Anti-inflammatory and Anti-mitotic activities of lupeol isolated from the leaves of Ixora Coccinea Linn.

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Lupeol was isolated from the petroleum ether fraction of ethanol extract of the leaves of Ixora coccinea (Fam. Rubiaceae). Pharmacological studies showed that lupeol is having anti-inflammatory activity in carrageenan induced paw oedema in albino rats and anti-mitotic activity in a preliminary cytotoxic stuy using the "Allium test" of Levan.

PETROLEUM ether extract of the root of Ixora coccinea and the saponifiable fraction of the petroleum ether extract were found to possess anti-inflammatory activity on our previous studies.[1,2] In the present study, leaves of Ixora coccinea were subjected to chemical and pharmacological evaluation.

Plant Material and Extraction

Fresh leaves of Ixora coccinea were collected from Thiruvananthapuram, Kerala in June 1992. 2 Kg of the leaves were macerated with 4L of ethanol (90%) for about a week. Extract was taken and concentrated under vacuum. A thick, dark brown syrup was obtained as residue (125 gms); the yield being 6.25% w/w. The residue was partitioned between petroleum ether (60-80) and water. The petroleum ether fraction was concentrated under reduced pressure to get a dark brown residue (27.98 gms); the yield being 1.40% w/w. It gave positive tests for phytosterols and triterpenoids.

Adsorption column chromatography of the petroleum ether fraction

12 gms of the petroleum ether fraction was column chromatographed is neutral alumina CC, eluted with petroleum ether (60-80), mixture of petroleum ether-benzene, 100% benzene, mixture of benzene-chloroform and finally with 100% chloroform. Identical fractions were pooled together according to their homogenity judged from the TLC pattern (system-petroleum ether (60-80): diethyl ether ; acetic acid 70 : 30 : 2 detected by anisaldehyde- sulphuric acid spray or Liebermann-Burchard spray reagent).

Nine spots were separated out which the compound in maximum yield having Rf value 0.53, designated as compound E was selected for chemical characterization by IR, NMR and mass spectrum.

Acute Toxicity Studies of Compound E

Albino rats of either sex (188-200 gms) were used for the study.[3] Test drug in 5% acacia solution in doses of 100 mg/kg, 200 mg/kg and 600 mg/kg body weight were administered orally. The animals were closely observed for the first three hours for any toxic manifestations and mortality was noted 24 hours after drug administration.

Anti-Inflammatory activity studies on Compound E

The test was carried out on albino rats of either sex weighing between 100-220 gms. The animals

were fasted for 18 hours prior to the test and were divided into four groups of five animals each. The test groups were given orally 100 mg/kg and 200 mg/kg body weight of compound E in 5% acacia solution and the control group was given Indomethacin 5 mg/kg body weight as the standard drug. The paw oedema was induced as described by D'Arcy et al.[4] Oedema was induced one hour after drug administration by injecting 0.1 ml of a 1% suspension of carrageenan in normal saline into the plantar aponeurosis of the left hind paw of the rat. The paw thickness was measured by using vernier calipers in three planes and the average was recorded, immediately after carrageenan injection and three hours later.

Anti-mitotic activity studies of compound E: Allium Tesst

Freshly rooted bulbs of the common onion, Allium cepa (2n = 16) were used for studying the cytological effects using the 'Allium test' of Levan.[5]

The onion bulbs were grown in pots containing sand. When the roots were about 1-1.5 cm long, the bulbs were removed from the soil, washed well with water and the root system kept in bottles containing 4 different concentrations 10 μg/ml, 1.0 μg/ml, 0.1 μg/ml and 0.01 μg/ml of compound E prepared in distilled water. The roots of onion bulbs were kept in the drug solution for 24 hours from 1.30 pm. Control without the drug was prepared in distilled water and treated similarly. Standard drug used was Adriamycin in concentrations of 0.1, 1.0 and 10.0 μg/ml in distilled water. Soon after treatment, the root tips were collected and fixed in 3:1 alcohol-acetic acid mixture. Acetocarmine squashes were made out of these after hydrolysis in 1N HCl at 60°C. (1% acetocarmine prepared by boiling 1 gm of carmine in 99 ml of 45% acetic acid and filtered). The slides were examined under microscope. A minimum of 1000 meristematic cells were observed for scoring adnormalities induced by each treatment

RESULTS AND DISCUSSION

Compound E: is in the form of White needles (m.p 215-217°C) IR bands (KBr) 3311 cm (OH), 2953, 1461, 1039.

The proton NMR spectrum (90 MHz, $CDC1_3$) showed 7 tertiary methyl singlets at δ values 0.75, 0.80, 0.85, 0.95, 0.99, 1.05 and 1.65. Multiplet at δ 3.20 may be due to one proton of C-3 methine. Broad singlets at δ values 4.6 and 4.7 may be due to olefinic protons.

