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Antidiabetic Activity of Lupeol and Lupeol Esters in Streptozotocin-Induced Diabetic Rats

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Abstract

Currently, natural products have been shown to present interesting biological and pharmacological activities and are also used as chemotherapeutic agents. Plants have been used in treating cancer, cardiovascular disorders, tuberculosis and many other diseases. Therefore, the current study was designed to evaluate the antidiabetic potential of lupeol and its semisynthetic derivatives to get a new and potent antidiabetic agent. The ethanol extract of *Crataeva nurvala*, its hexane and chloroform soluble fractions and lupeol isolated from the extractives were evaluated.. Lupeol derivatives were prepared through a one-step reaction with acid chlorides, long chain fatty acids and aromatic moieties. A series of ester derivatives of lupeol were assayed for antidiabetic activity in (STZ-sucrose model rats. Few derivatives of lupeol showed more potent activity as compared to the basic molecule, lupeol. The results of the present study clearly indicated that the ethanol extract, fractions and lupeol isolated from *C. nurvala* and synthetic lupeol analogs possess significant antidiabetic activity. The models used for studying theantidiabetic activities have already been validated. In our studies, it was found that the ester derivatives of lupeol posses better antidiabetic potential when compared to lupeol. It is thus concluded that lupeol skeleton deserves further investigation for the development of more potent and non-toxic new antidiabetic agents for therapeutic applications.

Key words: *Crataeva nurvala*, antidiabetic activity, STZ-sucrose model, esters of lupeol.

Introduction

Diabetes mellitus is a serious chronic metabolic disorder that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system. The World Health Organization (WHO) has projected that the global prevalence of type 2 DM will more than double from 135 million in 1995 to 300 million by the year 2025. With the present population of 19.4 million diabetics and a projected increase of 300% and thereby leading to approximately 60 million by the year 2025, India would rank first in sharing global burden of diabetes (King et al. 1998). Traditional medicines most often applies to plants are being employed as adjuvants in the management of diabetes mellitus in many of the Asian countries including India. India has a rich history of using various potent herbs and herbal components for treating diabetes. Medicinal plant or herb have a variety of metabolites, aliphatic compound and aromatic compound, have basic skeleton of organic molecule and have various functional group that makes ability to alter the various metabolic pathway and makes them medicinally important. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh *et al.,* 2003). Therefore, investigation on such agents from traditional medicinal plants has become more important (Suba *et al*., 2004).

The plant *Crataeva nurvala* Linn. belongs to the family Capparidaceae is commonly known as Varuna in Sanskrit (Anonymous, 1950; Warrier *et al*., 1995). The bark has been used as sedative, stomachic, anthelmintic, anti-inflammatory, anti-tubercular, antipyretic (Anonymous, 1998) and in urolithiasis (Sharma *et al.*, 2001). The chemical constituents reported so far from the stem bark are lupeol which was identified as a major component in association with α and β-amyrin (Chakravarti *et al.,* 1975), lupeol acetate, spinasterol

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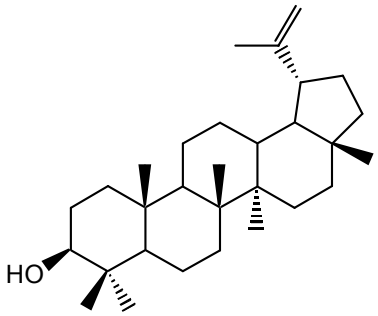
acetate (Lakshmi and Chauhan, 1975), taxarsterol (Lakshmi and Chauhan, 1976), 3-epilupeol (Sethi *et al*., 1978), cadabacine, cadabacine acetate (Ahmed *et al*., 1987), catechin, epicatechin-5-glucoside (Lakshmi and Chauhan, 1974), epifzelechin (Sethi *et al*., 1984) and glucocapparin (Sharma and Padhya, 1989). In continuation of our interest to develop drugs from natural sources, we selected the lupeol for evaluation of its antidiabetic property. Earlier work has shown that lupeol exhibits anti-inflammatory (Geetha and Varalakshmi, 1998) and cytoprotective (Sunitha *et al*., 2001) activities in experimental rat models. Topical anti-inflammatory effect of lupeol and its esters have been reported to be due to its effect on keratinocyte proliferation. Lupeol-3-palmitate and lupeol-3-linoleate, two synthetic long chain fatty acid ester analogues of the lupeol, were studied *in vitro* as potential inhibitors of serine protease activity (Hodges *et al*., 2003). In our studies, lupeol showed antidiabetic activity. Therefore, we planned to prepare ester derivatives of lupeol for a potent antidiabetic agent.

