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Lupeol and lupeol esters protect the gastric ulcer in rats

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ABSTRACT

Objective: The lupeol isolated from the bioassay guided fractionation of the ethanol extract from the stem bark of the *Crataeva nurvala,* showed promising antiulcer effect in our random screening programme of natural products. Therefore we planned to make some derivatives of the lupeol, which may further enhance the antiulcer activity. **Materials and methods**: A one-step synthesis of long chain fatty acid and aromatic esters derivative of the lupeol with different acid halides and acids were done. A series of esters derivatives of lupeol were prepared and bio assayed for the antiulcer activity in Cold restraint induced gastric ulcer(CRU). **Results**: Few derivatives of lupeol (Lupeol acetate, Lupeol toluate, Lupeol palmitate, Lupeol stearate,) showed more potent percentages of antiulcer activity in Cold restraint induced gastric ulcer model in rats (table-1) as compared to the basic molecule lupeol. **Conclusion**: In our studies it was found that the esters derivatives of lupeol posses better antiulcer 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 antiulcer agents for its therapeutic use.

KEYWORDS

Crataeva nurvala;
Antiulcer activity;
CRU- model;
Esters of lupeol.

INTRODUCTION

Gastric ulcer is a very common gastrointestinal disorder affecting a large number of people worldwide. It arises due to an imbalance between aggressive (acid, pepsin and *Helicobacter pylori* infection) and protective (mucin secretion, prostaglandin, epidermal growth factors and bicarbonate) factors in the stomach.The plant *Crataeva nurvala* Linn. (Figure 1) belongs to the Family Capparidaceae is commonly known as Varuna in Sanskrit[1,2]. The bark has been used as sedative, stomachic, anthelmintic, anti-inflammatory,antitubercular,antipyretic[3] and in urolithiasis[4]. 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[5] lupeol acetate, spinasterol acetate[6],taxarsterol[7], 3-epilupeol[8], cadabacine, cadabacine acetate[9] catechin, epicatechin-5-glucoside[10] epifzelechin[11] and glucocapparin[12]. In continuation of our interest to develop drugs from natural sources, we had selected the lupeol for the evaluation of its antiulcer property.

Since lupeol showed antiulcer activity against Cold restraint induced gastric ulcer model(CRU) in our initial bioassays, screened in four different antiulcer models (Cold restraint induced gastric ulcer, alcohol induced gastric ulcers model, aspirin induced gastric ulcer model, Pyloric ligation induced gastric ulcer model). Therefore

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Plant

Stem bark

Crataeva nurvala

Figure 1

we planned to make some derivatives of this major natural product to enhance the antiulcer property present in the molecule. Earlier work has shown that lupeol exhibits anti-inflammatory[13] and cytoprotective[14] effects 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[15]. In our studies it was found lupeol posses antiulcer activity, therefore we planned to prepare ester derivative of lupeol for a potent anti ulcer agent.

MATERIAL AND METHODS

Melting points were determined on a hot stage melting point apparatus and were uncorrected. IR spectra were measured on a Beckmann Acculab- 10 Spectrophotometer. ^{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). Elemental analysis was carried out on a Carlo Erba Strumentazone.

Collection of the plant material

Crataeva nurvula stem bark was purchased from the local market and authenticated by the botanists in Central Institute of Medicinal and Aromatic Plants, Lucknow.

Extraction and isolation

The air-dried powdered stem bark of *C. nurvula* (5.0 kg) was extracted at room temperature (5x5 lit.) with 95% ethanol. The combined ethanolic extract was filtered and concentrated in a rotavapor below 50^{0}C to a green viscous mass (74.5g). The green mass (70.0 g) was fractionated into 4 fractions (hexane, chloroform, n-butanol soluble and n-butanol insoluble) by maceration method successively and all the fractions were concentrated separately in rotavapour below 50^0 C which were finally dried under vacuum to get viscous masses of each fractions. All the four fractions were bio-assayed for antiulcer activity against CRU-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 (60.0 g) and chromatographed over a column of silicagel and the major compound lupeol was purified and crystallized from methanol (yield,1%).

