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Supplementary data

Comparative Binding Studies of Lupeol Compounds with Plasma Proteins and its Pharmacological Importance

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Fig. S1 Effect of Lupeol and Lupeol derivative on A) IL-1β expression B) IL-6 expression induced by LPS stimulation on mouse macrophages (RAW264.7). The inhibitory effect was determined by ELISA reader at 570 nm absorbance. Bar indicates mean ± SD of the concentration of cytokines (P = 0.005).

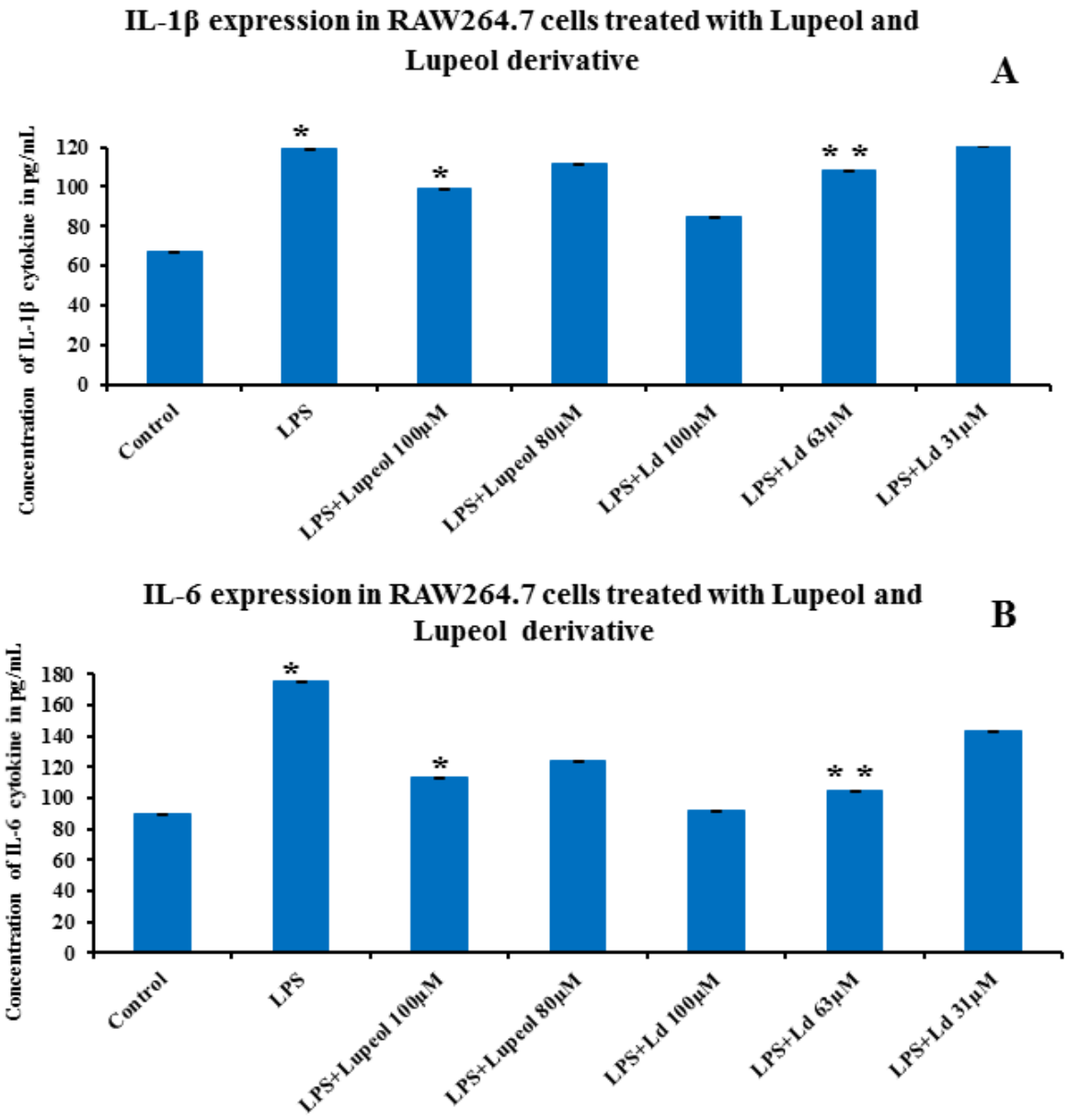