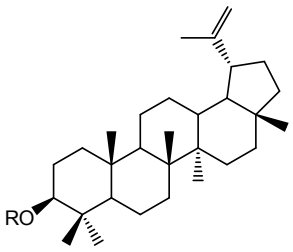
Materials and Methods

General experimental procedures: ^{1}H NMR spectra was recorded on a Bruker 300 FT NMR instrument using $CDCl_3$ as solvent and TMS as internal reference (chemical shifts in δ values). IR spectra were measured on a Beckmann Acculab-10 Spectrophotometer. Elemental analysis was carried out on a Carlo Erba Strumentazone. Melting points were determined on a hot stage melting point apparatus and are uncorrected.

Collection of the plant material: Stem bark of *C. nurvula* was purchased from the local market and authenticated by the botanists in Central Drug Research Institute, Lucknow, India.

Extraction and isolation: The air-dried powdered stem bark of *C. nurvula* (2.0 kg) was extracted with 95% ethanol at room temperature for 5 times. The ethanol extract was filtered and concentrated in a rotavapor below 50^0C to get a green viscous mass (74.5 g). The green mass (70.0 g) was fractionated into 4 fractions (hexane, chloroform, *n*-butanol soluble and *n*-butanol insoluble meterials). All four fractions were bio-assayed for antidiabetic activity against STZ induced rate model. The activity was localized in hexane and chloroform fractions only. TLC pattern of the hexane and chloroform fractions were found identical. Therefore these two fractions were mixed together (24.0 g) and was chromatographed over a column of silica gel, the major compound lupeol was purified and crystallized from methanol (yield; 1%).





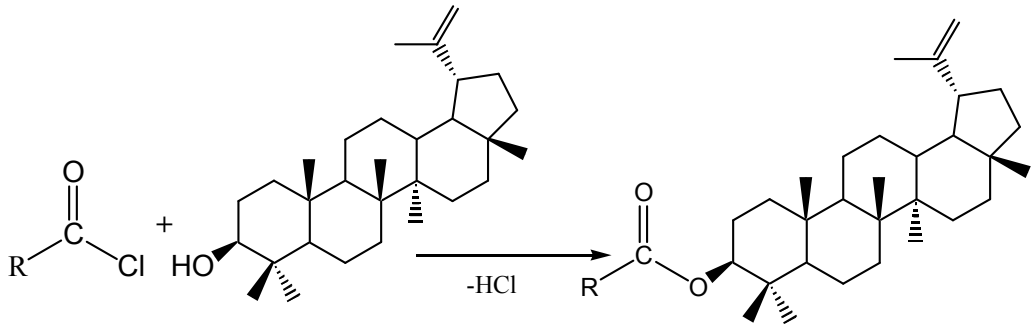
Lupeol		Parent Compound	
E-1	R= -acetoyl	**E-2**	R= -toluloyl
E-3	R= -salicyloyl	**E- 4**	R= -myristoyl
E-5	R= -palmitoyl	**E-6**	R= -stearoyl
E-7	R= -cinnamoyl	**E-8**	R= -*o*-Cl-benzoyl
E-9	R= - m-Cl-benzoyl	**E-10**	R= -*p*-Cl-benzoyl
E-11	R= -*o*-Br- benzoyl	**E-12**	R= -*m*-Br- benzoyl
E-13	R= -*p*-Br- benzoyl	**E-14**	R= -*o*-nitrobenzoyl
E-15	R= - m-nitro- benzoyl	**E-16**	R= -*p*-nitrobenzoyl
E-17	R= -*o*-methoxy- benzoyl	**E-18**	R= -*p*-methoxybenzoyl
E-19	R= -2-Cl-ethanoyl	**E-20**	R= -2-Cl-propanoyl
E- 21	R= -*o*-*p*-dichloro- benzoyl	**E-22**	R= -3,5-dinitrobenzoyl

