coupling constant (J = 2 Hz) between H-1 and H-2 of rhamnose indicated an α-configuration. The three proton doublet (J = 6 Hz) for the rhamnose methyl was located at δ 1.20, indicating that rhamnose was attached to position 7 [28]. In the ^{1}H-NMR spectrum of **1** the signal at δ 7.90 (d, 2H, J = 8.5 Hz) was assigned to H-2′, 6′, whereas the resonance at δ 6.85 (d, 2H, J = 8.5 Hz) was assigned to H-3′,5′. The singlet at δ 6.76 was assigned to H-3 and signals for H-8 and H-6 appeared as two doublets (J = 2 Hz), at δ 6.79 and 6.41. Enzymatic

hydrolysis of compound **1** with an equal volume of β-glucosidase afforded **1a**. The UV spectral studies of **1a** showed that position 7 is occupied. Acid hydrolysis of **1a** afforded rhamnose and apigenin. Therefore, compound **1** was identified as apigenin 7-*O*-α-L-rhamnoside-4′-*O*-β-D-glucoside, a new compound isolated for the first time from nature.

Compound **2** was isolated as a yellowish white powder. Acid hydrolysis using 2N HCl for 5 h at 100°C afforded two dark intermediate spots on PC using an S2 solvent system (Wessely–Moser rearrangement producing two isomers) indicating a C-glycosidic nature [26]. $FeCl_3$ degradation followed by cochromatography with authentic sugar samples yielded glucose. UV spectral studies indicated an apigenin with the free 4′-OH group and substitution at positions 6 and 7. ^{1}H-NMR spectrum of **2** showed two doublets at d 7.80 and 6.90 ppm (*J* = 8.5 Hz) assigned to H-2′, 6′ and H-3′, 5′, respectively. The observed down field position of H-8 at δ 6.60 ppm was due to the occupation of the hydroxyl group at position 7 by the OCH3 group. The anomeric proton of C-glucosyl moiety appeared as a doublet at 4.60 ppm (*J* = 8 Hz), whereas the singlet at 3.88 ppm was assignable to the methoxy group at 7-position. These assignments led to the identity of compound **2** as 7-methoxyapigenin-6-C-β-D-glucopyranoside [29], isolated from *C. longa* for the first time.

Compound **3** isolated as a brown amorphous powder appeared as a dark spot under UV (254 nm). R_f in S3 = 0.60. UV(MeOH) 245 nm. EI-MS showed $[M]^+$at *m/z* 165 corresponding to a molecular formula $C_9H_{11}NO_2$. ^{1}H-NMR displayed a singlet at δ 9.18 for N-H proton. Two doublets of doublets, each with one proton integration at δ 8.85 and 8.75 (*J* = 8.5 and 2.0 Hz), were assigned to H-4 and H-6, respectively. The proton of H-5 appeared as a multiplet at δ 8.15, and H-2 appeared as a doublet at δ 8.30 (*J* = 2.0 Hz). The three protons of C-8 appeared as a singlet at δ 1.95. The methoxy group at position 3 appeared as a singlet at δ 3.78. From the previously explained data, compound **3** was identified as *N*-(3-methoxyphenyl) acetamide [30]. Derivatives of phenylacetamide and related aromatic fatty acids have been shown to possess antiproliferative and differentiating effects on various human cancer cell lines such as leukemias, prostate and breast carcinomas [27] [Figure 1].

Diabetes causes the autoxidation of glucose. These changes accelerate the generation of ROS and increase oxidative stress. Oxidative stress may play an important role in the development of complications in diabetes, such as immune deficiency and bacterial infection. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed and developing countries, because plant-derived drugs have been reported to be safe and without side effects [31]. On this basis, we selected a natural antioxidant compound to test its effects on immune functions of diabetic rats and diabetic rats infected with *S. aureus*. Curcumin has previously been shown to reduce inflammation. For example, orally administered curcumin inhibited local TNF-α production and diminished the production of proinflammatory proteins in a murine lung model of infection. This reduction in inflammation and related cytokines occurs without reduction in bacterial load, indicating that the

Table 2 Effect of Cl-EtOH extract on different serum cytokines and serum IgE levels

Groups	IL-6 (pg/dl)	IL-1β (pg/dl)	TNF-α (pg/dl)	IgE (μg/ml)
Normal control	105.16 ± 13.08	37.23 ± 6.34	19.77 ± 0.99	18.41 ± 2.74
Diabetic infected control	141.89 ± 10.52[a]	56.33 ± 4.77[a]	34.31 ± 3.95[a]	28.67 ± 4.03[a]
Diabetic control	122.70 ± 10.17[a]	53.13 ± 1.95[a]	33.0 ± 2.96[a]	25.72 ± 3.28
Diabetic infected + Cl-EtOH extract	120.33 ± 4.97	41.72 ± 1.94	24.1 ± 2.61	21.82 ± 1.90
Diabetic + Cl-EtOH extract	110.1 ± 2.19	39.05 ± .95	21.62 ± 2.69	19.77 ± 1.76
F-ratio	6.83	4.41	5.71	4.92
P value	<0.001	<0.008	<0.002	<0.005