Lupeol

(Figure 2) was isolated from the hexane fraction of the stem bark *Crataeva nurvula* and It was named as compound-1. It displayed a molecular ion peak at m/z 426 for molecular ion $[M]^+$ of lupeol and a molecular formula $C_{30}H_{50}O$. The ^{1}H and ^{13}C NMR spectra were found exhibiting characteristic signals for lup-20(29)-en-3-ol[6]. The structure was confirmed by comparison of spectroscopic data of the compound-1 to those described for lupeol [6] and confirmation of the lupeol was also done by thin layer chromatography with authentic sample of lupeol (Co-tlc).

Lupeol

White microcrystalline powder; mp 213.0 C, $[\alpha]^{25}$

D +26.2 (c 0.67 in $CHCl_3$), UV(CHCl3) λ max ; 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).

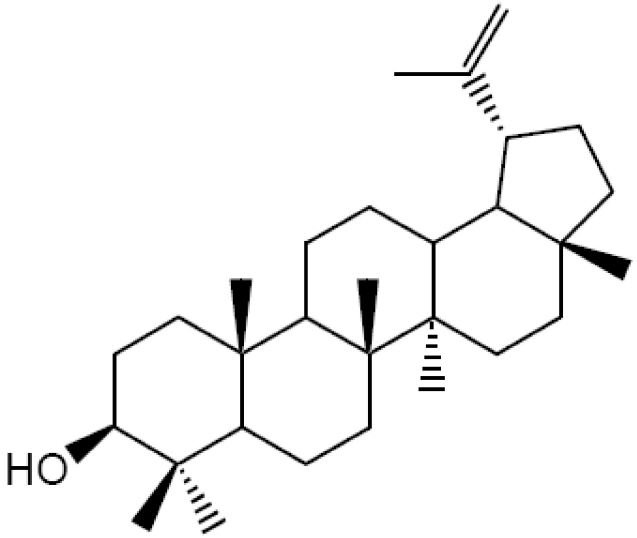


Figure 2

General method for preparation of lupeol esters with different acid chlorides

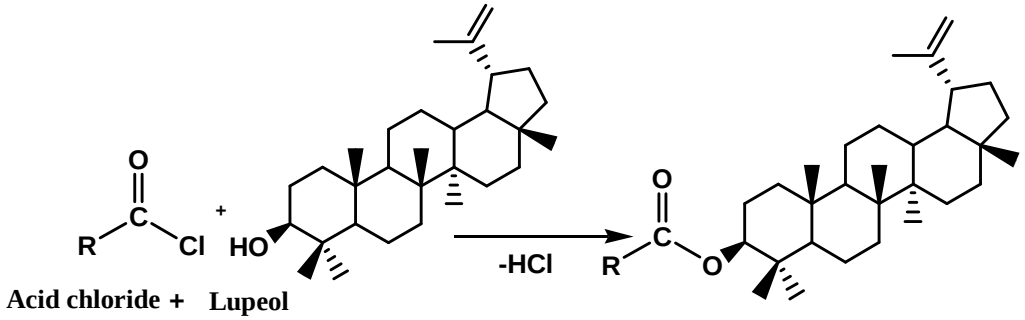
Lupeol was dissolved in dry DCM (CH_2Cl_2) and acid chloride and tri ethyl amine was added in the molar ratio (1:1.2:1.2) respectively to this solution. The reaction mixture was stirred at room temperature for 1-2 hr. The solvent was removed. TLC analysis ($CHCl_3$, 1% Vanillin/H_2SO_4) indicated the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

General method for preparation of lupeol esters with different acid

Lupeol was reacted with the acid loride, dicyclohexyl carbodimide (DCC) and dimethyl amino pyridine (DMAP) in dry dichloromethane (CH_2Cl_2) in the molar ratio (1:1.2:1.2:0.12) respectively. The reaction mixture was refluxed for 2-3 hrs. Formation of esters was checked by the TLC, the reaction mixture was worked up as usual. The esters were purified by silica gel 60 (230—400 mesh, Merck) column chromatography using hexane and ethyl acetate as eluent. The yield of esters were between 80 – 90 %.

