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CONTENTS

VIEWS

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Synthesis Of Ethyl 2-Methyl-2-[(5-Phenyl-4-{[(*E*)-Substituted) Benzylidene] Amino}-4*H*-1, 2, 4- Triazol-3-Yl)Sulfanyl] Propanoates

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ABSTRACT

In the present study an efficient method for the synthesis of ethyl 2-methyl-2-[(5-phenyl-4-{[(*e*)-substituted) benzylidene] amino}-4*h*-1, 2, 4- triazol-3-yl)sulfanyl]propanoates is described. About four such derivatives 5a to 5d are synthesised and characterized by physical data-melting point/boiling point, R_f valueand spectral studies-IR,^{1}HNMR.

Keywords: Synthesis, Propanoates, 1,2,4–triazole, Benzylidene, Fibric acid, Thiofibrates.

Introduction

1,2,4-triazole represents an important class of heterocyclic compounds and are found as versatile building blocks for the synthesis of pharmaceuticals substances[1]. These interesting groups of compounds have been found to possess a broad range of pharmacological applications such as antiinflammatory, antifertility, antiviral,antimicro bial,anticancer,antitubercular, ántioxidant, anticonvulsant activities[2] *etc.,*Schiff bases are also important medicinally active groups as they are the part of pharmaceutically active compounds [3]. However, literature review revealed that the presence of heteroatom in the schiffs bases enhances the activity[4].Further more fibric acid pharmacophore in fibrates and thiofibrates is of interest to medicinal chemist as these groups of drugs are found to have antihyperlipedemic activity[5,6] by lowering serum triglycerides and rising HDL- Cholesterol and remain current treatment of choice for patients with severe hypertriglyceridemia[6].These three biologically active molecules striked us to synthesise Schiff bases of 1,2,4 –triazole incorporated thiofibrates as there is a spacity of literature on such molecules so that they can be used in the treatment of dyslipedemia.

Materials and methods

Melting points / Boiling points of the synthesized compounds were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by TLC on pre-coated silica gel plates using n-hexane: ethylacetate (1:3) as mobile phase. The developed chromatographic plates were observed under UV at 254 nm and also in iodine chamber [7].IR spectral studies were carried out using ATR, and ^{1}H NMR and ^{13}C NMR spectra were recorded on DELTA2 NMR 500 MHz using $CDCl_3$ as solvent and TMS as internal standard[8].

Synthesis of Benzohydrazide(1)

In a dry 250 ml round bottomed flask fitted with a reflux condenser and a dropping funnel 1.45 ml (0.01mol) of ethyl benzoate in 5 ml of ethanol was placed and 1.0 ml (0.02mol) of hydrazine hydrate in 5ml of ethanol was added drop-wise under constant stirring. The reaction mixture was refluxed at 70^{0}C for about 12h and cooled. The solvent was then evaporated and the product obtained was collected, dried and recrystallized from ethanol[9].M.F: $C_7H_8N_2O$; M.W:136;%yield78; m.p 95^{0}C;R_f: 0.53; IR (cm^{-1}, KBr) : 3301(N-H),3217(NH_2)3108(C-H aromatic, 1639(C=O), 1495(C=C aromatic).

Synthesis ofpotassium 2-(phenylcarbonyl) hydrazinecarbodithioate(2):

To the above benzohydrazide(1) (1.36g, 0.01mol) in 50 ml of ethanol was added sufficient potassium hydroxide to make it neutral and then further additional 6.35g (0.1mol) of Potassium hydroxide pellets, 5.653g, 7ml (0.1mol) of Carbondisulphide was addedand refluxed for 3 h in a 250ml round bottomed flask. 3-4g of activated animal charcoal was added to the above refluxed mixture and further heated to 10 min, cooled and filtered. The filtrate obtained was then heated to 60-70^{0}C on a water-bath with sufficient quantity of aceticacid till the filtrate is acidic and 20ml of water where these were added under constant stirring of the filtrate. During this process glistering white crystals were formed and were allowed to crystallize in a refrigerator overnight. The product thus obtained was collected by filtration,

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recrystallized from ethanol and dried[10]. M.F: $C_8H_7N_2OS_2K$; M.W: 250;%yield75; m.p 228-230^{0}C;R_f: 0.51; IR (cm^{-1} , KBr) : 3141(NH), 3073(C-H aromatic), 1610 (C=O), 1573(C=C Aromatic).

Synthesis of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol(3):

To 0.01 mol (2.50 g) of potassium 2-(phenylcarbonyl) hydrazinecarbodithioate(2), 0.2 mol(1.0 g, 2 ml) of hydrazine hydrate was added in 250 ml round bottomed flask fitted with a reflux condenser and refluxed for about 5 h. The resulting solution was diluted with 100 ml of water and acidified with concentrated hydrochloric acid. The solid thus precipitated was filtered, washed with water and recrystallized from ethanol [11]. M.F: $C_8H_8N_4S$; M.W: 192;%yield 60; m.p195-200^{0}C;R_f: 0.81; IR (cm^{-1}, KBr): 3300(NH_2), 3193 (C-H Aromatic), 2754 (S-H), 1637(C=N), 1321(C-N).

Synthesis of 4-{[4-(substituted)benzylidene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol(4a-4d).

In a 250 ml round bottomed flask fitted with a reflux condenser and a dropping funnel 0.01 mol (1.92 g) 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol(3) was dissolved in ethanol. 0.01mol of 4- substitutedbenzaldehyde dissolved in ethanol was added drop wise to the above solution with stirring. The mixture was then refluxed on water bath for about 12h, cooled and poured in to ice cold water. The resulting solid was filtered and recrystallized from ethanol[11].

4a: 4-{[4-(dimethylamino)benzylidene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol.

4- dimethylaminobenzaldehyde 0.01mol(1.49 g); M.F: $C_{33}H_{32}N_{11}OS_4$; M.W: 766;%yield75; m.p: 185-190^{0}C;R_f 0.59 : IR (cm^{-1} , KBr) : 3112 (CH-Ar),2934 (C-H) 2826 (S-H), 1646 (C=N), 1271 (C-N)

4b:[(4-nitrobenzylidene)amino]-5-phenyl-4*H*-1,2,4-triazole-3-thiol

4- nitrobenzaldehyde 0.01mol(1.51 g); M.F: $C_{15}H_{11}N_5SO_2$; M.W: 325;%yield71; m.p222-225^{0}C; R_f: 0.56 IR (cm^{-1} , KBr) : 2842(C-H Aromatic), 2443 (S-H), 1518(C-H bend), 1675(C=N) 1291(C-N).

4c: 4-[(4-chlorobenzylidene)amino]-5-phenyl-4*H*-1,2,4-triazole-3-thiol

4- chlorobenzaldehyde 0.01 mol(1.56 g); M.F: $C_{15}H_{11}ClN_4S$; M.W:314;%yield60; m.p177-180^{0}C;R_f: 0.69; IR (cm^{-1} , KBr) : 3134(C-H Aromatic), 2996(CH) 2757 (S-H), 1486(C-H bend), 1625(C=N), 1294 (CN).

4d: 4-[(4-fluorobenzylidene)amino]-5-phenyl-4*H*-1,2,4-triazole-3-thiol

4- flurobenzaldehyde 0.01 mol(1.24 g);M.F: $C_{15}H_{11}FN_4S$;M.W: 298 ;%yield 61; m.p. 166-170^{0}C; R_f : 0.82; IR (cm^{-1} , KBr) : 3065(C-H Aromatic), 3068(CH) ,2590 (S-H), 1509(C-H bend), 1633(C=N), 1294 (CN).

Synthesis of ethyl 2-methyl-2-[(5-phenyl-4-{[(*E*)-4-substituted)benzylidene]amino}-4*H*-1,2,4-triazol-3-yl) sulfanyl]propanoate(5a-5d: TZTF1-TZTF4)

0.001mol of 4-{[4-(substituted)benzylidene]amino}-5-phenyl-4*H*-1,2,4- triazole-3-thiol (4a-4d) dissolved in 10 ml of dimethyl formamide and 4g (0.04mol) of anhydrous potassium carbonate were taken in a 100 ml round bottom flask attached with dropping funnel and calcium chloride guard tube. 2.56 ml (0.01mol) of Ethyl 2 bromoisobutyrate in 10 ml of dimethyl formamide was added drop-wise to the above solution with constant stirring at room temperature. The reaction mixture was then continuously stirred for about 24 h and progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was added to 300 ml of water and stirred well; the product was extracted with ethyl acetate (3 portions). From the combined extract ethyl acetate was distilled off using rotavapour and oily yellow colored product separated was then collected **(Scheme)**.

5a: ethyl 2-methyl-2-[(5-phenyl-4-{[(*E*)-4-dimethylamino) benzylidene]amino}-4*H*-1,2,4-triazol-3-yl)sulfanyl] propanoate(TZTF1)

4-{[4-(dimethylamino)benzylidene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol 0.001 mol (0.437g).M.F: $C_{23}H_{27}N_5O_2S$; M.W: 437; %yield55; m.p110^{0}C;R_f: 0.86; IR (cm^{-1}, KBr): 2971(C-H aromatic), 2930(C-H aliphatic), 1734 (C=O). ^{1}HNMR(δppm,$CDCl_3$); 8.1(s,1H,CH), 7.9-7.3 (m,5H,ArH),6.6-6.5(m,4H,ArH of dimethyl amino benzaldehyde), 4.1-3.9 (q,2H,CH_2), 2.9-2.7 [s,6H,$(CH_3)_2$ of dimethyl amino benzaldehyde], 1.6-1.3 (s,6H,$(CH_3)_2$) 1.1-0.9 (t,3H,CH_3);^{13}C NMR (δ ppm, $CDCl_3$): 172.2 (an ester carbon), 168 (one methyne carbon), 168 (carbon 3 in Triazole), 162 (carbon 5 in Triazole), 130,129,127,125,119,117,110(Twelve aromatic carbons); 60(one methylene carbon) 59(one quaternary carbon);35,35,30,29 (Four methyl carbon);13 (one methyl carbon).