Fig. S2 Modified Stern-Volmer plot. (A) Plot of log (dF/F) against log [Q] for HSA-lupeol. (B) Plot of log (dF/F) against log [Q] for HSA-lupeol derivative (Ld). In 0.1 mM phosphate buffer pH 7.4, λex = 285 nm, λem = 362 nm.

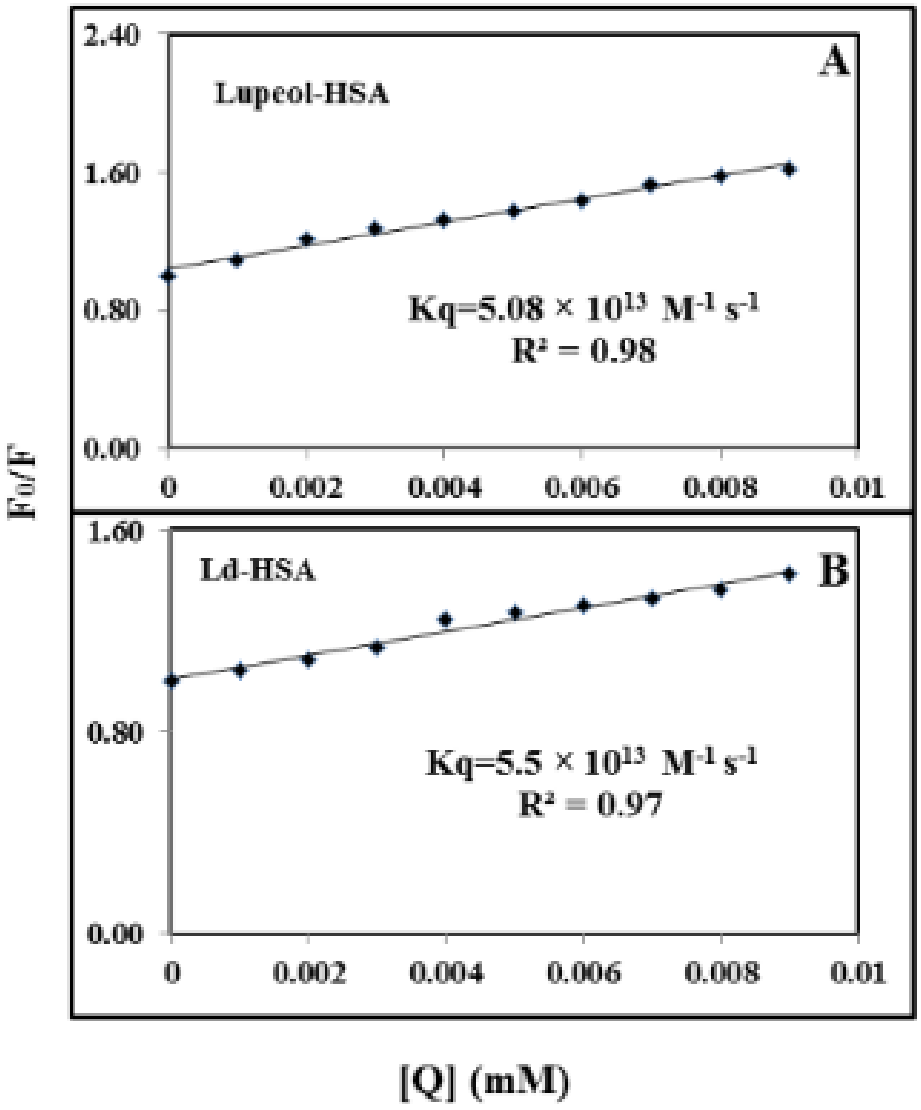

Fig. S3 Temperature dependent CD spectra. (A) HSA and HSA and HSA-lupeol complex (B) HSA and HSA-lupeol derivative complex. Secondary structure of HSA-complexes are stable upto 65°C, increase in temperature leads unfolding of HSA structure (C) Change in the percentage concentrations of lupeol and lupeol derivative (Ld).