Lupeol acetate (E-1)

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%). ^1H NMR (CDC_{l3}, 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 (CDC_{l3}, 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),



Acid chloride + Lupeol **Ester of Lupeol**

Scheme1: Reagents and conditions: (a) Dry dichloromethane (DCM), acid Chloride, triethyl amine, 1-2 hrs. at room temperature.

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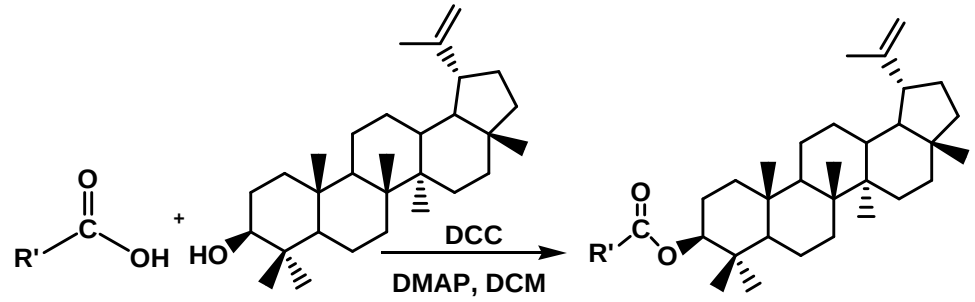

Scheme 2 : Reagents and conditions: (b) Dry dichloromethane (CH_2Cl_2), DCC, DMAP. dicyclohexyl carbodimide (DCC) and dimethyl amino pyridine (DMAP), 2-3 hrs

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, o-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 (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). 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, 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 (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). 118.9(C1'), 144.2(C2'), 134.5(C3'), 128.0(C4'), 128.8(C5'), 130.1(C6'), 166.83(C=O). IR (KBr, 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 (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'- 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, $CDCl_3$), 7.63 (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 (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). 118.9(C1'), 144.2(C2'), 134.5(C3'), 128.0(C4'), 128.8(C5'), 130.1(C6'), 166.83(C=O). IR (KBr, cm^{-1}), 1709 (C=O)

Lupeol o-chloro benzoate (E-8)

MS: (EIMS) [M$^+$] m/z 564, ^{1}H NMR (300 MHz, $CDCl_3$), 7.73 (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 (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). 131.9(C1'), 130.1(C2'), 133.7(C3'), 133.2(C4'), 129.8(C5'), 127.8(C6'), 167.0(C=O),IR (KBr, cm^{-1}), 1718 (C=O).

Lupeol m-chloro benzoate (E-9)

MS: (EIMS) [M$^+$] m/z 564, ^{1}H NMR (300 MHz, $CDCl_3$), 7.92 (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 (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). 131.9(C1'), 130.1(C2'), 133.7(C3'), 133.2(C4'), 129.8(C5'), 127.8(C6'), 167.0(C=O), IR (KBr, cm^{-1}), 1719 (C=O).

Lupeol p-chloro benzoate (E-10)

MS: (EIMS) [M$^+$] m/z 564, ^{1}H NMR (300 MHz, $CDCl_3$), 7.89 (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 (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). 131.9(C1'), 130.1(C2'), 133.7(C3'), 133.2(C4'), 129.8(C5'), 127.8(C6'), 166.4(C=O), IR (KBr, cm^{-1}), 1724(-C=O).

Lupeol o-bromo benzoate (E-11)

MS: (EIMS) [M$^+$] m/z 609, ^{1}H NMR (300 MHz, $CDCl_3$), 7.68 (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 (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).134.6(C1'), 121.8(C2'), 132.4(C3'), 131.6(C4'), 127.4(C5'), 131.3(C6'), 166.4(C=O). IR (KBr,cm^{-1}), 1723 (C=O).