5b: ethyl 2-methyl-2-[(5-phenyl-4-{[(*E*)-4-nitro) benzylidene]amino}-4*H*-1,2,4-triazol-3-yl)sulfanyl] propanoate (TZTF2)

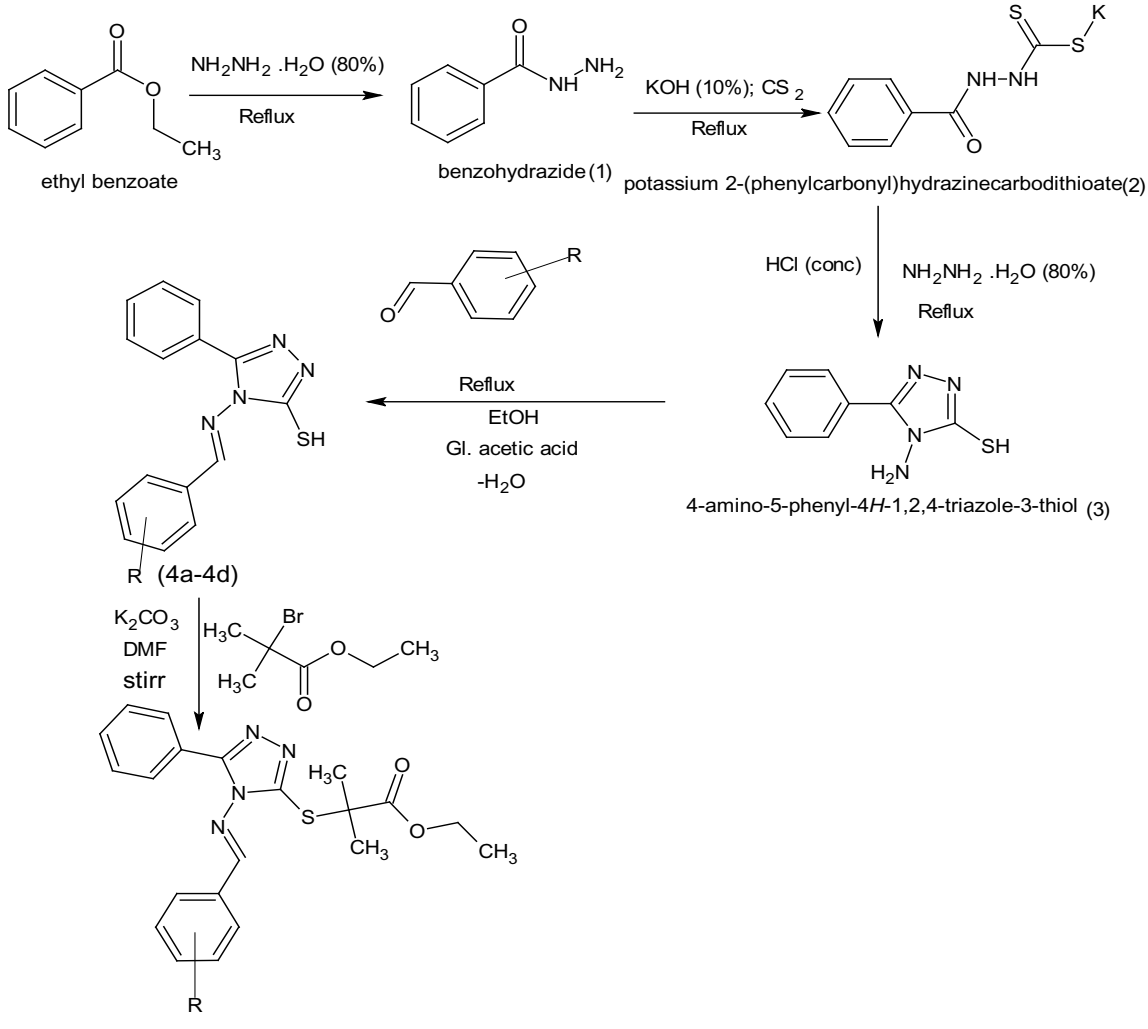
4-{[4-(nitro)benzylidene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol 0.001 mol (0.439 g) M.F: $C_{21}H_{21}N_5O_4S$;M.W: 439;%yield 43; m.p 135^{0}C; R_f : 0.62 ;IR (cm^{-1} , KBr) : 2982(C-H aromatic), 2906(C-H aliphatic), 1734 (C=O), 1386 (nitro);^{1}HNMR(δ ppm,$CDCl_3$):8.0(s,1H,CH), 7.9-7.3 (m,5H,ArH),6.8-6.9(m,4H,ArH of nitrobenzaldehyde), 4.1-3.8(q,2H,CH_2),1.6-1.3 (s,6H,$(CH_3)_2$) 1.1-0.9 (t,3H,CH_3);^{13}C NMR (δ ppm, $CDCl_3$): 172.0 (an ester carbon), 168 (one methyne carbon), 168 (carbon 3 in Triazole), 162 (carbon 5 in Triazole), 130,129,127,125,119,117,110(Twelve aromatic carbons); 60(one methylene carbon) 59(one quaternary carbon);35,30(Two methyl carbon);13.5 (one methyl carbon).

5c: ethyl 2-methyl-2-[(5-phenyl-4-{[(*E*)-4-chloro) benzylidene]amino}-4*H*-1,2,4-triazol-3-yl)sulfanyl] propanoate (TZTF3)

4-{[4-(chloro)benzylidene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol 0.001 mol (0.428 g)M.F: $C_{21}H_{21}ClN_4O_2S$;M.W: 429; %yield40;m.p:127 ^{0}C; R_f : 0.6 IR (cm^{-1} , KBr) : 2981(C-H aromatic), 2934(C-H aliphatic), 1734 (C=O), 763(chloro);^{1}HNMR(δ ppm,$CDCl_3$):8.0(s,1H,CH), 7.7-7.1 (m,5H,ArH),6.5-6.3(m,4H,ArH of chlorobenzaldehyde), 4.0-3.8(q,2H,CH_2),1.5(s,6H,$(CH_3)_2$) 1.0(t,3H,CH_3);^{13}C NMR (δ ppm, $CDCl_3$): 172 (an ester carbon), 168 (one methyne carbon), 168 (carbon 3 in Triazole), 162 (carbon 5 in Triazole), 130,129,127,125,119,117,110(Twelve aromatic carbons); 60(one methylene carbon) 59(one quaternary carbon);35,30 (Two methyl carbon);13 (one methyl carbon).

5d: ethyl 2-methyl-2-[(5-phenyl-4-{[(*E*)-4-fluoro) benzylidene]amino}-4*H*-1,2,4-triazol-3-yl)sulfanyl] propanoate (TZTF4)

4-{[4-(fluoro)benzylidene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol 0.001 mol (0.412 g) M.F: $C_{21}H_{21}FN_4O_2S$; M.W: 412; %yield 38; m.p: 60^{0}C;R_f : 0.88 IR (cm^{-1} , KBr) : 2982(C-H aromatic), 2899(C-H aliphatic), 1734 (C=O), 1386 (fluoro); ^{1}HNMR(δ ppm,CDCl3):8.1(s,1H,CH), 7.9-7.2 (m,5H,ArH),6.5-6.4(m,4H,ArH of fluorobenzaldehyde), 4.1-3.9(q,2H,CH_2),1.6-1.3 (s,6H,$(CH_3)_2$) 1.1-1.0 (t,3H,CH_3);^{13}C NMR (δ ppm, $CDCl_3$): 172.0 (an ester carbon), 168 (one methyne carbon), 168 (carbon 3 in Triazole), 162 (carbon 5 in Triazole), 130,129,127,125,119,117,110(Twelve aromatic carbons); 60(one methylene carbon) 59(one quaternary carbon);30,29 (Two methyl carbon);14 (one methyl carbon).



where

Compound	R
TZTF1	4-$N(CH_3)_2$
TZTF2	4-NO_2
TZTF3	4-Cl
TZTF4	4-F

Scheme: Synthetic route of ethyl 2-methyl-2-[(5-phenyl-4-{[(*e*)-substituted) benzylidene] amino}-4*h*-1, 2, 4- triazol-3-yl)sulfanyl]propanoates.

Results and Discussion

Benzohydrazide (1) was prepared using the regular protocol of refluxing ethyl benzoate with hydrazine hydrate in ethanol. Further conversion of this benzohydrazide into its potassium 2-(phenylcarbonyl) hydrazine carbodithioate (2) was achieved after making the mixture alkaline with potassium hydroxide, addition of CS_2 and refluxing for adequate amount of time and acidification with glacial acetic acid. This on refluxed with hydrazine hydrate and acidification with concentrated hydrochloric acid yielded the compound 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (3).Sodium fusion extraction test confirms the presence of the extra elements nitrogen and sulphur, the presence of amino group was confirmed by diazotization. This was further conformed by IR. The appearance of peak at 3300cm^{-1} and 2754cm^{-1} indicates the presence of amino and thiol group respectively.This intermediate was further confirmed by physical data m.p, R_f value and IR**.**Four substituted 4-{[4-benzylideneamino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol were synthesised by condensing 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol with different substituted aromatic aldehydesviz p-dimethylaminobenzaldehyde,p-nitrobenzaldehyde,p-chlorobenzaldehyde, p-fluorobenzaldehyde catalysed by glacial acetic acid. The reactions were completed in 4-5 h. and yield of the compounds range from 60-75%. The homogeneity of the compounds was checked by m.p and TLC. R_f value of the Schiff bases 4a-4d obtained using ethyl acetate: n-hexane (1:3) as developing solvent were in the range of 0.56-0.82. The structures of the Schiff bases 4a-4d were established on the basis of their IR spectra in which the peaks at 3300cm^{-1} were absent , indicating the Schiff base formation. Further, the absence of mercapto group at 2500-2800 cm^{-1} in the IR spectras of the thiofibrates (TZTF1-TZTF4) indicates the formation from their respective thiols.The appearance of protons in methylene group as quartet at δ 4.1-3.9ppm, methyl group as triplet at δ 1.1-0.9ppm, dimethyl group as singlet at δ 1.6-1.3ppm and aromatic protons as multiplets at δ 7.9-6.5 ppm in the ^{1}HNMR spectra of the compound ethyl 2-methyl-2-[(5-phenyl-4-{[(*E*)-4-dimethylamino)benzylidene] amino}-4*H*-1,2,4-triazol-3-yl)sulfanyl]propanoate(TZTF1) indicates its assigned structures. Similarly ^{13}C NMR spectra of this compound indicates the presence of an Ester carbon at δ 172.2ppm; at δ 168,162ppm for one methyne carbon and two heterocyclic carbons (1,2,4 triazol); at δ ppm 130 ,129,127,125,123,119,117,110for twelve aromatic carbons; at δ ppm 60 for one methylene carbon; at δ ppm59 for one quaternary carbon; at δ35,30,29 ppm for four methyl carbon and at δ ppm 13.8 for one methyl carbon. Also the spectral datas of the remaining compounds are in good agreement with the assigned structures.

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Acute Toxicity Studies of Petroleum Ether, Methanol and Aqueous Extracts of *Leptadenia Reticulata*

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ABSTRACT

The purpose of the study was to test the acute oral toxicity of the different extracts of the plant *Leptadenia reticulata*. Acute toxicity of petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata* was evaluated in Swiss mice. The acute toxicity studies were carried out based on OECD guidelines 423. The animals were orally administered with a single dose of 100, 250, 500, 750, 1000, 2000mg/kg body weight of each extract. Signs of toxicity and mortality were noted after 1, 4 and 24h of administration of the extract for 14 days. The highest dose administered (2000mg/kg body weight) did not produce mortality or changes in general behaviour of the test animals. These results indicate the safety of the oral administration of petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata.*

Introduction

Ayurveda, an ancient system of Indian medicine, has recommended a number of drugs for the treatment of various diseases, like anaphylaxis, bronchial asthma and allergic disorders [(1)]. Allergy is one of the common diseases that affect mankind with diverse manifestations and is responsible for significant morbidity and mortality [(2)]. Anaphylaxis is triggered by different substances like foods (nuts, fish, wheat etc), medications (Penicillin), venom from insects, latex from natural rubber, allergy shots and extreme temperature also act as stimuli for anaphylaxis [(3)]. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations owing to low efficacy, associated adverse events and compliance issues [(4)].

Leptadenia reticulata has been used in the Ayurvedic system of Indian medicine for the treatment of bronchial asthma, eczema, insect bites etc [(5)]. Plants or drugs must be ensured to be safe before they could be used as medicines. By conducting toxicity tests in appropriate animal models, acute toxicity studies, we ensuring the safety of drugs.

So, in the present study, the petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata* were analysed for their acute toxicity profile with reference to behavioural aspects, in Swiss Albino mice. The limit test dose of 2000mg/kg body weight was used following OECD guidelines [(6,7)].

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EXPERIMENTAL METHODOLOGY

Plant material collection

The plant material of *Leptadenia reticulata (Retz.)* was collected from Tirumala hills after taxonomic verification and were identified and authenticated in Department of Botany, S.V.University, Tirupathi. The plant materials were coarsely powdered using a rotary grinder and stored in airtight plastic containers. This powder was used for preparation of extracts.