Figure 2. Structures of the compound synthesized

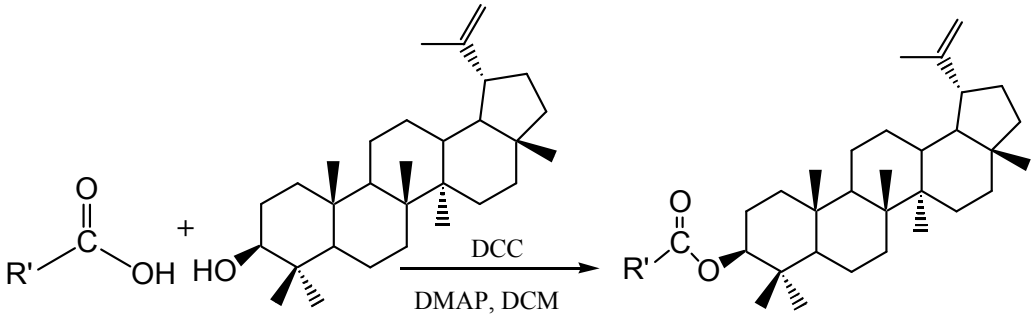
Lupeol: It was isolated from the combined hexane and chloroform fraction of the stem bark *C. nurvala* and we named it as compound-1. It displayed a molecular ion peak at *m/z* 426 for $[M]^+$ of lupeol and a molecular formula $C_{30}H_{50}O$. The ^1H and ^{13}C NMR spectra were found to exhibit characteristic signals for lup-20(29)-en-3-ol [9]. The structure was confirmed by comparison of spectroscopic data of the compound-1 to those described for lupeol (Lakshmi and Chauhan, 1975) and confirmation of the lupeol was also done by TLC with authentic sample of lupeol.

Lupeol: White microcrystalline powder; mp 213.0 C, $[\alpha]^{25}$ d +26.2 (*c* 0.67 in $CHCl_3$), UV ($CHCl_3$) λmax: 228 (60.1), 285 (31.8) nm; IR (KBr) cm^{-1}: 3326, 2931, 1631, 1450, 1377, 1035, 874; EIMS (70 ev) *m/z* (%): 425 (18) $[M^+ - H]$, 409 (23) $[M^+-OH]$ 218 (68), 207 (60), and 189 (100); ^1H NMR ($CDCl_3$, δ values): 0.77, 0.80, 0.84, 0.95, 0.97, 1.03 and 1.70 (each 3H, s, H-23, 24, 25, 26 27, 28, and 30), 2.38 (1H, dt, J = 4.0 and 9.6 Hz, H-19), 3.19 (1H, dd, J = 4.8 and 11.6 Hz, H-3), 4.57 (1H, brs, H-29b), 4.68 (1H, brs, H-29a); ^{13}C NMR ($CDCl_3$, δ values); 39.1, 27.8, 79.3, 39.2, 55.6, 18.7, 34.6, 41.2, 50.7, 37.5, 21.3, 25.5, 38.4, 43.2, 27.8, 35.9, 43.4, 48.3, 48.6, 151.1, 30.2, 40.4, 28.4, 15.8, 16.5, 16.3, 14.9, 18.4, 109.6, 19.7 (C-1 to C-30, respectively).

General method for preparation of lupeol esters with different acid chlorides: Lupeol dissolved in dry DCM (CH_2Cl_2) under nitrogen atmosphere and alkyl acid chloride and triethylamine were added in the molar ratio (1:1.2:1.2) respectively to this solution. The reaction mixture was stirred at room temperature for 1-2 hr (scheme 1). The solvent was removed. TLC analysis ($CHCl_3$, 1% Vanillin/H_2SO_4) indicated the formation of the desired product. The ester was purified by column chromatography over silica gel 60 (230-400 mesh, Merck) using hexane and ethyl acetate as eluent. The yield of ester was between the range of 75 - 90%.



Scheme 1: Reagents and conditions: Dry DCM (CH_2Cl_2), alkyl acid chloride, triethyl amine, 1-2 hrs, at room temperature.



Scheme 2. Reagents and conditions: (a) Dry CH_2Cl_2 , DCC, DMAP, 2-3 hrs.DCC = Di cyclohexyl carbodimide, DMAP = Di methyl amino pyridine.

General method for preparation of lupeol esters with different acid: Lupeol reacted with the acid, DCC and DMAP in dry CH_2Cl_2 in the molar ratio (1:1.2:1.2:0.12) respectively. The reaction mixture was refluxed for 2-3 hrs

(scheme 2). Formation of esters was checked by the TLC, the reaction mixture was worked up as usual. The esters were be purified by silica gel 60 (230-400 mesh, Merck) column chromatography using hexane and ethyl acetate as eluent. The yield of esters was checked.

Lupeol acetate (E-1) (Galoo *et al*., 2009)

