

Synthesis and Evaluation of Antipsychotic Compounds from Lupeol

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ABSTRACT

Aim: The current study was designed to evaluate the antipsychotic potential of lupeol and their semisynthetic derivatives to get a new and potent antipsychotic agent. **Methodology:** Lupeol was isolated from bark of *Crateva nurvala*. the current study was designed to evaluate the antipsychotic potential of lupeol and its semisynthetic derivatives. *C. Nurvala* stem bark was extracted by cold maceration with 95% ethanol and concentrated through rotary vacuum evaporator under reduced pressure. The concentrated ethanolic extract was defatted with petroleum ether and fractionated with chloroform successively. Lupeol derivatives were prepared through a three step reaction with different amines, aliphatic and aromatic moieties. A series of derivatives of lupeol were assayed for antipsychotic activity in actophotometer and compulsive behaviour (Stereotypy) in Plus Maze Model in rats. Few derivatives of lupeol showed more potent activity as compared to the basic molecule, lupeol. **Results:** The results of the present study clearly indicated that the derivatives and lupeol isolated from

C. Nurvala and synthetic lupeol analogs possess significant antipsychotic activity. **Conclusion:** Lupeol skeleton deserves further investigation for the development of more potent and non-toxic new antipsychotic agents for therapeutic applications.

Key words: Lupeol, Stereotype behaviour, Locomotor, *Crateva nurvala*, Antipsychotic activity.

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INTRODUCTION

Psychosis is the term given to the more severe forms of psychiatric disorder, during which hallucinations or delusions, violence and impaired insight may occur.¹ Psychotic disorders is reflected as significant psychological and social repercussions for everyday living.² Around three percent experience psychosis, more frequent than diabetes. Unlike infection, where the cause and effect are clear, most CNS ailments follow a complex biology, and have differing outcomes depending on predisposing factors. Even though the available drugs fulfil the requirements in the segment, there is need for more effective drugs that are better tolerated and cost effective to enhance the long term compliance.³

Psychosis is explained in terms of neurotransmitter dopamine. It is assumed that the dopamine of psychosis has been influential and states that psychosis results from an over activity of dopamine function in the brain, in particular the mesolimbic pathway. There exists a complex relationship in between dopamine and psychosis. The dopamine receptor D2 suppresses and D1 receptor increases adenylate cyclase activity. The blocked dopamine spills over to the D1 receptors during the administration of D2 blocking drugs. The increased adenylate cyclase activity affects genetic expression in the nerve cell; usually this process takes certain time period. Hence, antipsychotic drugs take a week or two to reduce the symptoms of psychosis.⁴

Antipsychotics are divided into two categories. The first category is called as typical antipsychotics and also referred to as first generation antipsychotics or classical neuroleptics or major tranquilizers or conventional antipsychotics. Second category drugs, known as atypical antipsychotics or second generation antipsychotics, are used to treat psychiatric conditions.⁵

MATERIALS AND METHODS

Materials

Instrumentation

The melting points of compounds were measured in open capillaries in electrical heated melting point apparatus of Jindal, S.M. Scientific Instruments Pvt. Ltd., New Delhi. IR spectra were recorded on Perkin Elmer FT-IR Spectrometer Spectrum Two and values are expressed in wave numbers (cm⁻¹).¹ H-NMR spectrums were recorded on JEOL, JNM-ECS 400 MHz using Chloroform-D (CDCl₃) as solvent. Tetramethylsilane (TMS, δ0.00 ppm) was used as internal standard in¹ H-NMR. Purity and Mass analysis were recorded on WATERS-Q-TOF Premier-HAB213 mass spectrometer. All the chemicals were procured from Aldrich, Qualikems and Merck Chemicals.

Animals

White Albino Rats of Wister strain weighing 150 ± 5 g and Swiss albino mice of both gender weighing 20 ± 5 g and studies on them. After observing the usual formalities lay down by IAEC as per provisions made by CPCSEA. All the animals were housed in laboratory cages in animal house maintained at 23 ± 2 °C under standard light/dark cycle. All the animals had free access to standard food pellets and filtered water.

Drugs

Diazepam (Calmtack, Indus Pharma Pvt. Ltd.) was dissolved in DMSO (2 mg/kg body weight), Apomorphine (Zyprima, Cadila Pharmaceuticals Ltd) (2.5 mg/kg body weight, ip), Haloperidol (Haldol, East West Pharma) (2.5 mg/kg body weight, ip) were dissolved in normal saline. Control animals were treated with distilled water in the same period.