Preparation of extracts

The freshly collected plant material was washed, dried at room temperature for 15-20 days under shade and was treated with a rotary grinder for size reduction. The fine powder was collected and was used for preparation of extracts. Dried plant material (100 g) was extracted with Soxhlet apparatus using 400 mL petroleum ether for about 48 h. After defatting, the marc was dried in hot air oven at 50°C, packed in soxhlet apparatus and further extracted with 400 mL of 95% Methanol until it does not show the presence of any residue on evaporation. The aqueous extract was prepared by cold maceration with 3% methanol-water for 7 days with occasional shaking. The solvents were removed from the extracts under reduced pressure by using rotary vacuum evaporator.

Experimental animals

Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423 [8]. Experiments were performed using healthy young adult Swiss albino mice weighing 25-35 g [9].

Housing and Diet

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm) in a standard condition of temperature (22 ± 2^0C) relative humidity (60 ± 5%). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. The animals were fed with standard laboratory animal food pellets with water ad libitum.

Grouping of animals

The animals were randomly divided into three batches. Each batch contains seven groups and each group containing four mice. Group 1 (Control Group), Group 2 : Receives 100 mg/kg, Group 3 : Receives 250 mg/kg, Group 4 : Receives 500 mg/kg, Group 5 : Receives 750 mg/kg, Group 6 : Receives 1000 mg/kg, Group 7 : Receives 2000 mg/kg of a specific extract of *leptadenia reticulata.*

Mode of administration

The test substance was administered orally in a single dose using specially designed mice oral needle. Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water).

Administration Dose

Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 100, 250, 500, 750, 1000 and 2000 mg/kg. After the administration of test substance, food for the mice was withheld for 2 h.

Test substance administration volume

The administration volume was 1ml/kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

Observation period

Animals were observed individually after atleast once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the mice were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes and for mortality if any.

Acute toxicity studies

Direct observation parameters include Alertness. Writhing, Torch response, Corneal reflux, Tremors, Righting reflux, Gripping strength, Pinna reflux, Skin colour, Urination, Pupils diameter, Subcutaneous swellings, and Abdominal distensions. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then they were observed for further 14 days and Percentage of Mortality was calculated.

Statistical Analysis

Data are presented as a mean ± SEM (Standard Error of the Mean). Comparisons were made between the treated groups by the use of single way ANalysis Of VAriance (ANOVA). $P < 0.05$ was considered as the level statistical significance.

RESULTS

The present study conducted as per the OECD guidelines 423 revealed that the said extracts did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 2000mg/ kg body weight. The oral LD50 was indeterminable being in excess of 2000mg/kg body weight. So, testing the extracts at a higher dose may not be necessary and the extracts were practically non-toxic.

Tables 1, 2, 3 indicates the parameters observed before and after the administration of the Petroleum ether, Methanolic and Aqueous extracts of *Leptadenia reticulata* respectively. The parameters observed were normal even at the highest dosage of 2000mg/kg body weight of the test animal. This clearly indicated that the above extracts of *Leptadenia reticulata* do not produce oral toxicity. The medium lethal dose (LD50) of the extracts is higher than 2000 mg/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect. Table 4 indicates the percentage of Mortality after 14 days of treatment with Petroleum ether, Methanolic and Aqueous extracts of *Leptadenia reticulata.*

DISCUSSION AND CONCLUSION

The non-toxic nature of petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata* is evident by the absence of mortality of the test animals at oral treatment of 2000mg/ kg body weight. The normal behaviour of the test animals during a period of 14 days suggests the non-toxic nature of the foresaid extracts. Hence *Leptadenia reticulata* could be safe up to the dose of 2000 mg/kg body weight of the animal. Further studies are warranted for determining chronic toxic symptoms.

Table - 1

Effect of petroleum ether extract of *Leptadenia reticulata* on acute oral toxicity test in mice

S.No	Response	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Torch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal
4	Corneal reflux	Present	Present	Present	Present	Present	Present	Present
5	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Righting reflux	Present	Present	Present	Present	Present	Present	Present
7	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Pinna reflux	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Pupils diameter	Normal	Normal	Normal	Normal	Normal	Normal	Normal
12	Subcutaneous swellings	Absent	Absent	Absent	Absent	Absent	Absent	Absent
13	Abdominal distensions	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 2

Effect of methanolic extract of *Leptadenia reticulata* on acute oral toxicity test in mice

S.No	Response	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Torch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal
4	Corneal reflux	Present	Present	Present	Present	Present	Present	Present
5	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Righting reflux	Present	Present	Present	Present	Present	Present	Present
7	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Pinna reflux	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Pupils diameter	Normal	Normal	Normal	Normal	Normal	Normal	Normal
12	Subcutaneous swellings	Absent	Absent	Absent	Absent	Absent	Absent	Absent
13	Abdominal distensions	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 3

Effect of Aqueous extract of *Leptadenia reticulata* on acute oral toxicity test in mice

S.No	Response	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Torch response	Normal	Normal	Normal	Normal	Normal	Normal	Normal
4	Corneal reflux	Present	Present	Present	Present	Present	Present	Present

5	Tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Righting reflux	Present	Present	Present	Present	Present	Present	Present
7	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Pinna reflux	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal	Normal
10	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Pupils diameter	Normal	Normal	Normal	Normal	Normal	Normal	Normal
12	Subcutaneous swellings	Absent	Absent	Absent	Absent	Absent	Absent	Absent
13	Abdominal distensions	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 4
% of mortality of mice after 14 days of treatment with different extract of *leptadenia reticulata*

Groups	No. of mice	Dose administered	Petroleum ether extract		Methanol extract		Aqueous extract	
			No. of mice died	% of mice died	No. of mice died	% of mice died	No. of mice died	% of mice died
1	6	Control	0	0	0	0	0	0
2	6	100	0	0	0	0	0	0
3	6	250	0	0	0	0	0	0
4	6	500	0	0	0	0	0	0
5	6	750	0	0	0	0	0	0
6	6	1000	1	16	0	0	0	0
7	6	2000	2	33	1	16	1	16

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Physicochemical Characterization and Antioxidant Activity of Extract of Epimedium Grandiflorum

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ABSTRACT

There are several studies on various species of Epimedium, however literature on the medicine first time describes proximate analysis, phytochemical investigations and antioxidant activity of powder extracts of different solvents. Standard procedures were used to conduct this study. Proximate analysis was conducted which showed moisture content 3.93%, total ash 9.86%, acid insoluble ash 3.24%, sulphated ash 3.76%, extractive value of alcohol 3.76% and extractive value of water 3.55%. Proximate analysis helps in further standardization, storage, pharmaceutical development and stability issues of the material. The antioxidant activity was performed by standard procedures of phosphomolybdenum, DPPH and Ferric reducing assays. The methanol extract showed maximum antioxidant activity 129.49% by phosphomolybdenum method, 67.53% by DPPH and 137.77% by ferric reducing method. The study showed that the concentration of secondary metabolites was directly related with antioxidant activity.

KEYWORDS: *Epimedium grandiflorum*, antioxidant activity, DPPH, ferric reducing assay, antioxidant.

INTRODUCTION

The genus Epimediumconsists of 52 species. It belongs to familyBerberidaceae. It is commonly known as Rowdy Lamb Herb, Horny Goat Weed, Yangheye, Xianlinpi, Barrenwort, Fairy Wings,Yin Yang Huo and Bishop's Hat, at different places [1]. 20 species of Epimedii are very famous for their common use in market [2].Horny goat weed is rarely used as a single ingredient. It is traditionally used as an ingredient intonic to promote health. The leaves of as many as 15Epimedium species are used to make*yin yang huo*, an herb in traditional Chinese medicine. The name literally means "obscene goat leaves of pulse plants," which is translated as "horny goat weed" in English.Epimedium species that are used to make horny goat weed grow in China and Korea.Leaves are the most common part of the plant used in medicine, although other parts may also be used. TheEpimedium genus has antimicrobial, antioxidant and anti-inflammatory activities. The medicinal potential of this genus is increasing consistently [3]. It is evidenced that *Herbaepimedii* is used in osteoporosis, cardiovascular, sexual and neurological diseases [4]. The aerial parts are very famous for strengthening the reproductive and skeletal system with wide pharmacological actions which include anti-tumor, anti-depressant, anti-oxidation, anti-aging, anti-atherosclerosis activities and immunological functions. Its main biological activities include, antiviral, antifungal [5], anticancerous [6], antiangiogenic [7], anti-inflammatory [8] and antioxidant activities [9].

Flavonoids have antioxidative properties due to prevention of endogenous free radical decomposing enzymes and electron-donating ability [10]. Free radicals are very dangerous to cell membranes, tissue proteins and DNA. It is related to oxidative stress which results in ageing, cancer and heart diseases [11]. Endogenous anti-oxidative enzymes prevent the accumulation of free radicals in human body. Natural anti-oxidants help our body against oxidative stress due to free radicals.

Phenylalanine and tyrosine like aromatic amino acids from plants are major building blocks of flavonoids. Flavonoids play their role as an anti-oxidant due to the presence of electron-donating groups [11]. Icariin is a major flavonoid along with vitamin C and polysaccharides [12]. It is verified from various studies which indicate that the antioxidant activity of icariin on DNA damage, β-amyloid mediated neurotoxicity, endothelial cell injury and exercise-induced oxidant stress in liver are of great importance [13].

The genus Epimedii also improves the menopausal symptoms and bone health. The combined use of Epimedium grandiflorum and Estradiol prevents osteoporosis of femur and mandible of ovariectomized rats. It also increases the bone density of rats with osteoporosis to some extent [14].

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It was reported that Icariin with other flavonoids enhance the osteogenic differentiation of rat primary bone marrow stromal cells [15]. Similarly, it increased the osteoblastic proliferation [16]. The osteoclastic bone resorption was reduced [17]. It prevented osteoporosis in ovariectomized rats and increase mineral content [18]. Epimedium extracts are now standardized with icariin and epimedin A, B and C [19].

Horny goat weed is traditionally used to increase fertility. It was reported that EpimediumkoreanumNakai (EKN) helps in promotion of stamina and improves the sexual activity of humans in oriental countries including Korea and China.Icariin is an important flavonoid which increases the sexual activity in goats. Epimedium extracts with aglycones were found to be helpful in improving specific estrogenic activity [20].

Importance of our work

Need of our work: Recent discoveries about Epemedium species have shown very wide uses of it. Ranging from general wellness to specific use, it has wide variety of traditional uses. Moreover, it is a natural product and is used as herbal medicine in China.

AIM AND OBJECTIVES

The present study was designed to characterize *Epimedium grandiflorum* which include proximate analysis, phytochemical determination and antioxidant activity by standard procedures.Since there is lack of study on the medicinal properties of *Epimedium grandiflorum*, the following study principally aims to address the gap in the study by highlighting the antioxidant activity of the plant with following objectives,to prepare extracts of *Epimedium grandiflorum* powder,to perform Proximate analysis on *Epimedium grandiflorum*,to determine the antioxidant activity of *Epimedium grandiflorum*,to establish relationship between phenolic contents, flavonoids and antioxidant activity.

MATERIALS

Plant Material and Chemicals

The plant material was purchased from Chungsha Organic herb in China against Batch #EGFES10-140715 and the following chemicals were used during this study: Methanol (BDH, England), Chloroform (BDH, England), Ethyl acetate (BDH, England), Hexane (BDH, England), Ascorbic acid, DMSO, 28 Mm Sodium phosphate (NaH_2PO_4, Mol. wt. 137.99 g/mol), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Apparatus

The following apparatus was used in this study. Incubator (Memmert -W. Germany), Oven (Memmert -W. Germany), Refrigerator (2-8°C), Ultraviolet spectrophotometer, UV-1700 (Shimadzu – Japan), UV analyzer (UVGL-58), Electric Carbolite Furnace (Sheffield).