Lupeol m-bromo benzoate (E-12)

MS: (EIMS) [M$^+$] m/z 609, ^{1}H NMR (300 MHz, $CDCl_3$), 8.07 (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 (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). 132.7(C1'), 133.0(C2'), 123.0(C3'), 136.1(C4'), 130.6(C5'), 128.7(C6'), 166.7(C=O). IR (KBr, cm^{-1}), 1721(C=O)

Lupeol p-bromo benzoate (E-13)

MS: (EIMS) [M$^+$] m/z 609, ^{1}H NMR (300 MHz, $CDCl_3$), 7.88 (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 (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). 129.5(C1'), 131.9(C2'), 131.7(C3'), 127.4(C4'), 166.4(C=O). IR (KBr, cm^{-1}), 1721(C=O)

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Lupeol o-nitro benzoate (E-14)

MS: (EIMS) [M^+] m/z 597, ^{1}H NMR (300 MHz, $CDCl_3$), 7.78 (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 (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). 125.6(C1'), 149.6 (C2'), 123.5(C3'), 133.7(C4'), 134.5(C5'), 130.6(C6'), 166.6(C=O), IR (KBr, cm^{-1}), 3422,1726 (C=O).

Lupeol m-nitro benzoate (E-15)

MS: (EIMS) [M^+] m/z 597, ^{1}H NMR (300 MHz, $CDCl_3$), 8.80 (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 (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). 131.4(C1'), 124.8(C2'), 148.3(C3'), 127.9(C4'), 129.3(C5'), 135.8(C6'), 166.4(C=O),IR (KBr, cm^{-1}) 3426,1720.

Lupeol p-nitro benzoate (E-16)

MS: (EIMS) [M^+] m/z 597, ^{1}H NMR (300 MHz, $CDCl_3$), 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 (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). 136.6(C1'), 130.6(C2'), 123.5(C3'), 152.7(C4'), 123.5(C5'), 130.6(C6'), 166.8(C=O), IR (KBr), 3433,1721.

Lupeol -o-methoxy benzoate (E-17)

MS: (EIMS) [M^+] m/z 560, ^{1}H NMR (300 MHz, $CDCl_3$), 7.80 (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 (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). 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) 1724.

Lupeol- p-methoxy benzoate (E-18)

MS: (EIMS) [M^+] m/z 560, ^{1}H NMR (300 MHz, $CDCl_3$), 7.99 (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 (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). 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, cm^{-1}), 1721.

Lupeol -2-chloro ethanoate (E-19)

MS: (EIMS) [M^+] m/z 502, ^{1}H NMR (300 MHz, $CDCl_3$), 4.36 (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 (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). 48.6(C1'), 166.4(C=O).IR (KBr, cm^{-1}), 1721 (C=O).

Lupeol 2- chloro propionate (E-20)

MS: (EIMS) [M^+] m/z 516, ^{1}H NMR (300 MHz, $CDCl_3$),4.36(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 (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). 57.7(C1'), 18.0(C2'), 166.4(C=O).IR (KBr cm^{-1}), 1721 (C=O).

Lupeol, o, p-di chloro benzoate (E-21)

MS: (EIMS) [M^+] m/z 600, ^{1}H NMR (300 MHz, $CDCl_3$), 7.87 (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 (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). 129.0(C1'), 136.4(C2'), 129.2(C3'), 139.5(C4'),126.9(C5'), 132.5(C6'), 166.4(C=O). IR (KBr, cm^{-1}), 1718 (C=O)

Lupeol,3, 5 dinitro benzoate (E-22)

MS: (EIMS) [M^+] m/z 620, ^{1}H NMR (300 MHz, $CDCl_3$), 9.15 (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 (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). 132.3(C1'), 130.9(C2'), 149.2(C3'), 123.0(C4'), 149.2(C5'), 130.9(C6'), 166.2(C=O), IR (KBr, cm^{-1}), 1725 (C=O).

Materials and reagents

Omeprazole and other chemicals were procured from Sigma (St. Louis, MD, USA). Sucralfate was obtained from Meranani Pharmaceuticals, India, whereas all other reagents used were of analytical grade.

Experimental animals

Adult Sprague Dawley rats of either sex, weighing 180-200g were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms (25 + 2°C, 12 hours light and dark cycle). Animals were fed with standard laboratory food pellets and water was provided *ad libitum*. Guinea pigs of either sex, weighing 300–350 g were used for histamine-induced ulcer model, which were also housed under standard conditions as described above. All experimental protocols were approved by our Institutional Ethical Committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals with the approval number 34/99/CPCSEA, 11.03.1999).) which complies with International norms of INSA (Indian National Science Academy). Sucralfate was obtained from Meranani Pharmaceuticals, India, whereas omeprazole and other chemicals were obtained from M/s. Sigma Chemicals,USA.