Plant material

Stem bark of *Crataeva nurvala* was authenticated from Chandra Shekhar Azad University of Agriculture & Technology, Kanpur (CSA/DOH/2015-16/ 31). The plants were selected on the basis of their folk medicinal value. Lupeol was isolated from the hexane fraction of the stem bark of *Crataeva nurvala* 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 1H 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 and confirmation of the lupeol was also done by thin layer chromatography (TLC).

METHODS

EXTRACTION OF LUPEOL (L-1)

Coarsely powdered (2 kg) raw material of *C. nurvala* stem bark was extracted by cold maceration with 95% ethanol and concentrated through rotary vacuum evaporator at 40 °C under reduced pressure. The concentrated ethanolic extract (130 g) was defatted with petroleum ether and fractionated with chloroform successively. The chloroform fraction was concentrated under reduced pressure to afford chloroform soluble light brownish residue (30 g). The compounds were isolated from chloroform fraction through column chromatography using gradient elution technique. The progress of separation was monitored by TLC (silica gel G 60 F254 plates, Merck). Fractions eluted with n-hexane and EtOAc (8:2) resulted an amorphous yellowish white residue which after crystallization with methanol provides colorless crystalline substance termed as Lupeol (L-1) (16 g) (Figure 1).

L-1: M.P.: 214-216 °C, FT-IR spectra hydroxyl group (3302.6 cm^{-1}), Vinylidene group $3068.1, 1636.8, 880\text{ cm}^{-1}$. 1H -NMR (400 MHz, $CDCl_3$, δ , ppm) 4.68 and 4.56 (s, 3H). MS (m/z) 426.

CHEMICAL MODIFICATIONS OF LUPEOL (L-1)

Step-1: Chemical modification at C-30

Lupeol (1 gm, 2.35 mm) was refluxed with selenium dioxide in dioxane with 3-4 drops of distilled water for 10-12 hours. After consumption of all of lupeol, reaction mixture was treated with 2.5% aq. KOH, and Organic layer was washed with distilled water till it became neutral, then dried over sodium sulphate, and was evaporated under vacuum. This reaction mixture was chromatographed over silica gel column and was eluted with n- hexane/ ethyl acetate with (9:1) (8:2) (7:3). Finally required pure lupeol aldehyde (LA-1) was obtained.

LA-1: M.P.: 222-226°C. FT-IR spectra showed 3283 cm^{-1} (OH), 2938 cm^{-1} (CH), 1680 cm^{-1} (CHO), 1463 cm^{-1} (-C-H) Bending. 1H -NMR (400MHz, $CDCl_3$, δ , ppm) $\delta 9.48$ (s, 1H-CHO), 6.25 and 5.87 (2s, 1H each, H29), 3.13 (m, 1H,H3), 2.76 (m, 1H), 2.10 (m, 1H), 1.65-1.27 (bunch, 24 H), 1.01 (s, 3H, -Me), 0.96 (s, 3H, -Me), 0.92 (s, 3H, -Me), 0.81 (s, 6H), 0.75 (s, 3H, -Me). MS (m/z) 441.

Formation of LA-1 generated an additional functional group (Aldehyde) in lupeol which can be used to build different heterocycles on this