METHODS

Preparation of different extracts of *Epimedium grandiflorum* powder

Hot extraction

The continuous hot extraction process was used to prepare different extracts of powdered drug with solvents such as chloroform, ethyl acetate, n-hexane and methanol in a soxhlet apparatus. A filter bag of 50 g powdered material was prepared and transferred in the thimble of soxhlet apparatus. 500 ml methanol was added to prepare first extraction. Same procedure was used to obtain extracts of other solvents, respectively. The extracts were dried in rotary evaporator.

Cold extraction

Aqueous extract was obtained by decoction method. For this 50 g of powdered material was dissolved in 500 ml water for 24 hours withcontinuous stirring. After that it was filtered and dried for further study.

Proximate Analysis

In proximate analysis, pH, moisture content, total ash, acid insoluble ash, acid soluble ash, sulphated ash, extractive values in alcohol and water of *Epimedium grandiflorum* were measured according to standard methods (Indian Pharmacopoeia, 1996).

pH of 1 % solution

1g material was dissolved in 100 ml distilled water. This solution was filtered and its pH was measured with the help of pH meter previously calibrated.

pH of 5 % solution:

5 g material was dissolved in 100 ml distilled water. This solution was filtered and its pH was measured using a pH meter previously calibrated.

Moisture Content

Initial weight of clean and dry crucible was noted on an analytical balance. 2 g material was taken in a crucible and placed in an oven at 105 °C till constant weight was achieved. This was repeated five times to attain accuracy. Moisture content and dry matter were calculated as:

Dry matter (%) = × 100

Moisture content (%) = 100 - Dry matter (%)

Total Ash

Initial weight of the clean and dry crucible was noted on an analytical balance. 2 g material was taken in a crucible and placed in the furnace at 450°C till carbon free ash was obtained. The crucible was then cooled till constant weight was obtained. The weight of the crucible was noted and total Ash was calculated as:

Total Ash (%) = × 100

Acid Insoluble Ash

Initial weight of clean and dry silica crucible was noted on an analytical balance. Total ash content was placed in a beaker and treated with 25 ml dilute HCl for approx. 5 min. Ash less filter paper was used to filter the above material. The filter paper was put in pre weighed silica crucible. The crucible was shifted to muffled furnace till carbon free ash was obtained. The percentage of acid insoluble ash was calculated as follow:

Acid insoluble Ash (%) = × 100

Acid soluble ash:

The value of acid soluble ash was determined by subtracting the weight of acid insoluble ash from total ash. The contents obtained were expressed in percentage.

Acid soluble Ash = Total Ash - Acid insoluble Ash

Water Insoluble Ash

Initial weight of the clean and dry silica crucible was noted on an analytical balance. Total ash content was placed in a beaker and treated with 25 ml distilled water for approx.5 min. Ash less filter paper was used to filter the above material. The filter paper was placed in pre weighed silica crucible and it was shifted to muffled furnace till carbon free ash was obtained. The percentage of water insoluble ash was calculated as follow:

Acid insoluble Ash (%) = × 100

Water soluble ash:

The value of water soluble ash was measured by subtracting the weight of water insoluble ash from total ash. The contents obtained were expressed in percentage.

Water soluble Ash = Total Ash – water insoluble Ash

Sulphated Ash

Initial weight ofclean and dry silica crucible was noted on an electric balance. 2 g material was transferred into the crucible. This material was moistened with 1ml sulphuricacid and ignited at 500-600 °C on a burner. The crucible was cooled and treated again to obtain constant weight. The percentage of sulphated ash was calculated by:

Sulphated Ash (%) = × 100

Extractive Value in alcohol

5 g powder was taken in a closed conical flask. 100 mlof 95% alcohol was added into the flask to macerate it. The flask was let to stand till 24 hours without shaking. After 24 hours it was filtered and evaporated at 105°C by putting it in an oven. The weight of the dried extract was measured and alcohol soluble extractive value was calculated as:

Alcohol soluble Extractive value (%) = × 100

Extractive Value in water

5 g powder was taken in a closed conical flask. 100 ml chloroform water (few drops of chloroform in 100ml water) was added into the flask to macerate it. The flask was allowed to stand for 24 hours without shaking. After 24 hours it was filtered and evaporated at 105°C by putting it in an oven. The weight of the dried extract was measured and water soluble extractive value was calculated as:

Water soluble Extractive value (%) = × 100

Antioxidant Activity

Total antioxidant capacity assay

The extracts were investigated for their total antioxidant capacity by phosphomolybdenum method as described by [21].1 ml reagent was mixed with0.1 ml sample. After incubation at 95ºC for 90 minutes, the mixture wascooled and used as a micro-plate reader for absorbance of the reaction mixture at 695nm. Ascorbic acid was tested at 125µg/ml, 100µg/ml, 75µg/ml, 50µg/ml, and 25µg/ml to prepare the calibration curve. This curve was used to measure the total antioxidant capacity of the samples.

DPPH assay

The scavenging potential of the samples was observed by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [22].

Preparation of stock solutions

The stock solution of plant material was prepared by dissolving 4mg of each extract in 1 ml DMSO. 3.32mg of solid DPPH was weighed and dissolved in 100ml methanol to make stock solution.4mg ascorbic acid was weighed and dissolved in 1 ml DMSO to prepare stock solution.

Procedure

20µl plant extract was mixedwith180 µl DPPH reagent in a microplate and volume was made upto 200µl.The mixture was incubated at 37ºC for about 1 hour.Ascorbic acid was used as a positive control while methanol was used as a negative control.After incubation the reading was taken by using micro plate reader at 517 nm. Triplicate samples were used and the final scavenging percentage was calculated by using the formula,

Inhibition % = ×100

- Ac is the absorbance of the control
- As is the absorbance of the sample

Ferric reducing assay

The procedure used to determine the reducing power of plant extracts was described by [21].200 µl of each sample was mixedwith 500 µl buffer.500 µl potassium ferricyanide was added intothe mixture and was incubated at 50ºC for 20min. After incubation500 µl Trichloroacetic acid was added and the mixture was centrifuged at 3000rpm for 10min. Upper layer from the centrifuged mixture measuring 100 µl was poured into the well of 96 well plate and 0.1

% ferric chloride was added to it along with 20µl distilled water. Results were obtained at 630nmon microplate reader.

Preparation of different extracts of *Epimedium grandiflorum* powder

Different extracts of *Epimediumgrandliflorium* were prepared and their physical properties such as odor, color, consistency as well as percentage yield of each extract was checked. These properties are given in Table 1 and Table 2. Methanolic extract showed highest yield with semi solid texture and brownish colorfollowed by chloroform and ethyl acetate extract with same color but different consistencies. n-hexane and water showed light and dark brown color with same consistency. The presence of various chemical constituents was indicated by different color and consistencies of different extracts.

Proximate analysis of different extracts of *Epimedium grandiflorum*

Proximate analysis was conducted on*Epimedium grandiflorum* extracts. The results of pH, moisture content, total ash, acid insoluble ash, acid soluble ash, water insoluble ash, water soluble ash, sulphated ash and extractive values are given in Table 3. pH of 1 % and 5 % aqueous solutions was found out to be 5.4 and 5.6 respectively. The pH value helps about the acidic and basic constituents (Agarwal *et al*., 2007). Moisture content value was found out to be 5.4 % that would prevent hydrolysis. Low levels of moisture content reduce the degradation and spoilage by microorganisms. It also helps in calculating the weight of materials for pharmaceutical drug development in different dosage forms and to improve stability.

The diagnostic purity index was determined by total ash value which was found to be 9.86%. There are two types of ashes, physiological and non-physiological ash. Physiological ash is due to biochemical processes while environmental contaminants generate non-physiological

Table - 1
Physical characteristics of extracts of *Epimedium grandiflorum*

Sr. No	Name of Extracts	Odor	Color	Consistency
1	Chloroform extract	Characteristic	Brownish black	Solid
2	Ethyl acetate extract	Characteristic	Brownish black	Solid
3	Methanol	Characteristic	Brownish black	Semi solid
4	n-hexane extract	Characteristic	Light brown	Solid
5	Aqueous extract	Characteristic	Dark brown	Solid

Table 2
Percentage yield of *Epimedium grandiflorum*extracts

Sr. No	Name of extract	Percentage Yield (% w/w)
1	Chloroform extract	**18.63**
2	Ethyl acetate extract	**14.40**
3	Methanol extract	**35.97**
4	n-hexane extract	**12.55**
5	Aqueous extract	**10.23**

Table 3
Proximate analysis of extracts of *Epimedium grandiflorum*

Sr. No	Physicochemical properties	Percentage Yield (% w/w)
1	pH 1% solution	5.4
2	pH 5% solution	5.6
3	Moisture content	3.93
4	Total ash	9.86
5	Acid insoluble ash	3.24
6	Acid soluble ash	1.54
7	Water insoluble ash	3.87
8	Water soluble ash	1.12
9	Sulfated ash	3.76
10	Extractive value in alcohol	3.55
11	Extractive value in water	2.67

ash (Kunle, 2000). The acid insoluble ash was 3.24% which is physiological ash. The water insoluble ash was 3.87% and sulphated ash was found 3.76% which is helpful in determining the identity of constituents.

The amount of active ingredients was figured out by extractive values which were achieved withthe help of suitable solvents (WHO, 1998). Ethanol and water are commonly used for the preparation of herbal products. The extractive value of ethanol and water was found out to be 3.55 % and 2.67 % respectively. These values provide information about the chemical constituents present (Ozakar, 2005). The extractive values showed that ethanol is a better solvent for extraction than water.

Antioxidant activity

Epimedium grandiflorum is an important antioxidant and a remedy for various diseases caused by free radicals. Free radicals can cause damage to the cellular DNA and cause oxidative stress that produces various disorders. Antioxidants are very necessary to control the excessive production of free radicals which cannot be controlled by body's natural biological system.

Investigation of total antioxidant capacity by Phosphomolybdenum Method

In phosphomolybdenum method molybdenum VI reduces to molybdenum V with the formation of green phosphate Mo (V) complex. The results calculated from the standard Ascorbic acid are shown in Table 4.

Investigation of antioxidant activity by DPPH assay

The DPPH method is also used to determine the antioxidant activity of variousextracts of *Epimedium grandiflorum* (Brand William, *et al,* 1995). DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an important antioxidant assay that produces a violet solution based on electron-transfer. This method provides an easy and rapid way to evaluate the chemical reactions involving radicals (Sharma and Bhat, 2009). DPPH is a trap ("scavenger") for other radicals and an indicator for rate reduction of radical reactions. The total antioxidant activity of various extracts of *Epimedium grandiflorum* are shown in Table 5.

Determination of antioxidant activity by Ferric reducing assay

The totalantioxidantactivity of theseextracts by using ferric reducing power (FRP)methodis shown in Table 6. The methanol extract showed maximum scavenging capacity (137.77± 0.06µg /mg) followed by chloroform extract (127.71± 0.037µg /mg), ethyl acetate extract (126.71 ±0.00µg /mg), n-hexane extract (122.12 ±0.03 µg /mg) and aqueous extract (88.33 ± 0.03µg /mg)

Conclusion

The antioxidant ,peroximate analysis ,and relation between phenolic contant flavonoids and antioxidant study was performed and its results shows the objects are achived and this plant can be used medicinally in herbal industry .

Table - 5

Scavenging capacity in the extracts of *Epimedium grandiflorum*

Name of extract	Percentage scavenging capacity
Chloroform extract	63.15 ±0.039
Ethyl acetate extract	49.80 ±0.005
Methanol extract	67.53 ±0.033
n-hexane extract	37.12 ±0.039
Aqueous extract	31.59 ±0.040

Each value is expressed as mean ± S.D. (n=3) of three separate values.