White needles (MeOH), m.p. 145^{0}C, EIMS for $C_{32}H_{52}O_2$ *m/z* (rel. int.): 468 [M^+] (17.2%), 453 (2.9%), 408 (1.7%), 357 (3.9%), 218 (15.2%), 189 (46.4%), 109 (29.1%), 43 (100%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.69 (1H, s, H-29b), 4.57 (1H, s, H-29a), 4.47 (1H, dd, J = 4.4, 12.8 Hz, H-3), 2.05 (3H, s, H-2/), 1.69 (3H, s, H-30), 1.03 (3H, s, H-25) 0.94 (3H, s, H-28), 0.85 (3H, s, H-23),0.84 (3H, s, H-24), 0.83 (3H, s, H-26), 0.79 (3H, s, H-27). ^{13}C NMR ($CDCl_3$, 100MHz): δ 171.3 (C-1'), 151.2 (C-20), 109.6 (C-29), 81.2 (C-3), 55.6 (C-5), 50.5 (C-9), 48.5 (C-18), 48.2 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.2 (C-22), 38.6 (C-1), 38.0 (C-4), 37.3 (C-10), 36.2 (C-13), 35.8 (C-16), 34.4 (C-7),30.0 (C-21), 28.2 (C-2'), 27.6 (C-23), 25.3 (C-15), 24.0 (C-12), 21.7 (C-2), 21.1(C-11), 19.5 (C-30), 18.4 (C-6), 18.2 (C-28), 16.7 (C-24), 16.4 (C-25), 16.2 (C-26), 14.7 (C-27).

Lupeol toluate (E-2)

White powder from methanol, MS: (EIMS) [M^+] *m/z* 544; ^{1}H NMR (300 MHz, $CDCl_3$), 7.85 (d, 2H, Ha), 7.15 (d, 2H, Hb), 4.65 (m, 1H, C (3)-H), 2.29 (m, 1H, C (19)H, 4.50 (1H, brs, H-29b), 4.61 (1H, brs, H-29a), 2.30 (S, 3H, CH_3);^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 131.2 (C1'), 138.9 (C2'), 129.1 (C3'), 132.7 (C4'), 125.4 (C5'), 129.6 (C6'), 167.0 (C=O), 14.1 (CH_3). IR (KBr) υ_{max}cm^{-1}, 1715 (C=O)

Lupeol salicylate (E-3)

White powder from methanol, MS: (EIMS) [M^+] *m/z* 546; ^{1}H NMR (300 MHz, $CDCl_3$), 7.77 (d, 1H, Hd), 7.37 (t, 1H, Hb), 6.91 (d, 1H, Ha), 6.81 (t, 1H, Hc), 4.68 (dt, 1H, C(3)H),4.51(1H, brs, H-29b), 4.64 (1H, brs, H-29a),2.32 (m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively). 118.9 (C1'), 144.2 (C2'), 134.5 (C3'), 128.0 (C4'), 128.8 (C5'), 130.1 (C6'), 166.83 (C=O). IR (KBr) υ_{max}cm^{-1}, 1718 (C=O)

Lupeol myristate (E-4)

White crystals from methanol; MS: (EIMS) [M^+] *m/z* 636; ^{1}HNMR (300 MHz, $CDCl_3$,). ð 1.26 (3H, s, term. CH_3 ester), 1.68 (3H, s, H-30), 2.29 (lH, t, *d,* H-19), 2.50-2.60 (2H, m, -$COCH_2$-), 4.42 (lH, *dd,* H-3), 4.58 (lH, brs, H-29a), 4.69 (lH, brs, H-29b); ^{13}C NMR (CDCl3) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 32.7 (C1'), 24.8 (C2'), 28.9 (C3'), 29.6 (C4'- C10'), 31.6 (C11'), 22.8 (C12'), 14.2 (C13'), 171.3 (C=O).

Lupeol palmitate (E-5)

MS: (EIMS) [M^+] *m/z* 664; ^{1}HNMR (300 MHz, $CDCl_3$,). ð 1.26(3H, s, term. CH_3 ester), 1.68 (3H, s, H-30), 2.29 (lH, t, d, H-19), 2.50-2.60 (2H, m, -$COCH_2$-), 4.42 (lH, dd, H-3), 4.58 (lH, brs, H-29a), 4.69 (lH, brs, H-29b); ^{13}C NMR (CDCl3) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 32.7 (C1'), 24.8 (C2'), 28.9 (C3'), 29.6 (C4'- C12'), 32.1 (C13'), 22.9 (C14'), 14.0 (C15'), 171.5 (C=O).

Lupeol stearate (E-6)

MS: (EIMS) [M^+] *m/z* 692; ^{1}HNMR (300 MHz, $CDCl_3$): ð 1.26(3H, s, term. CH_3 ester), 1.68 (3H, s, H-30), 2.29 (lH, t, d, H-19), 2.50-2.60 (2H, m, -$COCH_2$-), 4.42 (lH, dd, H-3), 4.58 (lH, brs, H-29a), 4.69 (lH, brs, H-29b); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 32.7 (C1'), 24.8 (C2'), 28.9 (C3'), 29.6 (C4'- C14'), 32.1 (C15'), 22.9 (C16'), 14.0 (C17'), 171.6 (C=O).