Treatment schedule of anti-ulcer studies

The ethanol extract of the stem bark of *Crataeva nurvula* and its hexane, chloroform fractions, omeprazole (10 mg/kg) and sucralfate (500 mg/kg) were prepared in 1% carboxymethyl cellulose (CMC) as suspension. These were administered orally to the animals at a volume of 1ml/200g of body weight 45 min. prior to exposure of ulcerogens was. All the animals were deprived of food for 16 h before ulcerogens exposure and were divided into three groups, (n=6).

(i) Control group of animals were treated with vehicle 1% CMC.

(ii) 2. Graded doses of ethanol extract of *Crataeva nurvula* (100,40,20,10 mg/kg, p.o.) and its hexane and chloroform fractions (10, 20 and 40 mg/kg, p.o.) were tested against Cold restraint ulcer (CRU) model to identify the effective dose and selected for further studies in other ulcer models.

(iii) Experimental group was treated with standard anti-ulcer drugs such as Omz (10 mg/kg, p.o.) in **(**CRU), ulcer model.

Cold restraint induced gastric ulcer (CRU)

Animals were subjected to cold restraint stress after 45 min. of treatment with ethanol extract, hexane, chloroform fractions and omeprazole. All the animals were immobilized in restraint cage and kept at 4°C in an environmental chamber[16]. The animals were sacrificed and stomachs were observed and scored under Magnascope for ulcers after 2 hours.

Alcohol induced gastric ulcers model (AL)

Animals for induction of gastric ulcer[17] were given chilled absolute alcohol (1ml/200g, body weight). Test samples and sucralfate (SUC) were administered 45 minutes before alcohol treatment. After 1 hour of alcohol administration, animals were sacrificed and stomachs were cut open along the greater curvature to observe the gastric lesions appearing as hemorrhagic bands along the mucosal ridges of the stomach. Lengths of the lesions were measured using Biovis image analyzer software and summated to give a total lesion score.

Aspirin induced gastric ulcer model (AS)

Test samples and reference drug omeprazole (Omz) were administered 45 mins before the treatment of aspirin (150 mg/kg body weight). Animals were sacri-

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ficed after 5 hours of aspirin treatment and the stomachs were dissected out, incised along the lesser curvature and the lesions were scored[18].

Pyloric ligation induced gastric ulcer model (PL)

After 45 min. of administration of test samples and omeprazole (Omz), ulcer was induced by pyloric ligation under chloral hydrate anesthesia (300mg/kg, i.p.). Abdomens were opened and pyloric part of stomach from each rat was ligated avoiding any damage to the adjacent blood vessels[19]. Stomachs were replaced carefully and the animals were allowed to recover with

TABLE 1: Effect of *Crataeva nurvala* extract and fractions and standard drug omeprazole on percentage protection against cold restraint induced gastric ulcer in rats. Data expressed as mean % protection ± S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test. **P< 0.01, in comparison to control. n = 6 in each group.

S.Nos.	Compounds	% Protection in Cold Restrain Ulcer model			
		(100mg/kg.p.o.)	(10 mg/kg, p.o.)	(20 mg/kg, p.o.)	(40 mg/kg, p.o.)
1	CrudeEtOHext.	45.6*	0	24.5	25
2	Hexanefr.		50	50.2**	55.0**
3	$CHCl_3$fr.		42.5	55.2**	60.5**
4	Omeprazole		77.4**	-	-

TABLE 2 : Effect of Lupeol and its derivatives and standard drug omeprazole on percentage protection against cold restraint induced gastric ulcer in rats. Data expressed as mean % protection ± S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test. **P< 0.01, in comparison to control. n = 6 in each group