Table - 6

Ferric reducing capacity in the extracts of *Epimedium grandiflorum*

Name of extract	Reducing capacity (µg /mg)
Chloroform extract	127.71 ±0.37
Ethyl acetate extract	126.71 ±0.00
Methanol extract	137.77 ±0.06
n-hexane extract	122.12 ±0.03
Aqueous extract	88.33 ±0.03

Each value is expressed as mean ± S.D. (n=3) of three separate values.

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Synthesis Of 2-Substituted Isonicotinoyl Chlorides In A Regioselective Manner

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ABSTRACT

Five nuclear substitution derivatives of 4- acetyl pyridine (**2a** to **6a**) were prepared via N-oxidation followed by either nitration or arylation in the yield of 60-80%. Substitution at 2- position is promoted due to the electron withdrawing and meta- directing ability of acetyl group at 4- position making the pyridine N-oxide vulnerable to attack at C-2.

Key words: 4- acetyl pyridine, N-oxidation, nitration, arylation, nuclear substitution.

Introduction

Substituted N-heterocycles are important structural motifs of bio-active compounds and advanced materials [1]. Hence, methods that allow for regioselective construction of C–C bonds to N-heterocycles have attracted continuous attention [2]. In particular, introduction of substituents in the C-2 position of pyridines, quinolines and related six-membered nitrogenous heterocycles is an important strategy in heterocyclic synthesis that represents a significant synthetic challenge [3]. One of the most commonly used strategies is based on transition metal-catalyzed coupling reactions of organometallic reagents with 2-haloazines [4], which are prepared from the corresponding N-oxides [5]. In recent years, direct arylation has emerged as an attractive alternative to typical cross-coupling reactions [6]. In direct arylation, one of the preactivated cross-coupling partners (typically the organometallic species) is replaced by an un functionalized arene. Consistent with an electrophilic aromatic substitution (SEAr) pathway, electron-rich heterocyclic arenes have been featured prominently in recent developments [7]. While some simple arenes can now be used [8,9], direct arylation reactions with π-electron deficient heteroarenes, such as pyridine, remain a challenging goal [10]. Bakke and coworkers were the first to report a remarkable reaction of pyridines with dinitrogen pentoxide in sulfur dioxide solution, to give N-nitropyridinium ion intermediates which, on treatment with water, gave 3-nitropyridines in good yield [11, 12]. They proposed that this reaction proceeds by a[1,5] sigma tropic shift of the nitro group from the 1- to the 3-position in the pyridine ring rather than an electrophilic aromatic substitution. A mixture of 3-nitropyridine and 3, 5-dinitropyridine was obtained in low yield by Suzuki and coworkers from the reaction of pyridine with dinitrogen pentoxide generated in situ from nitrogen dioxide and ozone [13, 14]. A similar attempt was made recently to generate dinitrogen pentoxide, the anhydride of nitric acid, from nitric acid itself using phosphorus pentoxide [15], for the in situ reaction with pyridine. Some 3-nitropyridine was obtained, but in low yield. Katritzky *et al.* have reported the preparation of nitropyridines by nitration of pyridines with nitric acid using nitric acid– TFAA system [16].

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Results and Discussion

As per our plan to synthesize some new pyridine containing compounds, we have prepared a set of 4- acetyl pyridine derivatives (table 1) as described in scheme 1. Many electrophilic substitutions on pyridine either do not proceed or proceed only partially; however, the heteroaromatic character can be activated by electron-donating functionalization. Common alkylations and acylations, such as Friedel–Crafts alkylation or acylation, usually fail for pyridine because they lead only to the addition at the nitrogen atom. Substitutions usually occur at the 3-position, which is the most electron-rich carbon atom in the ring and is, therefore, more susceptible to an electrophilic addition.

Substitutions to pyridine at the 2- or 4-position result in an energetically unfavorable σ complex. They can be promoted, however, using clever experimental techniques, such as conducting electrophilic substitution on the pyridine-N-oxide followed by deoxygenation of the nitrogen atom. Addition of oxygen reduces electron density on the nitrogen atom and promotes substitution at the 2- and 4-carbons. The oxygen atom can then be removed via several routes, most commonly with compounds of trivalent phosphorus or divalent sulfur, which are easily oxidized [17]. As the nuclear

substitution on pyridine ring itself is relatively difficult, we attempted to perform substitutions on pyridine N-oxide which is more nucleophilic and electrophilic, having higher dipole moment (4.37D for pyridine N-oxide *vs.* 2.03D for pyridine), much weaker base (pKa 0.79 for pyridine N-oxide *vs.* pKa 5.2 for pyridine). It exists as many mesomeric forms [18] (fig. 1).

The N-O moiety of pyridine N-oxides possesses a unique functionality which can act effectively as a push electron donor and as a pull electron acceptor group. This strong push-pull property has an essential chemical consequence; it accounts for the equally easy synthesis of 4-substituted derivatives of pyridine N-oxides with donor as well as acceptor groups. The contribution of the resonance forms I and II depends on the nature of the substituent at position 4. The moderate electron-acceptor acetyl group favors the charge transfer form II. Thus, acetyl group being electron withdrawing and meta directing group removes electron density from a π system, making the π system more electrophilic, as well as directs the substitution on the ring, meta to it, i.e. at position 2 [19-22](fig. 2).

Further, we were interested in the production of nitro pyridines using nitric acid, which is readily available, cheap and overcomes the problem of handling the unstable and difficult-to-obtain reagent, dinitrogen pentoxide. We sought to generate dinitrogen pentoxide easily in situ, under conditions in which it would react with pyridines immediately. These requirements led us to select the nitric acid– TFAA system as reported in the literature [16]. As a result of nitration of 4-acetylpyridine-N-oxide (**i_1**) we got 2-nitro-4-acetylpyridine N-oxide which was reduced by Pd/C to the base, 2-nitro-4-acetylpyridine (**2a**). The nitro group of compound **2a** was then reduced to amino group through Sn/HCl system to get 2-amino-4-acetylpyridine (**3a**) which through Sandmayer reaction produced corresponding chloro compound (**4a**). As a parallel path to some more 2- substituted derivatives, Arylation of N-oxide was carried out as per the reported procedure of Fagnou *et al.* [23]. Reaction development was carried out with pyridine N-oxide and 4-bromotoluene. From these studies, palladium acetate in combination with tri-tert-butylphosphine (added to the reaction mixture as the commercially available and air-stable HBF_4 salt) emerged as the optimal metal-ligand combination. Potassium carbonate was deemed the optimal base, and toluene the optimal solvent. While the reaction with bromobenzene under these conditions lead us **5a**, the combination with 4- bromotoluene gave compound **6a**. The structures of all the compounds were confirmed through IR and proton NMR spectroscopy (table 2) and elemental analysis carried out (table 3).

Conclusion

As a part of our synthetic strategy to get new series of heterocyclic compounds, we have prepared five new derivatives of 4-acetylpyridine either via nitration or N-oxidation which were confirmed through IR, NMR and Mass spectroscopy.

Acknowledgement

Authors are highly thankful to sophisticated analytical instrument facility (SAIF), Punjab University, Chandigarh, India, for spectrophotometric and elemental analysis.

Experimental

All the chemicals and reagents used were of Synthetic grade and purchased from Alfa Aesar or Sigma Aldrich and used as such without purification. Melting point was determined on Veego digital melting point apparatus and was uncorrected.IR spectra were recorded using Bruker Alpha FTIR Spectrometer equipped with ZnSe ATR crystal .^{1}H NMR spectra were recorded on Bruker

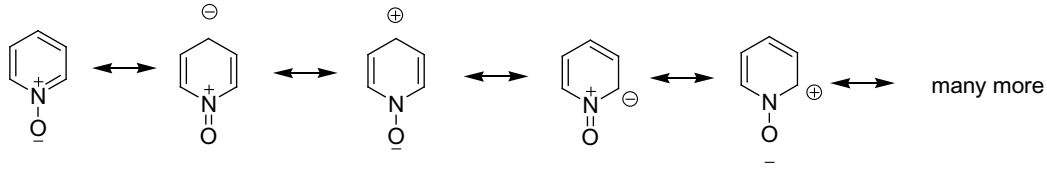


Fig. 1 : Mesomeric forms of pyridine N-oxide.

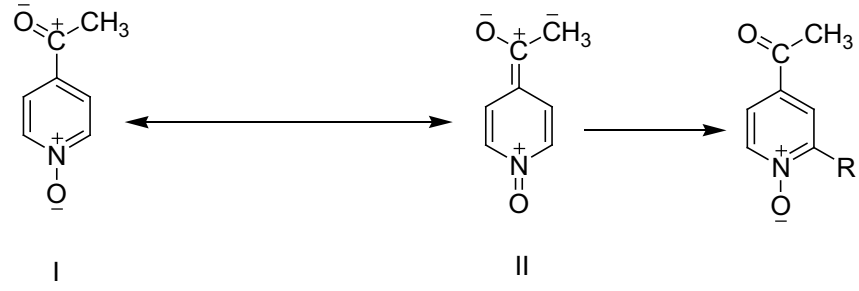


Fig. 2 : Substitution at 2- position on 4- acetyl pyridine

Spectrometer (400 MHz) in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on LC-MSD Trap-SL 2010A-Shimadzu. Micro analysis was performed on a Perkin Elmer-240 CHN elemental analyzer.

Preparation of 4-acetylpyridine-N-oxide (i_1): For N-oxidation, 4-acetylpyridine (1 equivalent) was taken in a round bottom flask, and added to it, from a dropping funnel, 1.2 equivalent 30% w/v solution of hydrogen peroxide at a temperature of 0-5ºC with constant stirring. The stirring of reaction mixture at the same temperature for 15 min yielded N-oxide almost quantitatively as a white hygroscopic solid (M.P 140-142ºC). The N-oxide was filtered and dried under vacuum and stored in vacuum desiccators for future use.

2-Nitro-4-acetylpyridine[1-(2-Nitro-4-pyridinyl) ethanone(2a)]. 4-Acetylpyridine (2.0 g, 18.2 mmol) N-oxide was added slowly to TFAA (10.6 mL, 76.4 mmol) at 0 °C and the mixture was stirred at 0 °C for 1 h. conc. HNO_3 (2.4 mL, 38.2 mmol) was added to the mixture dropwise and the mixture stirred at 0 °C for 8 h. The reaction mixture was added dropwise to a stirred solution of $Na_2S_2O_5$ (2.54 g, 18.2 mmol) in water (20 mL) at 0 °C and the mixture stirred at 0 °C for 16 h. The pH of the solution was adjusted to 6-7 with 1 M NaOH solution and the mixture was extracted with DCM (3 x 50 mL). The combined organic fraction was washed with water (50 mL), washed with brine (50 mL), dried and the solvent evaporated. The residue on reduction with Pd/Cu in ethyl formate and MeOH gave ketone (1.66 g, 55%) as oil.

2-Amino-4-acetylpyridine [1-(2-aminopyridin-4-yl) ethanone (3a)]. 2-Nitro-4-acetylpyridine (1g, 6.01 m mol) was added to 2.5g tin (16.84 m mol) and then 20 ml. conc. HCl was added under reflux. The reaction mixture was cooled and added to it, 10-15 ml. of water. 20% NaOH was then added to dissolve the tin hydroxide completely and make the solution sufficiently alkaline so as to be extracted with ether thrice. The combined ether extract was washed with water, dried over Na_2SO_4, and solvent was removed by distillation and the residue was recrystallized to give 0.64g (78%) of pale yellow product (M.P. 138ºC).