Lupeol cinnamate (E-7)

MS: (EIMS) [M^+] m/z 556; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.63 (d, 1H, Hb), 7.53 (m, 2H, Hd), 7.37 (m, 3H, Hc & He), 6.44 (d, 1H, Ha), 4.58 (m, 1H, C(3)H), 4.59 (1H, brs, H-29b), 4.68 (1H, brs, H-29a), 2.38 (td, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4,

35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 118.9 (C1'), 144.2 (C2'), 134.5 (C3'), 128.0 (C4'), 128.8 (C5'), 130.1 (C6'), 166.83 (C=O). IR (KBr) υ_{max}cm^{-1}, 1709 (C=O)

Lupeol *o*-chlorobenzoate (E-8)

MS: (EIMS) [M$^+$] *m/z* 564; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.73 (d, 1H, Hb), 7.36 (dd, 2H, Ha,Hc), 7.23 (m, 1H, Hd), 4.68 (m, 1H, C(3)-H), 4.51 (1H, brs, H-29b), 4.62 (1H, brs, H-29a), 2.30(m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 131.9 (C1'), 130.1 (C2'), 133.7 (C3'), 133.2 (C4'), 129.8 (C5'), 127.8 (C6'), 167.0 (C=O), IR (KBr) υ_{max}cm^{-1}, 1718 (C=O).

Lupeol *m*-chlorobenzoate (E-9)

MS: (EIMS) [M$^+$] *m/z* 564; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.92 (S, 1H, Ha), 7.84 (d, 1H, Hc), 7.44 (dd, 1H, Hd), 7.33 (d, 1H, Hb), 4.65(dd, 1H, C(3) H), 2.29 (m, 1H, C(19)H), 4.50 (1H, brs, H-29b), 4.62 (1H, brs, H-29a); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 131.9 (C1'), 130.1 (C2'), 133.7 (C3'), 133.2 (C4'), 129.8 (C5'), 127.8 (C6'), 167.0 (C=O), IR (KBr) υ_{max}cm^{-1}, 1719 (C=O)

Lupeol *p*-chlorobenzoate (E-10)

MS: (EIMS) [M$^+$] *m/z* 564; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.89 (d, 2H, Hb), 7.33 (d, 2H, Ha), 4.6 (m, 1H, C(3)H), 2.31 (m, 1H, C(19)H), 4.51 (1H, brs, H-29b), 4.62 (1H, brs, H-29a); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 131.9 (C1'), 130.1 (C2'), 133.7 (C3'), 133.2 (C4'), 129.8 (C5'), 127.8 (C6'), 166.4 (C=O), IR (KBr) υ_{max}cm^{-1}, 1724 (-C=O).

Lupeol *o*-bromobenzoate (E-11)

MS: (EIMS) [M$^+$] *m/z* 609; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.68 (1H, dd, Hd), 7.56 (1H, dd, Hb), 7.28 (1H, dd, Ha), 7.26 (1H, m, Hc), 4.62 (m, 1H, C(3)H),4.52 (1H, brs, H-29b), 4.68 (1H, brs, H-29a), 2.29-2.32 (m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 134.6 (C1'), 121.8 (C2'), 132.4 (C3'), 131.6 (C4'), 127.4 (C5'), 131.3 (C6'),166.4 (C=O). IR (KBr) υ_{max}cm^{-1}, 1723 (C=O).

Lupeol *m*-bromobenzoate (E-12)

MS: (EIMS) [M$^+$] *m/z* 609; ^{1}H NMR (300 MHz, $CDCl_3$): δ8.07 (s, 1H, Ha), 7.89 (d, 1H, Hb), 7.60 (d, 1H, Hd), 7.24 (dd, 1H, Hc), 4.60(d, 1H, C(3)H), 4.50 (1H, brs, H-29b), 4.64 (1H, brs, H-29a); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 132.7 (C1'), 133.0 (C2'), 123.0 (C3'), 136.1 (C4'), 130.6 (C5'), 128.7 (C6'), 166.7 (C=O). IR (KBr) υ_{max}cm^{-1}, 1721 (C=O).

Lupeol *p*-bromobenzoate (E-13)

MS: (EIMS) [M$^+$] *m/z* 609; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.88 (d,2H, Ha), 7.54 (d, 2H, Hb), 4.60(d, 1H, C(3)H), 4.50 (1H, brs, H-29b), 4.62 (1H, brs, H-29a); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 129.5 (C1'), 131.9 (C2'), 131.7 (C3'), 127.4 (C4'), 166.4 (C=O). IR (KBr) υ_{max}cm^{-1}, 1721 (C=O)

Lupeol *o*-nitrobenzoate (E-14)

MS: (EIMS) [M$^+$] *m/z* 597; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.78 (d,1H, Ha), 7.68 (d, 1H, Hd), 7.56 (m, 2H, Hb, Hc), 4.67 (dd, 1H, C(3)H),4.50 (1H, brs, H-29b), 4.62 (1H, brs, H-29a);^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively); 125.6 (C1'), 149.6 (C2'), 123.5 (C3'), 133.7 (C4'), 134.5 (C5'), 130.6 (C6'), 166.6 (C=O), IR (KBr) υ_{max}cm^{-1}, 3422, 1726 (C=O).