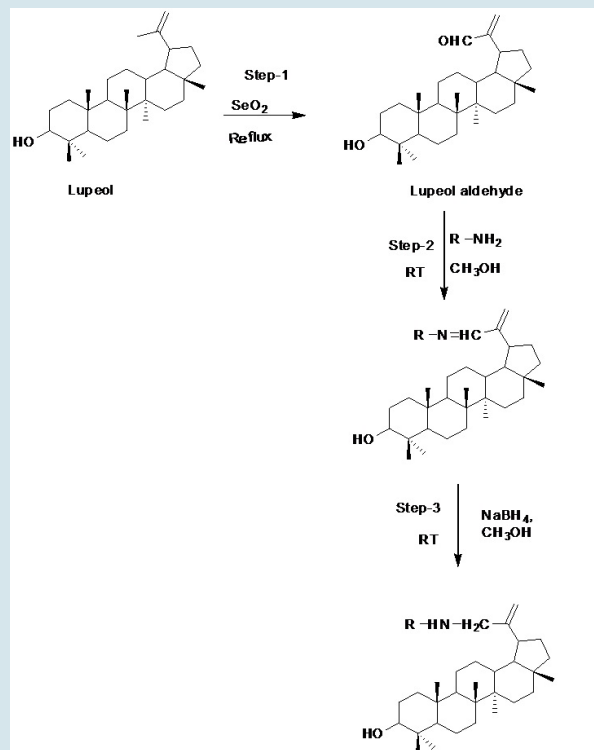
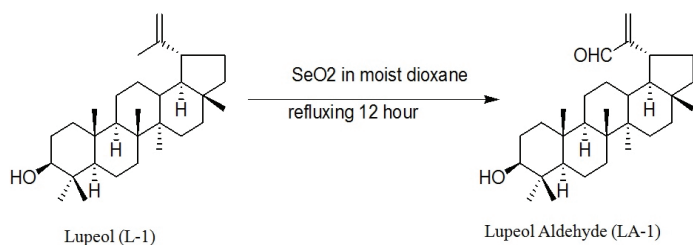


Figure 1: Synthetic scheme for extraction of Lupeol (L-1).

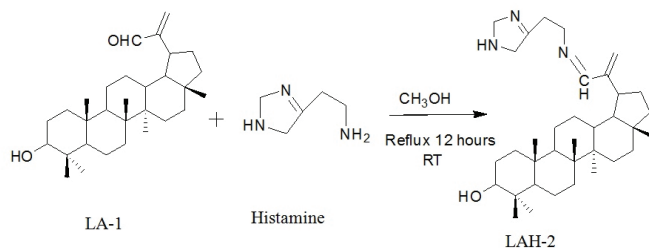
bi-functional three carbon unit. In NMR spectrum, a new proton at 9.5 appeared along with olefinic proton.

Step- 2: Chemical modification at C- 30 aldehyde

Second step was accomplished by doing changes in the isopropenyl side chain of LA-1.

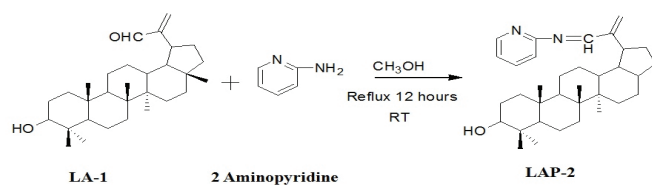
Reaction with histamine: LA-1 (1 mole) was refluxed with Histamine (1 mole) in methanol for 12 h. After that reaction product was separated by ethyl acetate and water. Then sodium sulphate was added in organic layer and filtered in order to get LAH-2.

LAH-2: M.P.: 217-218°C. FT-IR spectra showed 3400 cm^{-1} (OH), 2952 cm^{-1} (CH), 2379 cm^{-1} (C=N), 1655 cm^{-1} (CHO), 1559 cm^{-1} (NH Bending), 880 cm^{-1} (=CH). 1H -NMR (400 MHz, $CDCl_3$, δ , ppm) $\delta 4.8$ (d, 1H, J=16.8, OH), $\delta 2.0$ (s, 1H, NH), $\delta 4.8$ (d, 1H, J=10.8, OH), $\delta 2.8$ (m, 1H), $\delta 2.1$ (m, 2H, H-2), $\delta 1.65$ (bunch 24H), $\delta 1.01$ (s, 3H, Me), $\delta 0.92$ (s, 3H, CH_3), $\delta 0.81$ (s, 6H), $\delta 0.75$ (s, 3H, CH_3).



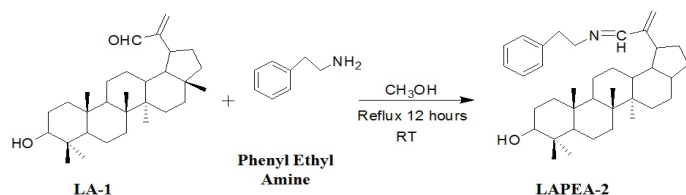
Reaction with 2- Aminopyridine: LA-1 (1 mole) was refluxed with 2- Aminopyridine (1 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. Then sodium sulphate was added in organic layer and filtered in order to get LAP-2.