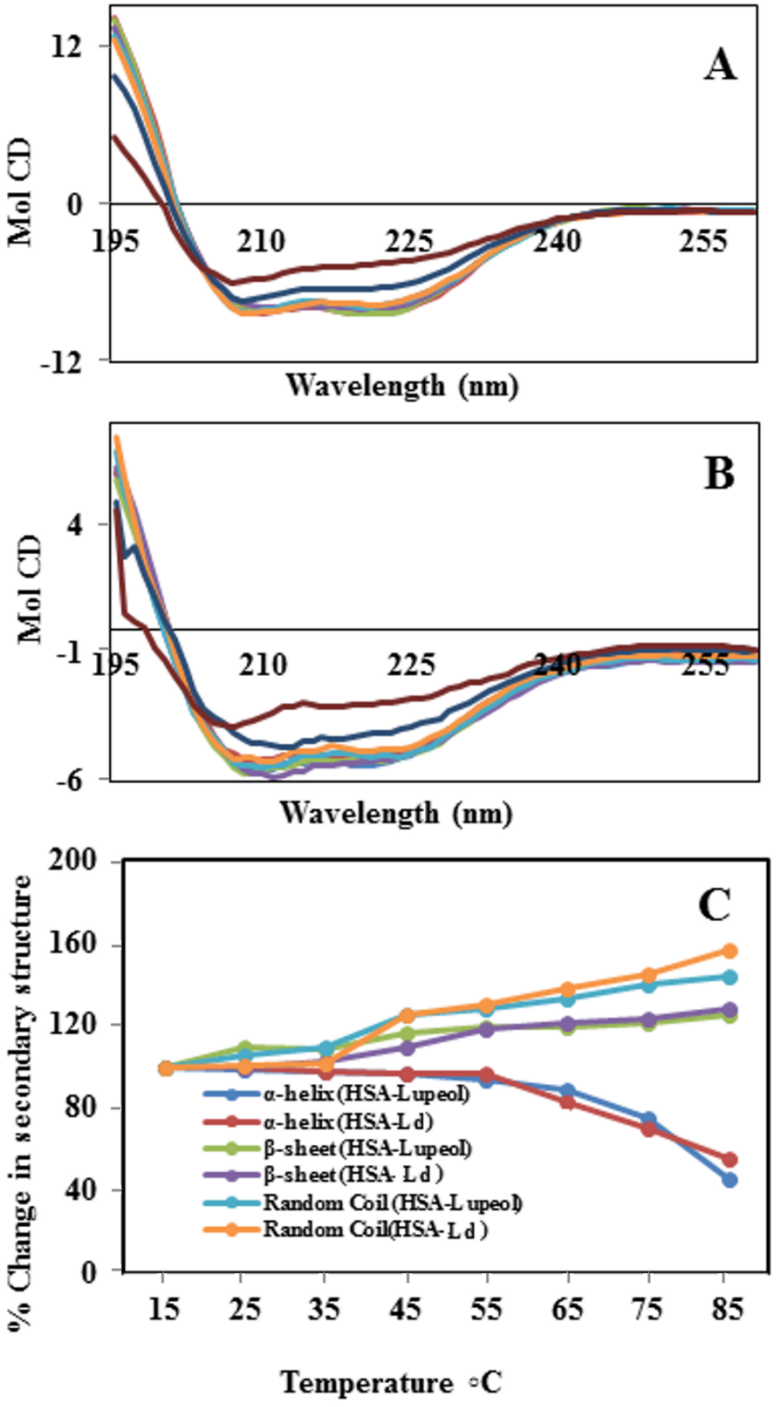

Fig. S4 Graphical representation of the sequence of conformational changes of lupeol around binding pocket. Ligplot of HSA-Lupeol showing hydrophobic interactions with subdomain IIIA at different ns (0.1 – 10 ns).