Lupeol *m*-nitrobenzoate (E-15)

MS: (EIMS) [M$^+$] *m/z* 597; ^{1}H NMR (300 MHz, $CDCl_3$): δ8.80 (s,1H, Ha), 8.31 (d, 1H, Hd), 8.29 (d, 1H, Hb), 7.57 (dd, 1H, Hc), 4.72(m, 1H, C(3)H), 4.49 (1H,

brs, H-29b), 4.61 (1H, brs, H-29a), 2.31(m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 131.4 (C1'), 124.8 (C2'), 148.3 (C3'), 127.9 (C4'), 129.3 (C5'), 135.8 (C6'), 166.4 (C=O), IR (KBr) $\upsilon_{max}cm^{-1}$, 3426, 1720 .

Lupeol *p*-nitrobenzoate (E-16)

MS: (EIMS) [M^+] *m/z* 597; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22 (d, 2H, Hb), 8.14 (d, 2H, Ha), 4.71 (dd, 1H, C (3) H), 4.51 (1H, brs, H-29b), 4.63 (1H, brs, H-29a), 2.31(td, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 136.6 (C1'), 130.6 (C2'), 123.5 (C3'), 152.7 (C4'), 123.5 (C5'), 130.6 (C6'), 166.8 (C=O), IR (KBr) $\upsilon_{max}cm^{-1}$, 3433, 1721.

Lupeol *o*-methoxybenzoate (E-17)

MS: (EIMS) [M^+] *m/z* 560; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.80 (d, 1H, Ha), 7.45 (t, 1H, Hc), 6.98 (m, 2H, Hc, Hd), 4.58 (1H, brs, H-29b), 4.70 (1H, brs, H-29a), 4.73 (m, 1H, C(3)H);^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 116.1 (C1'), 163.2 (C2'), 114.0 (C3'), 133.8 (C4'), 120.7 (C5'), 130.7 (C6'), 167.0 (C=O), 56.0 (OCH_3). IR (KBr) $\upsilon_{max}cm^{-1}$, 1724.

Lupeol- *p*-methoxybenzoate (E-18)

MS: (EIMS) [M^+] *m/z* 560; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.99 (d, 2H, Ha), 6.92 (d, 2H, H_b), 4.69 (m, 1H, C(3)H), 4.57 (1H, brs, H-29b), 4.66 (1H, brs, H-29a), 2.38 (m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 122.8 (C1'), 130.7 (C2'), 114.0 (C3'), 166.3 (C4'), 114 (C5'), 130.7 (C6'), 167.0 (C=O), 56.0 (OCH_3). IR (KBr $\upsilon_{max}cm^{-1}$, 1721.

Lupeol -2-chloroethanoate (E-19)

MS: (EIMS) [M^+] *m/z* 502; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.36 (q, 1H, H-2'), 5.2 (br m, 1H, C(3)H), 2.27 (S, 3H, H-3'), 4.46-4.61 (br m, 2H, C(29)H,=CH_2), 2.36 (m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 48.6 (C1'), 166.4 (C=O). IR (KBr) $\upsilon_{max}cm^{-1}$, 1721 (C=O).

Lupeol 2-chloropropionate (E-20)

MS: (EIMS) [M^+] *m/z* 516; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.36 (q, 1H, H-2'), 5.2 (br m, 1H, C(3)H), 2.27 (S, 3H, H-3'), 4.46 (1H, brs, H-29b), 4.61 (1H, brs, H-29a), 2.36 (m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 57.7 (C1'), 18.0 (C2'), 166.4 (C=O). IR (KBr) $\upsilon_{max}cm^{-1}$, 1721 (C=O).