LAP-2: M.P.: 232°C, FT-IR spectra showed 3443 cm^{-1} (OH), 2933 cm^{-1} (CH), 2318 cm^{-1} (C=N), 1712 cm^{-1} (CHO), 1645 cm^{-1} (NH-Bending). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 7.29 (s,1H,NH), δ 4.92 (s,1H, CN) δ 4.91(d,2H,J=14.68,CH₂), δ 4.8 (d,1H,J=12.8,OH), δ 2.8(m,1H), δ 2.1(m,2H,H-2), δ 1.65 (bunch 24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



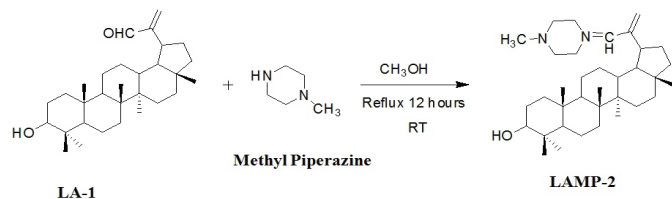
Reaction with Phenyl Ethyl Amine: LA-1 (1 mole) was refluxed with Phenyl Ethyl Amine (1 mole) in methanol for 12 hours. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and sodium sulphate was added into it. Finally it has been filtered in order to get LAPEA-2.

LAPEA-2: M.P.: 219°C, FT-IR spectra showed 3316 cm^{-1} (OH), 2933 cm^{-1} (=CH), 2883 cm^{-1} (CH), 1693 cm^{-1} (NH-Bending), 1453 cm^{-1} (C=C), 1378 cm^{-1} (C-N). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 5.22 (m,1H,CN), δ 5.25 (s,1H,CN), δ 4.8 (d,1H,J=10.8,OH), δ 3.4 (m,2H,CH₂), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch 24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



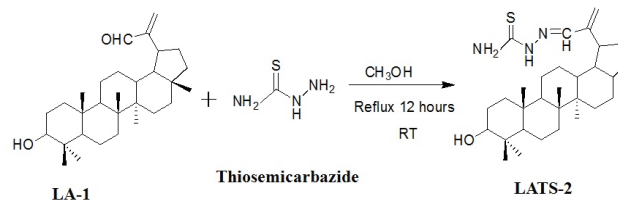
Reaction with Methyl Piperazine: LA-1 (1 mole) was refluxed with Methyl Piperazine (1 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product LAMP-2.

LAMP-2: M.P.: 224°C, FT-IR spectra showed 3420 cm^{-1} (OH), 2924 cm^{-1} (=CH), 2853 cm^{-1} (CH), 2359 cm^{-1} (C=N), 1693 cm^{-1} (NH-Bending), 1383 cm^{-1} (C-N). $^1\text{H NMR}$ (400MHz, CDCl_3 , δ , ppm) δ 6.27 (s,1H,CN), δ 5.89 (s,1H,CN), δ 3.13 (d,2H,J=5.04,CH₂), δ 4.8 (d,1H,J=12.8,OH), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch-24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



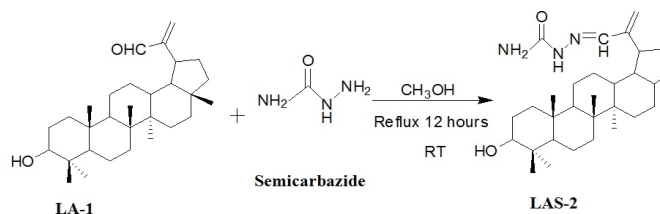
Reaction with Thiosemicarbazide: LA-1 (1 mole) was refluxed with Thiosemicarbazide (1 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to obtain LATS-2.

LATS-2: M.P.: 220°C. FT-IR spectra showed 3431 cm^{-1} (OH), 2990 cm^{-1} (=CH), 2879 cm^{-1} (CH), 2348 cm^{-1} (C=N), 1662 cm^{-1} (NH-Bending), 1260 cm^{-1} (C-N). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 7.34 (s,2H,NH₂), δ 5.42 (s,1H,CN), δ 5.18 (s,2H,CH₂), δ 4.8 (d,1H,J=9.8,OH), δ 2.2 (m,1H,NH), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch-24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



Reaction with Semicarbazide: LA-1 (1 mole) was refluxed with Semicarbazide (1 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to obtain LAS-2.