2-Chloro-4-acetylpyridine[1-(2-chloropyridin-4-yl) ethanone(4a)]

a. Preparation of copper (I) Chloride solution: 3.5g (0.014 mol) of Copper Sulphate pentahydrate and 0.92 g (0.0157 mol) of pure Sodium Chloride was dissolved in 12.5 ml. of water with warming. A solution of 0.84g (0.0044 mol) of sodium metabisulphite in 9 ml. water was added to the hot solution during about 5 min. by constant shaking. The reaction mixture was cooled to room temperature and the supernatant liquid was decanted from the colorless copper (I) chloride. The precipitated Copper (I) chloride was washed twice with SO_2 dissolved water (to prevent oxidation), dissolved in 6 ml. conc. HCl and used within 24 hrs of its preparation.

b. Preparation of Chloro- compound via Sandmayer reaction: The freshly prepared Cu(I)Cl solution in HCl was cooled in an ice-salt mixture whilst the diazotization is being carried out.

1g (7.3m mol) of 2-amino-4-acetylpyridine was dissolved in 2.5 ml. of conc. HCl and 2.5 ml water in a flask. The mixture was cooled to 0ºC in an ice-salt bath with vigorous stirring and the addition of little crushed ice. The hydrochloride salt was separated as finely divided crystalline precipitate. Added during 10-15 min, a solution of 0.5 g (0.0075mol) of Sodium Nitrite in 5 ml of water (1); at a temperature of 0-5ºC by the addition of little crushed ice from time to time. When all the Nitrate solution had been introduced, the solution contained a trace of free nitrous acid which was tested with potassium iodide-starch paper.

The cold diazonium chloride solution was poured slowly by shaking into the cold Cu(I)Cl solution (2). The mixture became very thick owing to the separation of an addition product between the diazonium salt and Cu(I)Cl ($MeCOC_5H_4N.\ N_2^+\ Cl^-.CuCl$). Without external heating, the mixture was allowed to warm up to room temperature with occasional shaking (3).

When the temperature reached to about 15ºC, the solid addition complex broke down with the liberation of Nitrogen. The mixture was warmed on a water bath to about 60ºC to complete the decomposition of the double salt, with occasional shaking. When the evolution of Nitrogen has ceased, the reaction mixture was transferred to 1M NaOH and extracted with three equal portions of EtoAc. The combined organic layer was washed with brine solution, then with water, dried over Na_2SO_4 and evaporated under *vacuo* to yield after recrystallization with 2-propanol afforded 0.78g (68.4%) pale white powder (155.58) with M.P. 85-86ºC.

2-Phenyl-4-acetylpyridine [1-(2-phenylpyridin-4-yl)ethanone (5a)]

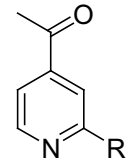
a. Preparation of 2-Phenyl-4-acetylpyridine N-oxide: K_2CO_3 (2 equiv.), $PtBu_3 - HBF_4$ (0.15 equiv.), $Pd(OAc)_2$ (0.05 equiv.) and 4-acetylpyridine N-oxide (4 equiv.) are weighed to air and placed in a round bottom flask with a magnetic stir bar. The reflux condenser was capped with rubber septa. The reaction is evacuated and backfilled with Nitrogen. Bromobenzene (1 equiv.) is then added via syringe as a stock solution in toluene (0.3M). The mixture is then heated to 110ºC for 6 hours. The reaction mixture is filtered, (wash with Me_2CO and DCM) then evaporated under reduced pressure and purified by silica gel column chromatography using DCM/Acetone (1:1) mixture as mobile phase. The N-oxide so obtained was then reduced to the base by following procedure:

b. Reduction of N-oxide to the base 2-Phenyl-4-acetylpyridine (5a): Ammonium formate (~10 equiv.) is added to a stirring solution of 2-Phenyl-4-acetylpyridine

N-oxide (1 equiv.), Pd/C (0.1 equiv.) in MeOH (0.3M) in a round bottom flask. The flask is then capped with rubber septa and purged with Nitrogen. The mixture is then stirred under an atmosphere of Nitrogen at room temperature. When the reaction is deemed complete by TLC analysis, the reaction is filtered and evaporated under reduced pressure. The residue is then purified via silica gel chromatography using DCM/Acetone mixtures. 2-Phenyl-4-acetylpyridine was obtained as yellow amorphous solid (yield 69%) with melting point 150 ºC.

2-Toluoyl-4-acetylpyridine [1-(2-p-tolylpyridin-4-yl) ethanone; 6a] 4-acetylpyridine N-oxide on reaction with *p*- Bromotoluene (described in 7.2.4 b) and heating to 110 ºC for 8 hrs afforded 2-Toluoyl-4-acetylpyridine N-oxide, which on subsequent Reduction by Pd/C in MeOH (by the procedure described in 7.2.4 c) yielded 2-Toluoyl-4-acetylpyridine as pale yellow solid (72%, M.P.170 ºC) after recrystallization with hot ethanol.

Table - 1
Nuclear substitution derivatives of 4-acetyl pyridine



Compound	R	% yield
1a	H	Purchased
2a	NO_2	55
3a	NH_2	78
4a	Cl	68.4
5a	C_6H_5	69
6a	$C_6H_4CH_3$	72

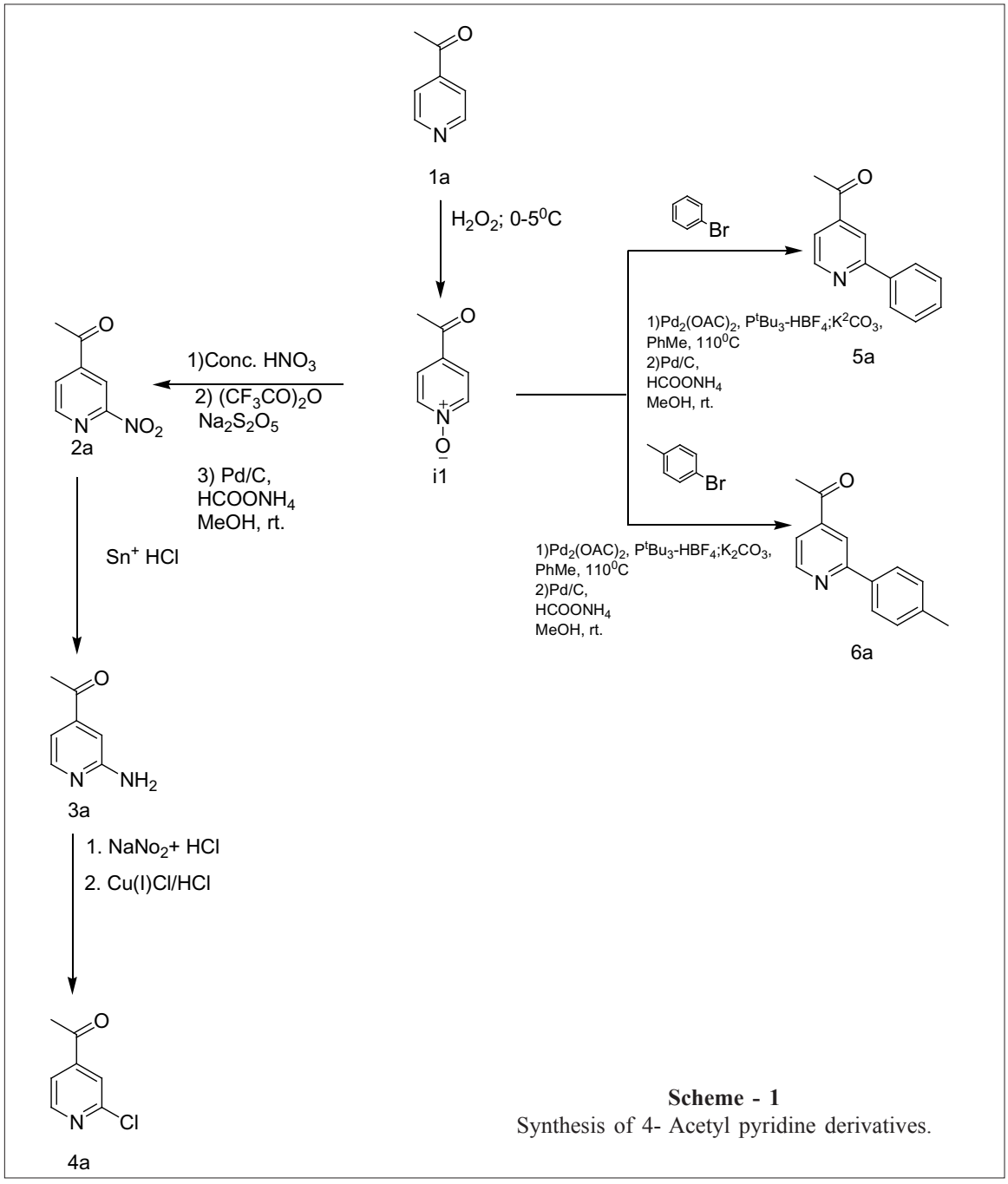
Table - 2

Physical constants and spectral data for 4-acetyl pyridine derivatives

Compound	M.P. (uncorrected) or B.P./mmHg	1H –NMR δ (ppm) ($CDCl_3$)
1a	212.8°C at 760 mmHg.	^{1}H NMR (DMSO-d_6, 400 MHz,): δ= 8.46-8.44 (d, 2H, Ar–H), 7.38-7.36 (d, 2H, Ar–H), 2.19 (s, 3H, CH_3). IR (νmax /cm^{-1}): 2923, 1692, 1596, 1556, 1492, 1407. LCMS m/z $[M]^+$ 122.1 Commercially available - CAS # 1122-54-9
2a	oil	^{1}H NMR (DMSO-d_6, 400 MHz,): δ= 8.72-8.71 (d, 1H, Ar–H), 8.37 (d, 1H, Ar–H), 8.09-8.07 (d, 1H, Ar–H), 2.23 (s, 3H, CH_3). IR (νmax /cm^{-1}): 1696,1540, 1335, 855, 759, 695. LCMS m/z $[M]^+$ 167.2
3a	142-144°C	^{1}H NMR (DMSO-d_6, 400 MHz,): δ= 8.44-8.42 (d, 1H, Ar–H), 7.19-7.15 (m, 2H, Ar–H), 2.61 (s, 3H, CH_3), 1.58 (s, 2H, NH_2); IR (νmax /cm^{-1}): 3381,3405, 3036, 1697, 1590, 688,759, 855. LCMS m/z $[M]^+$ 136.1
4a	35-40 °C	^{1}H NMR (DMSO-d_6, 400 MHz,): δ= 8.80-8.79 (d, 1H, Ar–H), 8.02-7.89 (m, 2H, Ar–H), 2.43 (s, 3H, CH_3). IR (νmax /cm^{-1}): 2913, 1698, 1615, 1698, 1475, 696,760. LCMS m/z $[M]^+$ 157.1
5a	128-132 °C	^{1}H NMR (DMSO-d_6, 400 MHz,): δ= 8.78-8.76 (d, 1H, Ar–H), 8.11 (s, 1H, Ar–H), 7.91-7.93 (d, 1H, Ar–H), 7.47-7.30 (m, 5H, Ar–H), 2.49 (s, 3H, CH_3): IR (νmax /cm^{-1}): 3047, 1698, 1615, 760. LCMS m/z $[M]^+$ 197.1
6a	179-182 **°C**	^{1}H NMR (DMSO-d6, 400 MHz,): δ= 8.59 (d, 1H, Ar–H), 7.93 (s, 1H, Ar–H), 7.71-7.69 (d, 2H, Ar–H), 7.29-7.27 (d, ^{1}H, Ar–H), 7.10-7.09 (d, 2H, Ar–H), 2.31 (s, 3H, CH3), 2.13 (s, 3H, CH3). IR (νmax /cm^{-1}): 3049, 2353, 1697, 759. LCMS m/z [M]+ 212.1

Table - 3
Analytical data for Acetyl pyridines

Compound	Formulae	Analyses (%)					
		Calculated			Found		
		C	H	N	C	H	N
1a	C_7H_7NO	69.41	5.82	11.56	69.68	5.36	11.26
2a	$C_7H_6N_2O_3$	50.61	3.64	16.86	50.85	3.47	16.56
3a	$C_7H_8N_2O$	61.75	5.92	20.58	61.58	5.68	20.18
4a	C_7H_6ClNO	54.04	3.89	9.00	54.12	3.74	9.08
5a	$C_{13}H_{11}NO$	79.16	5.62	7.10	79.28	5.76	7.18
6a	$C_{14}H_{13}NO$	79.59	6.20	6.63	79.65	6.29	6.55



Scheme - 1
Synthesis of 4- Acetyl pyridine derivatives.