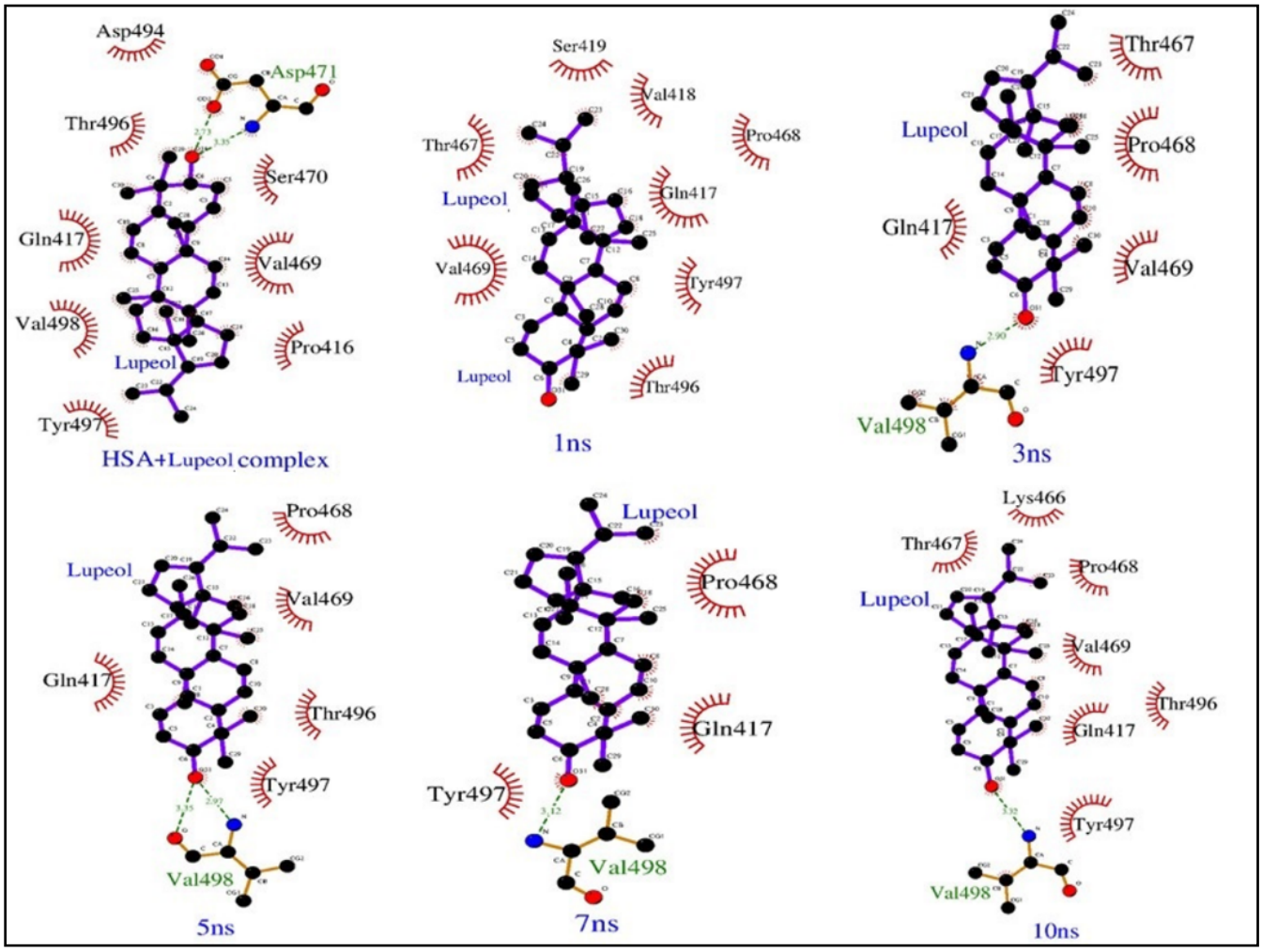

Fig. S5 Graphical representation of the sequence of conformational changes of lupeol derivative around binding pocket. Ligplot of HSA-Lupeol derivative (Ld) showing hydrophobic interactions with subdomain IIIA at different ns (0.1 – 10 ns).