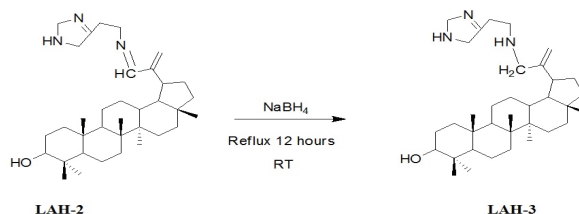
LAS-2: M.P.: 232°C. FT-IR spectra showed 3356 cm^{-1} (OH), 3010 cm^{-1} (=CH), 2881 cm^{-1} (CH), 2346 cm^{-1} (C=N), 1650 cm^{-1} (NH-Bending), 1230 cm^{-1} (C-N). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 7.49 (s,2H,NH₂), δ 6.45 (s,1H,NH), δ 4.8 (d,1H,J=13.8,OH), δ 5.55 (s,1H,H), δ 5.35 (s,1H,H), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch-24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



Step- 3: Further modification of synthesized modified products

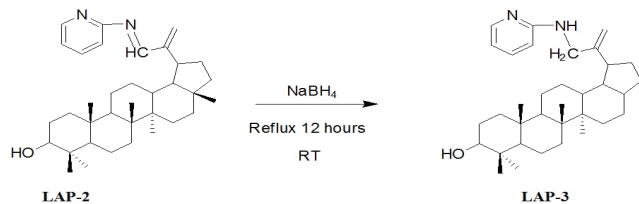
LAH-2 (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 h. Then the reaction product was separated by ethyl acetate and water. The organic layer was taken and sodium sulphate was added and filtered to obtain LAH-3.

LAH-3: M.P.: 226 °C, FT-IR spectra showed 3454 cm^{-1} (OH), 2946 cm^{-1} (=CH), 2870 cm^{-1} (CH), 2374 cm^{-1} (C=N), 1609 cm^{-1} (NH-Bending), 1361 cm^{-1} (C-N). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 4.8 (d,1 H,J=16.8,OH), δ 2.0 (s,1H,NH), δ 4.8 (d,1H,J=16,OH), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



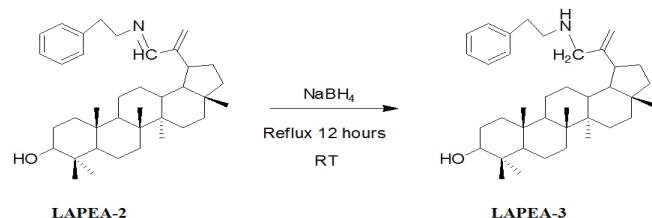
LAP-2 (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to obtain LAP-3.

LAP-3: M.P.: 228°C. FT-IR spectra showed 3310 cm^{-1} (OH), 2924 cm^{-1} ($=\text{CH}$), 2853 cm^{-1} (CH), 2340 cm^{-1} (C=N), 1707 cm^{-1} (NH-Bending), 1452 cm^{-1} (-C-H Bending), 1232 cm^{-1} (C-N). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 7.29 (s,1H,NH), δ 4.92 (s,1H, CN), δ 4.91 (d,2 H,J=14.68,CH₂), δ 4.8 (d,1H,J=12.4,OH), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch-24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).

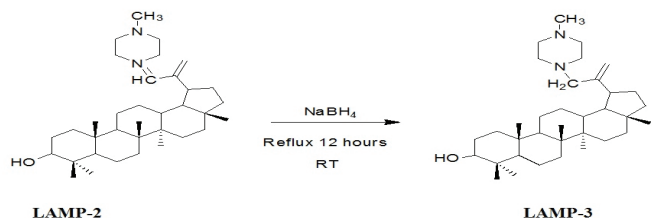


LAPEA-2 (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to obtain LAPEA-3.

LAPEA-3: M.P.: 223°C. FT-IR spectra showed 3516 (OH), 2922 cm^{-1} ($=\text{CH}$), 2854 cm^{-1} (CH), 2343 cm^{-1} (C=N), 1720 cm^{-1} (NH-Bending), 1359 cm^{-1} (-C-H Bending). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 5.22 (m,1H,CN), δ 6.2 (s,1H,CN), δ 4.8 (d,1H,J=12.8,OH), δ 3.4 (m,2H,CH₂), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch-24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