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Synthesis and Antibacterial Activity of 2-(4-Nitro Phenyl)-5-Aryl-1, 3, 4-Oxadiazole Analogues

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ABSTRACT

Novel 2-(4-Nitro Phenyl)-5-Aryl-1, 3, 4-Oxadiazole Analogues" (4a-g) are synthesized, characterized by the IR spectra and screened for Antimicrobial activity by Agar diffusion method. The synthetic route involves the 4-Nitro benzoic acid was dissolved in excess of ethanol the reaction mixture was acidified and neutralized with sodium bicarbonate to obtain Ethyl-4-nitro benzoate. Equimolar mixture of Ethyl-4-Nitrobenzoate and Hydrazine hydrate was refluxed for 12 hr to obtain 4-Nitro benzo hydrazide. 4-Nitro benzo hydrazide and Substituted Aromatic aldehydes was irradiated by microwaves in Micro oven for 10-50 Sec using 20 mol% $NaHSO_3$ and Ethanol-Water system (1:2, v/v) solvent to give 7 novel 2-(4-Nitro phenyl)-5-aryl-1, 3, 4-Oxadiazole analogues (4a-g).The compounds show the mild to moderate anti microbial activity when compared with standard Amoxicillin.

Keywords: Antimicrobial activity, Neutralization, Equimolar mixture, Refluxation.

INTRODUCTION

Oxadiazole, a heterocyclic nucleus has attracted a wide attention of the chemist in search for the new therapeutic molecules. Literature survey reveals that out of various isomers particularly 1, 3, 4-oxadiazole derivatives exhibit wide range of biological activities [1]. Also various route for the synthesis of 1, 3, 4-oxadiazole have been reported1-7. Acidhydrazides8-11 have been in general use as the starting-materials in some 1, 3, 4-oxadiazole. In view of theseobservations, in the present study we have used acid hydrazide as one of the starting material. These acid hydrazides on condensation with highly reactive intermediate 4-chlorophenyl isocyanodichloride resulted in the formation of some new derivatives of 1, 3, 4-oxadiazole.

MATERIALS AND METHODS

All chemicals and solvents used in this study were supplied by Merck (Darmstadt, Germany), Aldrich Chemicals Co. (Steinheim, Germany) and SD Fine Chemicals, Mumbai. Melting points were determined in open capillaries on a Heco melting point apparatus and are uncorrected. The purity of the compound was assessed by Thin Layer Chromatography (TLC) on silica gel, using the developing system Chloroform: Ethanol (9: 1). Spots were detected by UV radiation using UV radiation chamber. The chemical structures were confirmed by elemental and spectral analysis. IR spectra were recorded on a SHIMADZU as KBr disc (γ, cm-1).

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EXPERIMENTAL PROCEDURE

Synthesis of Ethyl-4-nitro benzoate (2)

4-Nitro benzoic acid **(1)** (1.67 g, 0.05 mol) was dissolved in excess of ethanol (50 ml) the reaction mixture was acidified and refluxed for 8-10 h. The layer of ester is separated by separating funnel and neutralized with sodium bicarbonate to obtain Ethyl-4-nitro benzoate [2]. Once the reflux is completed it is then cooled at room temperature and poured in to ice cold water. The cream colored crystals separated out. Filter, dry and recrystallized with absolute ethanol as a solvent

Synthesis of 4-nitro benzo hydrazide (3)

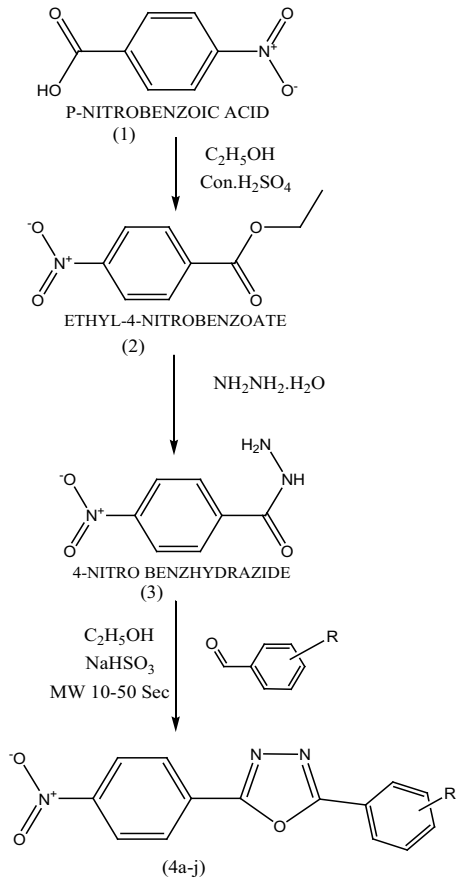
Equimolar mixture of ethyl-4-Nitrobenzoate (2) and hydrazine hydrate was refluxed for 12 hr and the excess solvent removed under vacuum and poured into the crushed ice [3]. The yellow colored crystals separated out. Filter, dry and recrystallized with absolute ethanol as a solvent to obtain 4-nitro benzo hydrazide (3).

General method for the Synthesis of 2-(4-nitro phenyl)-5-aryl-1, 3, 4-oxadiazole analogues (4a-g)

4-Nitro benzo hydrazide (0.85 g, 0.005 mol) (3) and

substituted aromatic aldehydes was irradiated by microwaves in Micro oven using 20 mol% $NaHSO_3$ and ethanol-water system (1:2, v/v) solvent [4]. After completion of reaction the mixture the excess solvent removed and the concentrate was poured into crushed ice washed with water, dried and recrystallized with absolute ethanol. The reaction was monitored throughout by TLC using Chloroform-Methanol (9:1) and Acetone: n-Hexane (8:2) as mobile phase.

Scheme



The schematic representation of the procedure is as follows

Antibacterial activity Screening

In vitro Antibacterial activity of all synthesized compounds was evaluated against two strains of microorganisms namely S.aureus (Gm+ve), and E. coli (Gm–ve) by MIC (Agar diffusion method). Nutrient Agar medium was used as nutrient medium to grow and dilute the drug suspension for the test bacteria [5]. This method depends upon the minimum concentration of drugs which inhibits the growth of microbial culture in a serial dilution solution of antibacterial in a fluid medium that is favorable to its rapid growth in the absence of the antibacterial agent. In this method minimal inhibitory concentration (MIC) of the lowest concentration of an antibacterial agent that inhibits the growth of test organism can be detected. Inoculum size for test strain was adjusted to 108 Cfu [Colony Forming Unit] per milliliter by comparing the turbidity.

Preparation of Nutrient Agar Medium

Media Used: Peptone-10 g, NaCl-10g, Yeast Extract 5g,Agar 20g in 1000 ml of distilled water.

Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. The control wells with Gentamycin were also prepared. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted

The solid ingredients were dissolved in water and pH adjusted to neutral at 25 0C and the medium was sterilized by autoclaving at 15 lb for 15 min.

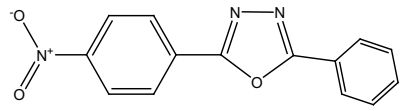
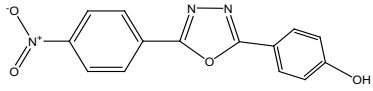
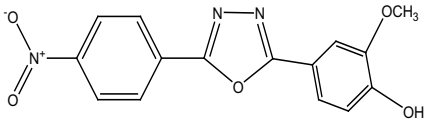
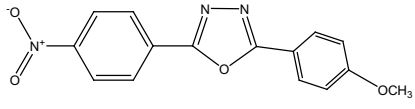
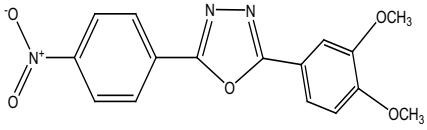
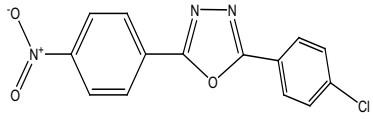
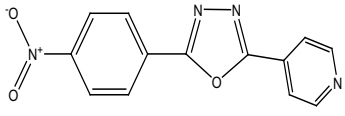
CONCLUSION

Generally for the synthesis of various 2-(4-Nitro phenyl)-5-aryl-1, 3, 4-Oxadiazole Analogues it requires more time to occur reaction between 4-Nitro benzo hydrazide(3) and substituted aromatic aldehydes. By the microwave irradiation, it offers significant improvements over normal existing procedures in the literature. The yields of different synthesized compounds were found to be in the range of 60-85%. Characteristic IR spectra show several functional groups. Seven derivatives were prepared, identified and screened for antibacterial activity,

Among synthesized compounds 4b & 4f shown equipotent activity that is MIC-600µg and zone of inhibition of 3mm respectively however 4a show MIC 400 µg and zone of inhibition is 3mm against gram–ve E.coli but none of the compounds shown potent antibacterial activity than standard Amoxicillin.

All the three compounds were also screened for antibacterial activity against gram + ve S. aureus the result reveled that 4a posse's greater activity when compared to 4b & 4f respectively, but however the MIC and zone of inhibition of 3mm the compounds shown lesser antibacterial activity than standard Amoxicillin.

RESULTS AND DISCUSSION

Compound Code	Structure &Chemical Name	Mol. Formula	Mol. Wt	% Yield	M.P °C	Solubility
4a	 2-(4-nitrophenyl)-5-phenyl 1,3,4-0xadiazole	$C_{14}H_9N_3O_3$	267	84	104	Benzene, Chloroform, Methanol
4b	 4-(5-(4-nitrophenyl)-1,3,4-oxadiazil-2 yl)phenol	$C_{14}H_9N_3O_4$	283	75	110	Benzene, Chloroform, Methanol
4c	 2-methoxy-4(-5(-nitro phenyl)-1,3,4-oxadiazol-2yl)phenol	$C_{15}H_{11}N_3O_5$	313	69	153	Benzene, Chloroform, Methanol
4d	 2-(4-methoxy phenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole	$C_{15}H_{11}N_3O_4$	297	73	128	Benzene, Chloroform, Methanol
4e	 2-(3,4-dimethoxypropyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole	$C_{16}H_{13}N_3O_5$	327	75	120	Benzene, Chloroform, Methanol
4f	 2-(4-Chloro phenyl)-5-(nitrophenyl)-1,3,4-oxadiazole	$C_{14}H_8N_3O_3cl$	301.5	80	123	Benzene, Chloroform, Methanol
4g	 4-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)pyridine	$C_{13}H_8N_4O_3$	268	42	139	Benzene, Chloroform, Methanol

Results of Anti Bacterial Activity by MIC Method for the Synthesized Compounds

Gram positive bacteria S. aureus

S.NO.	SAMPLE	CONCENTRATION µg/ml						MIC µg/ml
		100	200	300	400	500	600	
1.	4a	0	0	0	0	0	3	600
2.	4b	0	0	0	0	3	5	500
3.	4f	0	0	0	0	4	5	500
4.	Standard	17	20	22	25	27	29	100

Gram negative bacteria E. coli

S.NO.	SAMPLE	CONCENTRATION µg/ml						MIC µg/ml
		100	200	300	400	500	600	
1.	4a	0	0	0	3	4	5	400
2.	4b	0	0	0	0	0	3	600
3.	4f	0	0	0	0	0	3	600
4.	Standard	17	20	22	25	27	29	100

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Study on Solid Waste Management In Nizamabad Muncipal Corporation Telangana State, India

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ABSTRACT

Now a day, we are facing a problem regarding the management of waste generated daily. This waste includes solid waste from industrial zone, commercial zone and residential zone. Among all these waste management from residential zone requires first priority because if this waste is not properly disposed off daily, then it will create problems to public health, affects the aesthetics. This paper gives the present status of solid waste management in Nizamabad Region and also suggests some methods to control the same. Urgent steps in this direction will reduce the water, air, soil pollutions and health hazards. The town is an important business centre. The study was carried out for one calendar year that is 2015. The work is a humble beginning to study the solid waste focusing on domestic waste or organic waste related to degradable products and non degradable recyclable wastes in commercial area or market yards. The solid wastes are collected on an average of 175 metric tons per day manually, and from where the wet and dry material was separated, the wet wastes are sent to the compost yard and the dry material will be sent to recycling place. As urbanization continues to take place the management of solid waste is becoming a major public health and environment concern in urban areas of many developing countries.