S.Nos.	Compounds	% Protection in Cold Restrain Ulcer model		
		(10 mg/kg, p.o.)	(40 mg/kg, p.o.)	(20mg/kg,p.o.)
1	Lupeol	0	74.5**	77.6**
2	Lupeol acetate (E-1)	50.0*	81.2**	80.0**
3	Lupeol toluate (E-2)	42.5	81.2**	78.5**
4	Lupeolsalicyliate (E-3)	13.2	14.2	15.1
5	Lupeol myristate (E-4)	17.2	12.5	16.2
6	Lupeol palmitate (E-5)	60.5**	64.2**	76.6**
7	Lupeol stearate (E-6)	40.2*	61.5**	60.0**
8	Lupeol cinnamate (E-7)	15.1	18.2	21.5
9	Lupeol- o-hlorobenzoate (E-8)	28.2	30.5	32.2
10	Lupeol-m-hlorobenzoate (E-9)	56.2*	64.2**	70.8**
11	Lupeol- p-hlorobenzoate (E-10)	46.2*	55.4**	72.2**
12	Lupeol -o-romobenzoate (E-11)	25.7	33.2	24.3
13	Lupeol-m-romobenzoate (E-12)	11.1	12.4	10.2
14	Lupeol-p-bromobenzoate (E-13)	8.5	12.3	15.
15	Lupeol -o-nitrobenzoate (E-14)	7.2	7.8	13.4
16	Lupeol -m-nitrobenzoate (E-15)	6.7	8.9	6.7
17	Lupeol -p-nitrobenzoate (E-16)	11.8	8.5	9.0
18	Lupeol -o-methoxybenzoate (E-17)	12.5	7.9	15.4
19	Lupeol -p-methoxybenzoate (E-18)	14.5	13.4	14.7
20	Lupeol –chloroethanoate (E-19)	21.5	15.5	17.5
21	Lupeol -2-chloropropionate (E-20)	20.5	23.0	22.5
22	Lupeol-3,5-chinitrobenzoate (E-21)	32.1	36.2	33.9
23	Lupeol-o,p-di-chlorobenzoate (E-22)	31.9	28.9	26.4
24	Omeprazole	77.4**		

***Statistically significant at *P<0.05, **P< 0.01 and ***P<0.001, in comparison to control. n = 6 in each group**

free access to water. After 4 hours, animals were sacrificed and stomachs were dissected out. Lesions were scored and gastric fluid was collected and centrifuged at 2000 rpm for 10 min. The supernatant was collected and used for estimation of gastric secretion and mucin level.

Measurement of ulcer index

Ulcer formed due to treatment with different ulcerogens were observed under Magnascope (5X magnification) and were scored according to the arbitrary scoring system[20]. The severity and intensity of the lesions were graded as following: i) Shedding of epithelium = 10; (ii) Petechial and Frank hemorrhages = 20; (iii) one or two ulcers = 30; (iv) more than two ulcers = 40; and (v) Perforated ulcers = 50.

Statistical analysis

All values shown in the figures (3-5) and tables (1&2) represent the means ± S.E.M. values with 95% confidence limits were estimated using Maximum Likelihord Iterative Procedure[21]. Statistical analysis was performed with Prism version 3.0 software using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSIONS

Initially Lupeol showed promising antiulcer activity at 10, 20 and 40 mg/Kg dose levels(50,0%, 81.2% 1nd 80.0% respectively) in CRU ulcer model in rats. Further 22 esters of lupeol (E-1 to E-22were were synthesized, which were when tested for antiulcer activity in CRU model in rats showed promising antiulcer effect only E-1, E-5,E-6,E-9 and E-10 (TABLE-2). Other compounds did not show activity in CRU ulcer model in rats. In our studies of antiulcer activity of lupeol in CRU model, it showed 74.5% protection at 20 mg/kg dose and enhancing the dose to 40mg/kg, it only showed 77.6% protection of ulcer in CRU model. On derivatization of lupeol to various esters ester E-1 showed maximum protection at 20 mg/kg (TABLE-2). E-5, E-6, E-9 and E-10 showed maximum protection at 40mg/kg dose. Further more derivatives other than ester of lupeol are being prepared in our laboratory for further enhancing the activity as compared to lupeol or its esters. Further results on other compounds will be reported in our second communication.

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