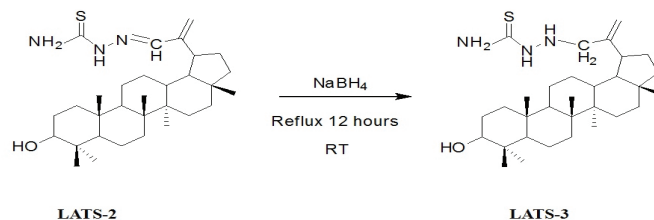
LAMA-2 (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product LAMA-3. **LAMA-3:** M.P.: 230°C. FT-IR spectra showed 3310 cm^{-1} (OH), 2914 cm^{-1} ($=\text{CH}$), 2844 cm^{-1} (CH), 1561 cm^{-1} (-C-H Bending). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 6.27 (s,1H,CN), δ 5.89 (s,1H,CN), δ 3.13 (d,2H,J=5.04,CH₂), δ 4.8 (d,1H,J=17.2,OH), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch-24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



LATS-2 (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product LATS-3.

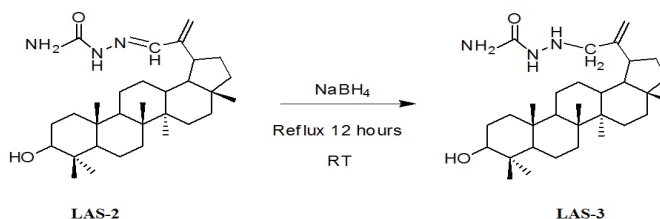
LATS-3: M.P.: 231°C. FT-IR spectra showed 3367 cm^{-1} (OH), 2990 cm^{-1} ($=\text{CH}$), 2881 cm^{-1} (CH), 2340 cm^{-1} (C=N), 1612 cm^{-1} (NH-Bending), 1549 cm^{-1} (-C-H Bending). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 7.34

(s,1H,NH₂), δ 5.42 (s,1H,CN), δ 5.18 (s,2H,CH₂), δ 2.2 (m,1H,NH), δ 4.8 (d,1H,J=10.1,OH), δ 2.8 (m,1H), δ 2.1 (m,2H,H-2), δ 1.65 (bunch-24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



LAS-2 (1 mole) was refluxed with Sodium Borohydride (1.5 mole) in methanol for 12 h. After that the reaction product was separated by ethyl acetate and water. The organic layer was taken and added sodium sulfate and then filtered it to give the product LAS-3.

LAS-3: M.P.: 227°C. FT-IR spectra showed 3420 cm^{-1} (OH), 2914 cm^{-1} ($=\text{CH}$), 2844 cm^{-1} (CH), 2266 cm^{-1} (C=N), 1561 cm^{-1} (-C-H Bending). $^1\text{H-NMR}$ (400 MHz, CDCl_3 , δ , ppm) δ 7.49 (s,2H,NH₂), δ 6.45 (s,1H,NH), δ 5.55 (s,1H,H), δ 5.35 (s,1H,H), δ 2.8 (m,1H), δ 4.8 (d,1H,J=13.4,OH), δ 2.1 (m,2H,H-2), δ 1.65 (bunch-24H), δ 1.01 (s,3H,Me), δ 0.92 (s,3H,CH₃), δ 0.81 (s,6H), δ 0.75 (s,3H,CH₃).



ACUTE TOXICITY STUDY

The acute toxicity study of potent compounds was performed as per the OECD guideline. The albino mice of either sex (body weight 20-25 g) were used. The potent compound was administered by oral route at different dose levels in group of 3 animals each. Animals were observed individually after administration at least once during the first 30 minutes, periodically during the first 24 hours and then they were sacrificed.

ANTI-PSYCHOTIC ACTIVITY (ACTOPHOTOMETER)

Experimental study protocol has been approved by the Institutional Animal Ethics Committee (IAEC) constituted as per the guidelines laid by the committee for the purpose of control and supervision of experiments on Animals (CPCSEA) (1273/AC/09). Actophotometer was performed by administering Diazepam (2mg/kg body weight) and synthesized molecules (LAH-3, LAP-3, LAPEA-3, LAMP-3, LATS-3, LAS-3) (n=3) into different groups of White Albino Rats of Wistar strain (150-170 g). Rats were maintained on laboratory stock diet. They were fasted for 24 h before starting the experiment. The rats were numbered and weighed. The Actophotometer was switched on and the rats were placed individually in the activity cage for 10 min. The basal locomotor activity score of all rats were noted. Standard, Test, Control were noted on each rat of proposed groups and after 30 min. each animal was retested for 10 min. They were divided into three groups each comprised of three rats. The groups are:- Control group - three rats received 0.8 ml of DMSO, Standard group- three rats received oral dose of Diazepam 2 mg/kg body weight, Test group- divided into first to six groups, each containing three rats, received 250 mg of test drug per kg body weight

dissolved in 1ml of DMSO. The synthesized compounds LAH-3, LAP-3, LAPEA-3, LAMP-3, LATS-3 and LAS-3 were selected and evaluated for their Anti-Psychotic Activity using Actophotometer.