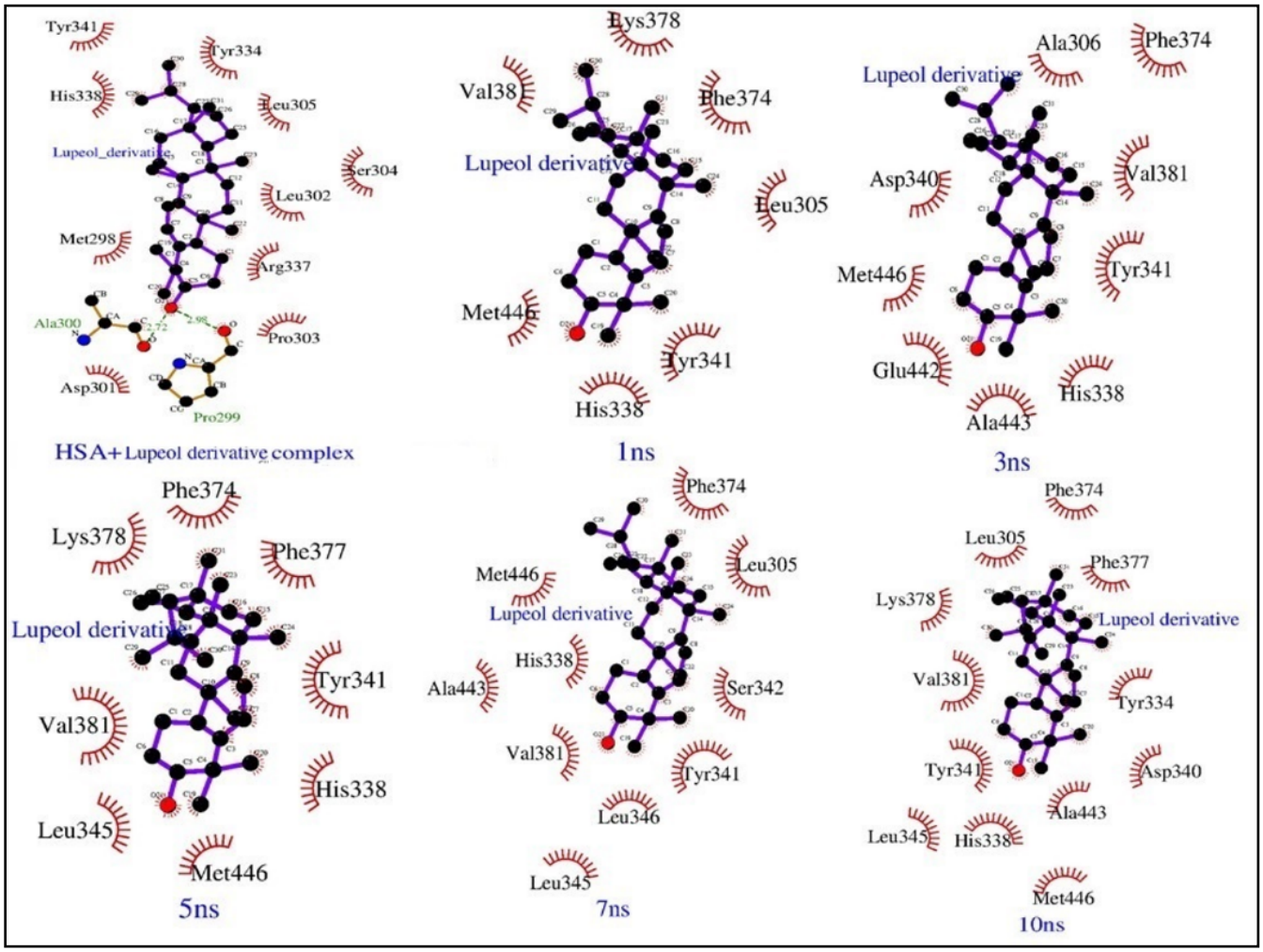

Table S1. Induction of HeLa cells apoptosis at 24 h by lupeol and lupeol derivative

Sample (μM)	% live cells	% dead cells	% early apoptosis	% late apoptosis
control	78.5	16.6	1.1	3.8
lupeol-15 μM	80.4	17.8	**0.8**	5.3
lupeol derivative-12 μM	83.8	11.1	**2.6**	2.6

Table S2. Secondary structural components of free HSA and HSA-lupeol and HSA-lupeol derivative complexes.

	HSA	**HSA-lupeol (mM)**			**HSA-lupeol derivative (mM)**		
	0.001	0.001	0.002	0.003	0.001	0.002	0.003
Helix (%)	58±2.5%	55.40±2.03	53.2±2.04	50.50±1.8	49.10±1.8	46.40±0.8	44.30±1.0
Beta turn (%)	20±0.73%	21.7±0.6	23.1±0.8	24.0±0.1	24.4±1.2	25.3±0.7	26.6±2.1
Random coil (%)	22±1.0%	22.90±0.6	23.7±0.4	25.50±0.8	26.50±0.9	28.30±0.2	29.10±2.03

Table S3. Table of 10 conformers from AutoDock4.2 showing rank, run, binding energy, Ki, Ka, cluster RMSD and reference RMSD.