Lupeol *o,p*-dichlorobenzoate (E-21)

MS: (EIMS) [M^+] *m/z* 600; ^{1}H NMR (300 MHz, $CDCl_3$): δ7.87 (d, 1H, Hc), 7.39 (s,1H, Ha), 7.26 (d, 1H, Hb), 4.68 (m, 1H, C(3)-H), 4.50 (1H, brs, H-29b), 4.62 (1H, brs, H-29a), 2.30(m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 129.0 (C1'), 136.4 (C2'), 129.2 (C3'), 139.5 (C4'), 126.9 (C5'), 132.5 (C6'), 166.4 (C=O). IR (KBr) $\upsilon_{max}cm^{-1}$, 1718 (C=O)

Lupeol -3,5-dinitrobenzoate (E-22)

MS: (EIMS) [M^+] *m/z* 620; ^{1}H NMR (300 MHz, $CDCl_3$): δ9.15 (S,1H, Hb), 9.06 (S, 2H, Ha), 4.78 (m, 1H, C(3)H), 4.51 (1H, brs, H-29b), 4.63 (1H, brs, H-29a), 2.31(m, 1H, C(19)H); ^{13}C NMR ($CDCl_3$) 38.3, 23.6, 83.2, 37.7, 55.3, 18.2, 34.1, 40.7, 50.2, 37.5, 20.9, 25.0, 37.9, 42.7, 27.4, 35.5, 42.9, 48.2, 47.9, 151.2, 29.8, 39.9, 27.9, 16.5, 16.1, 15.9, 14.4, 18.0, 109.2, 19.2 (C-1-C-30, respectively), 132.3 (C1'), 130.9 (C2'), 149.2 (C3'), 123.0 (C4'), 149.2 (C5'), 130.9 (C6'), 166.2 (C=O), IR (KBr) $\upsilon_{max}cm^{-1}$, 1725 (C=O).

Animals: Male albino rats of Sprague Dawley strain (8 to 10 weeks of age: body weight 120 ± 20 g) were procured from the animal colony of Central Drug Research Institute, Lucknow, India. Breeding colonies of animals were maintained under SPF (specific pathogen

free) environment in standard housing conditions. Research on animals was conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964.

Chemicals: Streptozotocin and metformin were purchased from Sigma Chemical Company, St. Louis, USA. All other chemicals were of highest purity grade.

STZ-s PROCEDURE: Male albino rats of Sprague Dawley strain of the body weight 160±20g were selected for this study. Animals, 3/ cage were kept for 7 days under standard experimental conditions before the experiment. Animals were given standard rat-pellet diet and tap water ad libitum. Day O-Day before experiment, animals were kept for overnight starvation. Day 1: STZ Streptozotocin (Sigma, USA) was dissolved in 100 m M citrate buffer pH 4.5 and calculated amount of the fresh solution was injected to overnight fasted rats (45 mg/Kg.) intraperitonially. Day 2: Rats remained as such in the same conditions. Food pellets were removed on the penultimate day at 5:00 P.M. in the evening and animals were kept on over night starvation. Day 3: Blood-glucose level was estimated between 9:30-10:00 A.M. in all animals. Blood was taken from tail of the rats by stab techniques and the glucose level was estimated using "Advantage Glucometer" of Boehringer Mannheim Co, USA. Blood was checked 48 hours later by glucostrips and animals showing blood glucose values between 160 to 270mg./dl. (8 to 15 mM) were included in the experiments and termed diabetic. The diabetic animals were divided into groups consisting of 5 to 6 animals in each group. Rats of experimental groups were administered suspension of the desired test samples orally (made in 1.0% gum acacia) at 500 mg/Kg body weight in the case of ethanol extract and at 250 mg/kg.in case of the hexane and chloroform fractions. In the case of the pure compound and standard drug Metformin, the dose was taken at 100 mg/kg. The animals of the control group were given an equal amount of 1.0% gum acacia. A sucrose load of the 2.5g/kg of the body weight was given after 30 minutes of the drug administration. After 30 minutes of the post sucrose load blood glucose level was again checked by glucostrips at 30, 60, 90, 120, 180, 240, 300 minutes and 24 hours respectively. The animals not found diabetic after 24 hours post treatment of the test samples were not considered and omitted from the calculations and termed as non responders. The animals which did not show any fall in blood glucose profile in a group while the others in that group showed fall in blood glucose profile were also considered as non responders. The food but not the water was withheld from the cages during the experimentation. Comparing the AUC of experimental and control groups determined the percent of antihyperglycemic activity. Statistical comparison between groups was made by the Student's- t test.

AUC method

AUC (Area under curve, data not shown) was determined by using Prism soft ware (Mishra *et al.*, 2013). The percentage lowering in AUC of standar drug/ test sample treated group compared with control groupdetermined the percentage of improvement on oral glucose tolerance (OGTT) post sucrose load.

Table 1. Antihyperglycemic activity profile of ethanol extract of *C. nurvala,* its hexane and chloroform soluble fractions, lupeol, and standard drug metformin in sucrose challenged streptozotocin-induced diabetic rats.