Statistical analysis

Data were analyzed by analysis of variance test followed by turkey's test. All the results were expressed as mean \pm SEM. $P < 0.05$ was considered significant. Percent reduction in activity score and fall off time were calculated with reference to respective basal recordings.

COMPULSIVE BEHAVIOUR (STEREOTYPY) IN PLUS MAZE MODEL

Compulsive behaviour (Stereotypy) in Plus Maze Model was performed by administering Apomorphine (2.5 mg/kg body weight, ip), Haloperidol (2.5 mg/kg body weight, i.p) and synthesized molecules (LAH-3, LAP-3, LAPEA-3, LAMP-3, LATS-3, LAS-3) ($n=3$) into different groups of Swiss albino mice (20-25 g). Animals were weighed, numbered and divided into different groups such as control, standard and test. Then animals were trained in elevated plus maze. Control, standard, test solutions were prepared and injected into different groups. The groups are: Control group - three mice received 0.65 ml of Apomorphine (ip), Standard group- three mice received of Haloperidol (ip) 2.5 mg/kg body weight, Test group- divided into first to six groups, each containing three mice, and received 250 mg of test drug per kg body weight. Noted the onset and intensity of rearing, sniffing, and licking behaviour at 0 times 15, 30, 45, 60 min after the Apomorphine, haloperidol and test drugs were injected. The severity of the response can be scored as + = Presence - = Absent.

Statistical analysis

Data were analyzed by analysis of variance test followed by turkey's test. All the results were expressed as mean \pm SEM. $P < 0.05$ was considered significant. Percent reduction in activity score and fall off time were calculated with reference to respective basal recordings.

RESULT AND DISCUSSION

Acute Toxicity Study

After Sacrifice infection was observed in intestine, stomach, liver and ulcer occurs in intestinal parts of animals at the various concentrations of drugs. The results of acute toxicity study showed no clinical signs of toxicity and mortality in the lupeol drug treated animals even after administration of 500 mg/kg dose (Table 1). Hence, as per OECD guidelines lethal dose was assigned to be more than 1000 mg/kg. One-fourth of this lethal dose (250 mg/kg) was taken as effective dose for the study.

Table 1: Lupeol extract at different concentration level

S. No.	Compound Conc. (mg/kg, oral)
1	L-1, no toxic > 250
2	L-1, no toxic <500
3	L-1, no toxic <1000
4	L-1, toxic <2000

ANTI-PSYCHOTIC ACTIVITY (ACTOPHOTOMETER)

Effect of lupeol derivatives on animal behaviour was observed and it was found that there is significant difference between LAH-3 and LAPEA-3 occurs ($P < 0.05$) (Table 2). Diazepam (2.5 mg/kg, oral) and drug derivatives (250 mg/kg, oral) treated groups showed significant locomotor

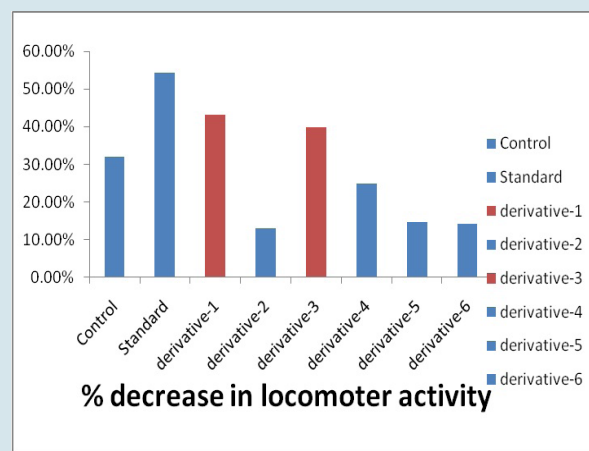


Figure 2: Locomotor activity of lupeol derivatives and standard drug.