	Rank	Run	Binding energy (kcal M^{-1})	K_i	K_a (M^{-1})	RMSD cluster	Reference RMSD
	1	1	-8.92	288.04nM	2×10^6	0.0	60.17
	2	7	-7.89	2.4 µM	1.2×10^6	0.0	53.55
Lupeol	3	9	-7.69	2.7 µM	1.01×10^6	0.0	47.25
	4	5	-7.65	3.47 µM	3.2×10^5	0.0	44.83
	5	6	-7.09	9.35 µM	4.2×10^4	0.0	40.35
	6	22	-6.75	15.30 µM	5.1×10^4	0.0	38.45
	7	**13**	**-6.45**	**18.67 µM**	**4.5×10^4**	**0.0**	**34.47**
	8	24	-6.02	25.48 µM	2.6×10^4	0.0	34.82
	9	26	-5.67	34.44 µM	7.2×10^3	0.0	31.44
	10	28	-5.16	42.7 µM	4.1×10^3	0.0	30.02
Lupeol	1	27	-8.54	551.52 nM	1.8×10^6	0.21	47.88
derivative	2	28	-8.11	1.13 µM	1.2×10^6	0.00	52.86
	3	7	-7.76	2.02 µM	3.3×10^5	0.00	54.69
	4	20	-7.70	2.64 µM	3.3×10^5	0.00	28.42
	5	10	-7.57	3.8 µM	3.0×10^5	0.0	57.27
	6	16	-7.55	4.92 µM	3.0×10^5	0.0	66.73
	7	3	-6.91	9.58 µM	4.3×10^4	0.00	60.30
	8	**11**	**-6.89**	**12.33 µM**	**4.2×10^4**	**0.13**	**61.02**
	9	14	-6.77	18.83 µM	2.6×10^4	0.0	25.79
	10	19	-5.93	25.25µM	7.1×10^3	0.0	70.15