The EIMS of the compound showed M^+ ion at m/z 426 and 7 characteristic peaks at 411 (M^+ - CH_3), 408 (M^+ - H_2O), 393 [M^+ - (CH_3 and H_2O)] 218, 207, 203 and 189. The peaks at m/z 218, 207, 203 and 189 can be explained by the retra-Diels-Alder fragmentation pattern[6] and the observed fragmentation pattern agress with the earlier observation of Djerassi[7]. From the results, the molecular mass of the compound could be assigned as 426.

The compound E was acetylated using acetic anhydride in pyridine. The acetyl derivative of compound E on co-TLC with authentic sample of lupeol acetate gave identical spot.

The compound E has been confirmed to be lupeol which is a triterpenoid, Lup-20 (29)-en-3β - ol with molecular formula $C_{30}H_{50}O$.

ACUTE TOXICITY STUDIES OF LUPEOL: (Compound E)

No mortality was observed for lupeol upto a dose level of 600 mg/kg body weight.

Table-1 Effects of Lupeol on Carrageenan Induced Paw Oedema:

Group	Drug & Dose	Mean increase in paw thickness ± S.D (mm)	Reduction in Paw thickness (mm)	% inhibition of oedema	*P-value
I	Control Acacia 5% Solution	1.794 ± 0.3944	-	-	
II	Lupeol 100mg/kg body wt.	0.3526 ± 0.0378	1.4414	80.35	< 0.001
III	Lupeol 200mg/kg body wt.	0.2336 ± 0.06688	1.5604	86.98	< 0.001
IV	Indomethacine 5mg/kg body wt.	0.5188 ± 0.1129	1.275	71.07	< 0.001

*P-value calculated by comparing with control value.

The results of the anti-inflammatory activity lupeol is shown in Table I

ANTI-INFLAMMATORY ACTIVITY STUDIES OF LUPEOL: (Compound E)

Table - 2 Cytological Effects of Lupeol on the Root Tip Meristem of Allium Cepa:

Drug	Concentration of drug (μg/ml)	Percentage of cells in				Percentage of cells with abnormalities				
		Inter phase	Pro phase	Meta-phase	ana-phase	Sticki-ness & clumping	tropo-kine-sis	Sticky bridges	pycno-tic nuclei	Nucl-ear les-ions
Control [Distill-ed water]		74.0	10.0	9.0	2.0	Nil	Nil	Nil	Nil	Nil
Lupeol	0.01	86.74	7.79	3.09	1.53	0.13	0.04	-	0.04	7.12
	0.10	87.64	3.84	1.61	1.14	0.35	0.12	-	0.08	9.25
	1.00	93.72	1.51	1.32	0.80	0.52	0.28	0.05	0.24	16.60
	10.00	94.89	0.72	0.92	0.48	0.63	0.39	0.09	0.34	19.61
Adria-mycin	0.10	98.08	0.37	0.46	0.09	0.52	0.22	0.06	0.06	15.63
	1.00	98.56	0.36	0.18	-	0.21	0.09	0.04	0.11	16.97
	10.00	99.24	0.19	0.09	-	0.22	0.02	-	0.19	19.70

Anti-Mitotic Activity Studies of Lupeol : (Compound E)

Table-2 shows cell divisions in the control were normal. Chromosome clumping at metaphase or late anaphase might have given rise to the pycnotic nuclei. Mitotic arrest was also observed in anaphase and early telophase. The appearance of pycnotic nuclei is suggestive of antimitotic arrest under the influence of antimitotic compounds after chromosomes had undergone prophasic condensation. The mechanism for antimitotic action of lupeol includes the possible inhibition of DNA replication prior to karyokinesis, depolymerization of DNA and nucleoproteins resulting in denatured chromosomes and the disassembly of microtubules resulting in spindle breakdown (8). It is interesting to note that cytological abnormalities such as pycnosis of nuclei, nuclear lesions, chromosome clumpings, tropokinesis, sticky bridges and stickiness have also been observed in onion root tip cells treated with adriamycin.

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Synthesis and Biological Activities of 1-[(3,4-dihydro-3-oxo-2h- 1,4-benzoxazin-2-yl) Acetyl]-3,4-disubstituted Pyrazoles and 3- Methyl-pyrazoliones

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Some new 1-[(3,4-dihydro-3-oxo-2H-1, 4-benzoxazin-2yl) acetyl]-3, 4-disubstituted pyrazoles (II) and 1-[(3,4-dihydro - 3 - oxo - 2H - 1, 4 - benzoxazin - 2yl) - 3 - methyl pyrazolin - 5 - ones (III) have been synthesized and tested for their (II) antimicrobial activity. Some of the II exhibit a significant antimicrobial activity, compound IIa has been found to possess antihistaminic activity.

IN continuation of our work on benzoxazinone derivatives[1,2] and having synthesized the oxobenzoxazinyl-acetic acid hydriazides, it has been considered worthwhile to employ them as "synthons" for obtaining new oxo-benzoxazinyl pyrazolines and pyrazolones. In view of the known pharmacological and biological importance of the pyrazoline and chromone moieties it will be interesting to evaluate these new compounds for their possible biological properties.