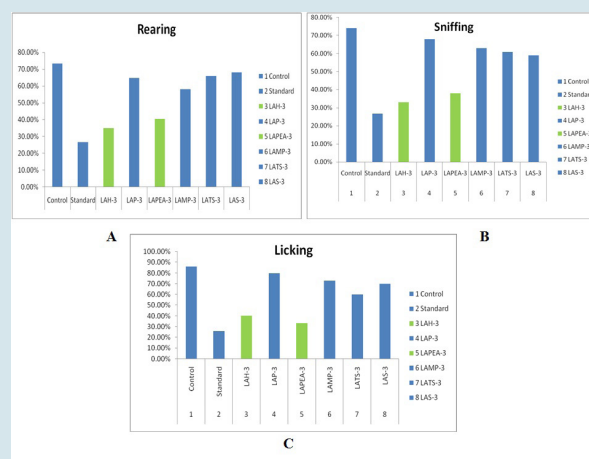


Figure 3: Behavioural symptoms of Swiss albino mice after administration of lupeol derivative compounds with respect to (A) Rearing, (B) Sniffing, (C) Licking.

activity when compared with control (Figure 2); however, this psychosis was less with drug derivatives treated group than diazepam-treated group.

COMPULSIVE BEHAVIOUR (STEREOTYPY) IN PLUS MAZE MODEL

All the drug derivative compounds were subjected to pharmacological evaluation to determine their behaviour symptoms, inhibition of apomorphine induced Rearing, Sniffing, Licking. Swiss albino mice (three mice in each group) of either sex (20-25 g) were used and housed per cage in standard laboratory conditions (12 h light/ dark cycle, 22 ± 2 °C room temperature). Food and water were available ad libitum. All experiments were approved by institutional ethical Committee. All synthesized compounds were suspended in 1% solution of apomorphine in distilled water and administered by the intraperitoneal (i.p) route. The changes in the behavior symptoms were noted down for an interval of 30 min for 3 h and then after 24 h, the cages were inspected for any mortality of the animals. Haloperidol (2 mg/kg i.p.) and drug derivatives (250 mg/kg, oral) significantly ($P < 0.001$) exhibited psychosis; as evident from decreased rearing, sniffing, and licking behaviour as compared with control (Figure 3).

SUMMARY AND CONCLUSION

A series of compounds were prepared using the pathway shown in synthetic schemes (3.5). The target compounds were prepared by three steps procedure. In series A, in the first step Lupeol (L-1) was reacted with selenium dioxide and few drops of water to give the product Lupeol aldehyde (LA-1). In the second step, LA-1 was reacted with the different amines to give the final products. At in the last step, the reduction double bond at C-30. All the target compounds were subjected to pharmacological evaluation for behaviorsymptoms, likes standing (rearing), continuous sniffing (touching the nose), and licking the body inhibition of apomorphine induced in elevated plus maze model .The semisynthetic derivatives were also subjected to locomotor activity and result shown that compound LAH-3 and LAPEA-3 was found to be active. The acute toxicity of the potent compound in a series was also performed. The pharmacological results suggested that the presence of heterocyclic amines in the ring increased the antipsychotic activity. This clearly demonstrate that 5 membered Histamine and aromatic amine such as phenyl ethyl amine increases the activity while other substituent has no effect on activity as compared to lupeol. Although inclusion of pyridine, piperazine, semicarbazide and thiosemicarbazide does not increase any effect. Only imidazoline and aromatic amine like phenyl ethyl amine shows the positive response. It is thus concluded that lupeol skeleton deserve further investigation for the development of more potent and

non-toxic new agents for therapeutic use.

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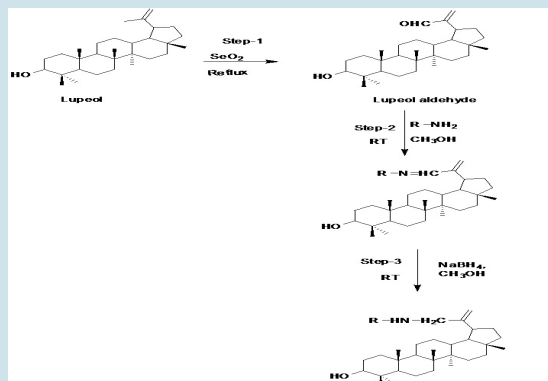
CONFLICT OF INTEREST

None

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PICTORIAL ABSTRACT



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