Sl .Number	Test sample	Dose mg/kg	% Activity in sucrose challenged STZ-induced diabetic rats
1	Crude EtOH ext.	500	21.5
2	Hexane fr.	250	20.2**
3	$CHCl_3$ fr.	20	25.2**
4	Lupeol	100	22.2**
5	Metformin (Standard drug)	100	26.4-35.8**

Table 2. Antihyperglycaemic activity of lupeol and lupeol esters and standard drug metformin on sucrose challenged streptozotocin-induced diabetic.

Sl. #	Compounds	Dose mg/kg	% Activity in sucrose challenged STZ-induced diabetic rats
1	Lupeol	100	22.2**
2	Lupeol acetate (**E-1**)	100	24.2**
3	Lupeol toluate (**E-2**)	100	25.5**
4	Lupeol salicyliate (**E-3**)	100	13.1
5	Lupeol myristate (**E-4**)	100	15.2
6	Lupeol palmitate (**E-5**)	100	23.6**
7	Lupeol stearate (**E-6**)	100	20.0**
8	Lupeol cinnamate (**E-7**)	100	21.5**
9	Lupeol- *o*-chlorobenzoate (**E-8**)	100	10.5
10	Lupeol -*m*-chlorobenzoate (**E-9**)	100	14.2**
11	Lupeol- *p*-chlorobenzoate (**E-10**)	100	15.4**
12	Lupeol -*o*-bromobenzoate (**E-11**)	100	13.2
13	Lupeol -*m*-bromobenzoate (**E-12**)	100	12.4
14	Lupeol -*p*-bromobenzoate (**E-13**)	100	12.3
15	Lupeol -*o*-nitrobenzoate (**E-14**)	100	7.8
16	Lupeol -*m*-nitrobenzoate (**E-15**)	100	8.9
17	Lupeol -*p*-nitrobenzoate (**E-16**)	100	8.5
18	Lupeol -*o*-methoxybenzoate (**E-17**)	100	7.9
19	Lupeol -*p*-methoxybenzoate (**E-18**)	100	13.4
20	Lupeol chloroethanoate (**E-19**)	100	15.5**
21	Lupeol -2-chloropropionate (**E-20**)	100	13.0
22	Lupeol -3,5-dinitr-benzoate (**E-21**)	100	16.2**
23	Lupeol-*o,p*-dichlorobenzoate (**E-22**)	100	28.9**
24	Metformin (Standard drug)	100	26.4 -35.8**

*Statistically significant at *P<0.05 and **P< 0.01 in comparison to control. n = 6 in each group.

Discussion

The results of the present study clearly indicate that the ethanol extract of *C. nurvala* has significant antidiabetic activity. The models chosen for studying these activities have already been validated. The currently available antidiabetic drugs for the management of diabetes mellitus have certain drawbacks and are cost effective for developing world (Yudkin, 2000; Yach *et al*., 2004). There is a need for a more widely applicable, safer and more effective antidiabetic drug therapy. India has a rich diversity of plants. These medicinal plants or herbs have a variety of metabolites, aliphatic compounds and aromatic compounds along with various functional groups that make ability to alter the various metabolic pathways make them medicinally important. To reduce the serious complications and negative outcome of this metabolic disorder, the control not only of blood glucose but also of lipid is necessary (Moller, 2001). In this study, the ethanol extract of *C. nurvala* showed blood glucose lowering effect in streptozotocin-induced sucrose challenged diabetic rat model. Since streptozotocin destruct pancreatic β cell and therefore develops insulin deficiency and hyperglycemia (Arora *et al*., 2009) and the antidiabetic activity of plants is dependent upon the degree of β cell destruction caused by the effective dose of the diabetogenic agents. The results of the present study indicate that lupeol was found to reduce the elevated glucose levels in animals made diabetic. In the present study the ethanol extract of the stem bark of the plant *C. nurvala* help in the reduction of blood glucose in STZ-induced and sucrose challenged diabetic rats as comparable with standard drug metformin. This lowering in blood glucose was supposed to be due to the inhibitory effect of Lupeol on glucosidase, an enzyme present on the brush border membrane of small intestine, which helps in the breakdown of carbohydrate and its transportation into the blood stream and therefore blocking the absorption of glucose from small intestine into blood stream, a major point of entry for glucose. Many medicinal plant species have glucosidase inhibitory activity (Shai *et al*., 2010).

Conclusions

In our studies it was found that the ester derivatives of lupeol posses better antidiabetic activity as compared to lupeol. It is thus concluded that lupeol skeleton deserve further investigation for the development of more potent and non-toxic new antidiabetic agents for its therapeutic use. Further optimization is needed to have lead antidiabetic agents for clinical trial.

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Conflicts of interest none

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