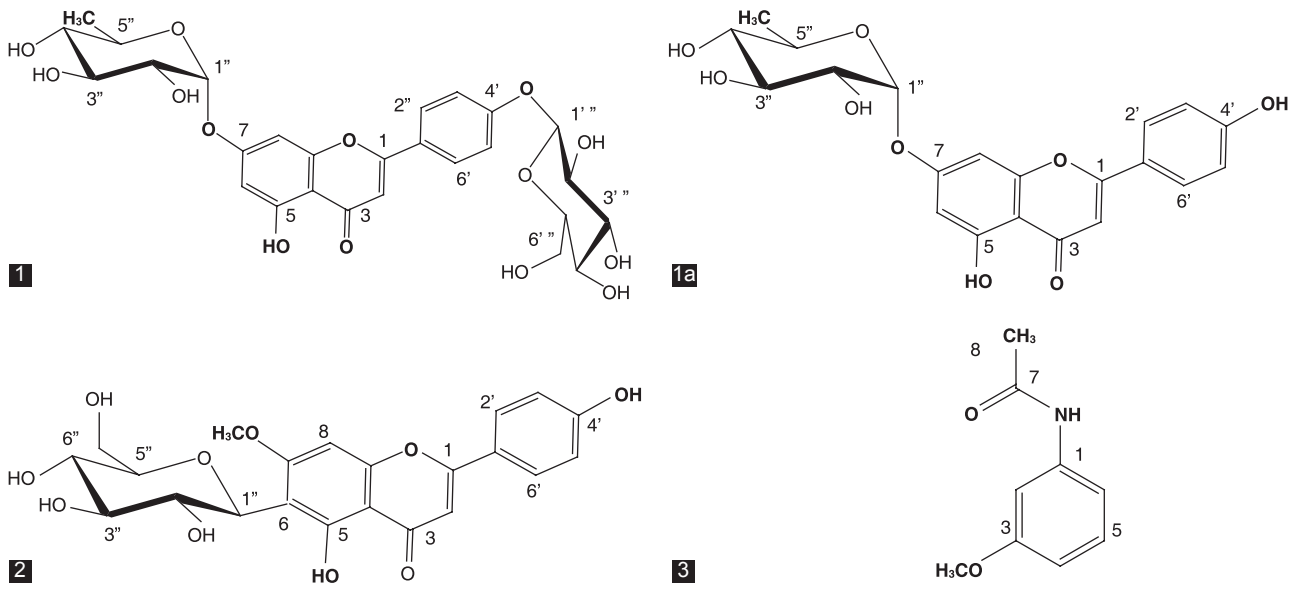
Data are expressed as mean ± SD (*n* = 6); [a]*P* < 0.001 versus normal control.

Table 3 Effect of Cl-EtOH extract on total leukocyte count and differential blood count

Groups	TLC	Lymph (%)	Segmented (%)	Monocyte (%)	Eosinophil (%)	Basophill (%)
Normal control	7.97 ± 1.01	58 ± 6.1	40.33 ± 5.89	1.5 ± 0.55	0.00 ± 0.00	0.00 ± 0.00
Diabetic infected control	13.22 ± 6.58[a]	78.83 ± 5.88[a]	19.67 ± 8.89	1.67 ± 1.03	0.17 ± 0.41	0.17 ± 0.41
Diabetic control	12.37 ± 3.13[a]	78.17 ± 9.5[a]	20.67 ± 4.37	1.67 ± 1.21	0.17 ± 0.41	0.00 ± 0.00
Diabetic infected + Cl-EtOH extract	10.71 ± 1.41	67.20 ± 7.13	31.62 ± 1.24	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Diabetic + Cl-EtOH extract	8.99 ± 2.12	61.92 ± 1.02	35.81 ± 3.61	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
F-ratio	2.96	8.2	8.22	0.83	0.67	1.0
P value	<0.04	<0.001	<0.001	NS	NS	NS

Data are expressed as mean ± SD (*n* = 6). TLC, total leukocyte count; [a]*P* < 0.001 versus normal control.

Figure 1



Chemical structure of isolated compounds from *Curcuma longa*

reduction in inflammation is a result of curcumin's anti-inflammatory activity and not due to any antimicrobial properties [9]. Diabetic rats showed symptoms of polyuria, polyphagia, polydipsia and reduced body weight, which were reversed on supplementation with curcumin extracts. Treatment with curcumin extracts reduced elevated blood glucose levels, which is in agreement with a previous study that showed that curcumin had an antihyperglycemic effect and improved insulin sensitivity; this action may be attributed at least in part to its anti-inflammatory properties, as evident by attenuating TNF-α levels in HFD-fed rats, and its antilipolytic effect, as evident from attenuating plasma free fatty acids. The curcumin effects are comparable to those of rosiglitazone, which indicates that they may act similarly. It could be a beneficial adjuvant therapy in patients with type 2 DM [32]. The curcumin treatment appeared to improve the overall function of β-cells, with very minor adverse effects. Its use in a prediabetic population may be beneficial.

In addition, curcumin impairs the T-cell stimulatory function of dendritic cells with reduced secretion of proinflammatory cytokines and NO and low surface expression of costimulatory molecules, leading to an overall diminished antigen-presenting cell activity. These in-vitro effects correlated with ex-vivo analysis of cells obtained from curcumin-treated mice during the course of autoimmune diabetes. These findings reveal an effective therapeutic effect of curcumin in autoimmune diabetes by its actions on key immune cells responsible for β-cell death [33]. In diabetes there is evidence of increased ROS formation (e.g. NO) as a result of oxidative stress. Also, during phagocytosis, the first step in macrophage response to the invading microorganism, NO is generated in excess as a result of host response against infections and inflammatory conditions [34]. In this study, the NO level increased in both diabetic and diabetic infected groups and reduced on supplementation with curcumin extracts.

NO regulates inflammatory responses, including cytokine production, depending on its concentration [35,36]. Cytokines are a class of signaling proteins that are used extensively in immune function. IL-1β, IL-6, and TNF-α are the most important immune response-modifying cytokines. Diabetes is a frequent underling medical condition among individuals with *S. aureus* infections, and diabetic patients often suffer from chronic inflammation and prolonged infections [37]. This complication correlated to deregulation of immune function during diabetes in the form of increased expression of inflammatory cytokines and enhanced generation of ROS [38]. Our results showed increase in the levels of total IgE in diabetic and diabetic infected rats as well as in the most important immune response cytokines (IL-1β, IL-6, and TNF-α), similar to that observed in diabetic patients [39,40]. Treatment of these rats with curcumin extracts, as an antioxidant, reduced total IgE and cytokines levels, suggesting an overall improvement of immune function by reducing levels of proinflammatory cytokines in diabetes and diabetic infection. This is in agreement with other studies that reported potential antioxidant and anti-inflammatory activities for curcumin [41].

One of the key steps during inflammation is leukocyte infiltration [35]. Peripheral blood leukocytes are

composed of polymorphonuclear cells, including monocytes and lymphocytes. They are activated and secrete cytokines in the diabetic state [42]. A number of studies have shown that diabetic patients have leukocytosis [43–46]. Our results revealed a significant increase in TLC with significant increase in lymphocyte percentage in both diabetic and diabetic infected rats compared with controls. Otton *et al*. [47] suggested that a high proportion of apoptotic lymphocytes in diabetic states may explain the impaired immune function in poorly controlled diabetic patients. Our study showed decreasing TLC and lymphocyte percentage on supplementation with curcumin returning to normal control levels. Thus curcumin supplementation improves immune function in diabetic rats.

Neutrophils are short-lived but abundant leukocytes. They are rapidly recruited to the site of a bacterial infection and are generally considered to be part of the 'first line of defense' of the host innate immune system. Because of their sheer numbers, as well as their toxic contents and elaboration of proinflammatory cytokines, neutrophil clearance is key to the resolution of the inflammatory response and hence tightly regulated [48]. Neutrophil apoptosis (either spontaneous or pathogen induced) is crucial for neutrophil uptake and subsequent elimination by macrophages at the site of infection, leading to resolution of the inflammatory process [49]. Previous studies have reported impaired bactericidal function and decreased phagocytic activity by neutrophils in diabetic hosts [50]. Recently, Hanses *et al*. [51] suggested that defects in neutrophil apoptosis may contribute to the chronic inflammation and the inability to clear staphylococcal infections observed in diabetic patients. Our results revealed decrease in neutrophil percentage during infection and diabetes, indicating decreased phagocytic activity by neutrophils — that is, a defect in immune response. These abnormalities might contribute to the increased susceptibility and severity of infections in diabetic patients. Our study revealed that curcumin supplementation decrease ROS and NO levels (improving oxidative stress) and increases the neutophil percentage, improving the bactericidal process.

Conclusion

C. longa extract exhibited a potent antihyperglycemic activity in STZ diabetic rats. Our results demonstrated that it has a role as an antioxidant in reducing oxidative stress in STZ diabetic rats and in diabetic staphylococcal infected rats in addition to improving immune functions by increasing total IgE, decreasing inflammatory cytokines (IL-6, IL-1β, and TNF-α), and decreasing total blood count with increasing neutrophil count. Thus, turmeric might serve as a hypoglycemic natural antioxidant compound and help in attenuating diabetic complications by reducing oxidative stress and improving immune functions.

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Conflicts of interest

There are no conflicts of interest.

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