Keywords: Solid Waste, Dumping yard, Solid waste management, Pollutions.

Introduction

The solid waste from Nizamabad Muncipal Corporation is rising day by day. Such rise in solid waste generation is observed because of increase in urbanization, population density and income, changing food habits, taste and pattern. The growth of industry, commercial units such as hotels, theaters, restaurants, malls are rising fast. Such units are positively contributing to the solid waste generation. Solid waste collection, segregation and disposal capacity of Municipal Corporations is low and inadequate with rising solid waste. Therefore Municipal Corporation must adopt scientific methods for collection, segregation and disposal of solid waste. Municipal corporations must accommodate private sector for investment and management of solid waste. Urgent steps in this direction will reduce the water, air, soil pollutions and health hazards. It will improve the quality life of people in Nizamabad Muncipal Region.

Review of Literature

Environmental impact of MSW can usually result from the run-off of the toxic compounds into surface water & ground water, which eventually lead to water pollution as a result of percolation of Leachate. (Beaven & walker, 1997), (Rjkumar Subrama & Elango, 2010) [1].The leachate generated from solid waste dumps may have the potential to pollute the surrounding water sources & and soil also. The most serious problem is ground water contamination (Sabahi, Rahim, Zuheli, Nazaily & Alshaebi, 2009)[2].

Solid waste management (SWM) has been an integral part of every human society. The study of environmental impacts of leachates in Nigeria (chian, 2009) (Akinbile & Yusff,2011) proved that the polluted ground water is unfit for drinking & causes many health problems like jaundice, nausea, asthma, miscarriage & infertility[3]

Study of the environmental quality in and around municipal solid waste dumpsite of Kolkata do evaluated (Arun Kanti Biswas, et al, 2010) [4]. In Chennai solid waste is assessed under tropical climate condition using land fill lysimeters by (S.Sri Shalini et al, 2009)[5] and suggested solutions to some of the major problems. An economic analysis was done on the recovering urban solid waste in Bangalore in 1993 by (Pieter van Beukering ,1994)[6].

Study Area

A study on the solid waste management was carried out at Nizamabad, Telangana State which is 170kms away from greater Municipal Corporation Hyderabad. Nizamabad

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occupies an area of 40 Sq.kms and it is located at latitude 18.672^0 N and longitude78.094^0 E with an elevation of 395m. (Fig.1)

The Godavari River enters into Telangana from Nizamabad district at Kandhakurthi. Nizamabad was founded in the year 1905 when Nizam's Dominion were recognized, where upto it was known as Indur which was supposed to have originated from the name of the King Indradatta who seems to have flourished it during the 5th century A.D. Later during the 18th century the Nizams ruled over the Deccan region and the district got its name from him.

Nizamabad is a city and a municipal corporation in Nizamabad district of Telangana state, India.It is headquarter of the district It is one of the major urban centre in TS and the third biggest in TS after Hyderabad and Warangal As of 2011 census, the population is recorded as3,11,152, with 66,670 house holds, by 2014 household survey by Govt of Telangana State recorded the population as 4,87,709 which makes it the third most populous city in TS after Hyderabad and Warangal.

Fig.1 : Map of Nizmabad city

Materlal and Methods

Collection of domestic waste from door to door is collected manually, in the collection vehicle and the commercial waste is also collected manually. Both commercial and domestic solid waste are transport to dumping yard situated at mallaram was done at regular intervals between 6AM to 10AM and 2.00 PM to 5.00 PM(Fig.3).The collected solid waste is separated in to two groups that is wet and dry components. (Fig.2) The waste collection methods vary among different countries or regions.

The Sources Of Muncipal Solid wastes

Sources	Examples
Residential	House Holds,Apartments
Commercial	Shops, Hotels, Offices, Hospital and Institutions
Agriculture	Field Scrap, Markets, Domestic wastes
Industrial	Factories, Small scale firms

Fig.2: Door to Door Collection of Garbage Through Try Cycle by Sanitation Worker

Fig. 3 : Nizamabad Dumping Yard at Mallaram

Results and Discussions

In Nizamabad Town the Solid waste is generated 175 metric tons per day but an average of 140 metric tons of solid waste is collected per day that means 35 metric tons per day is remained un collected which dumped in open and contaminates the ground and surface water.

As Urbanization and Modernization continues to take place, the management of solid waste is also becoming a major environmental and public health concern in urban areas of many developing countries. A typical solid waste management system in place like Nizamabad has lot of problems such as collection, open dumping, burning MSW and (water / Air / Soil) pollution control. The solid waste is dumped in open areas.

The public sanitation system is lacks because of inadequate planning in Nizamabad as well as unsustainable solid waste management. Indian cities are often characterized by poorly rendered services including Solid waste management.

In Mysore India the city is found to be highly efficient in collection of waste, transportation, dumping and Segregation of waste in to Dry &Wet .Solid waste Management activities practiced very effectively. The collection efficiency is also maximum extent.

Conclusion

Unscientific and Poorly designed or poorly managed landfills will create adverse environmental impacts; well managed, scientific landfill can be a hygienic and relatively inexpensive method for disposing waste material is used. Incineration is controversial method of waste disposal, due to amine as of gaseous pollutants. Nizamabad a town which has a proper dumping yard and need the collected solid waste should be properly handled or managed to see that ground water should not be contaminated with degradable or non degradable waste. A treatment plant is required for recycling. The municipality should have a proper plan and implement the system keeping in view of increasing population in the area. Public participation and co-operation awareness for the clean environment will be a successful operation in Nizamabad town. Education and Awareness in the area of waste and waste management is an important aspect from a global perspective of source management. Every urban domestic household be provided with bins for recyclable and non recyclable waste. House hold waste is segregated, recyclables be made in to new product like vermi compost, General waste such as non recyclable wastes will be shifted to landfill area. It is a common practice in most of the under developing countries that the disposing of waste in landfills of abandoned areas. Poorly managed landfills leads to a number of environmental impacts, for example the wind current will carry the litter to different clean places, attracting different types of insects, animals and also leads to the formation of methane gas and carbon dioxide. This creates a filthy odor problem for the near-by residential areas, the formed waste material that is organic in nature can be recycled. The waste gases from the process, such as methane can be used for cooking purposes, it also generate heat and electricity.

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Formulation and *in vitro* evaluation of Tolperisone HCl Gastro-Retentive Floating Tablets

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ABSTRACT

Aim of work: The aim of present study was to convert Tolperisone HCl (TH) into Gastro Retentive Floating Tablet (GRFT), which can extend its release up to 12 hrs in gastric pH and simultaneously to study the effect of combination of semi-synthetic polymer (HPMC K100M) with natural gums xanthan gum (XG) & guar gum (GG) in extending the release of TH **Methods:** The drug- excipient compatibility studies of TH and the polymers were carried by FTIR studies. The effervescent TH GRFT was prepared by direct compression. All Formulations were evaluated for pre-compression, post-compression, *in vitro* buoyancy studies. Accelerated stability studies were conducted for the optimized formulation (F11). **Results:** The drug- excipients compatibility studies reveals that combination of TH and the polymers used are compatible. Pre & post compression parameters were within the acceptable limits for all formulations. In-vitro dissolution studies, showed the formulation F11 (25% HPMC K100M and 12.5% GG) is exhibiting better extended release of TH up to 12 hrs, with a Floating Lag Time (FLT) of 58 s, Total Floating Time (TFT) and Matrix Integrity (MI) maintained up to 12 hrs. Drug release kinetics of formulation (F11) suggests it follows Zero order kinetics, drug release is predominantly by diffusion and the release mechanism is by super case-II transport. Comparative DSC & FT-IR studies of pure TH and optimized formulation (F11) further confirmed the integrity of drug. Accelerated stability studies of the optimized formulation (F11) in the final package, indicates it passes the test for stability as per ICH guidelines. It was finally concluded that a better twice daily TH GRFT was formulated & evaluated.

Key words: Tolperisone HCl (TH), Gastro retentive floating tablets (GRFT), Hydroxy Propyl Methyl Cellulose (HPMC K100M), xanthan gum (XG), guar gum (GG), *In vitro* buoyancy studies.

Introduction

Oral route is one of the most extensively utilized routes for administration of dosage forms. Drugs that have an absorption window in stomach or upper small intestine, have low solubility and stability at alkaline pH were suitable to convert as Gastro Retentive Dosage Forms (GRDFs). GRDFs significantly extend the period of drug release, and thereby decreasing the dosing frequency of drugs with shorter elimination half life ($t_{1/2}$ < 5hr) and will increase patient's compliance.[1, 2] Various approaches for GRDFs include: Floating Drug Delivery System (FDDS), bio adhesive systems, swelling, expanding systems and high density systems.[3] FDDS has a bulk density lower than gastric fluids and thus remain buoyant in the stomach for a prolonged period of time, without affected by gastric emptying rate. When the system is floating on the gastric fluids, the drug is released slowly at a desired rate from the system. Based on the mechanism of buoyancy, two different technologies for FDDS are effervescent systems and non-effervescent systems.[1,2] Effervescent systems contains carbonates (sodium bicarbonate) and organic acids (citric acid / tartaric acid) in their formulation to produce carbon dioxide (CO_2) gas when comes in contact with gastric fluids. The CO_2 gas entraps in the matrix system, which reduces its density and makes the system buoyant.[1, 2] The non-effervescent systems are based on the mechanism of swelling of polymer or bio-adhesion to mucosal layer in GI tract.[1, 2] Tolperisone HCl (TH), a centrally acting muscle relaxant agent, which has been in therapeutic use for more than three decades, has been widely used as spasmolytics of choice. It is mainly used for acute and chronic back pain and spasticity of neurological origin. It has also been used in treatment of condition which includes dysmenorrhoea, climacteric complaints, lockjaw, and neurolatyrism.[4] TH is a "Class-I drug" according to Bio pharmaceutics Classification System (BCS), possessing both high solubility and high permeability absorption characteristics. TH is rapidly and completely absorbed from the entire gastrointestinal tract.

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Peak plasma concentrations (C_{max}) is reached within 0.9-1.0 h after oral dosing and its elimination half-life ranges ($t_{1/2}$) from 1.5 to 2.5 h.[5] Conventional TH tablets are unable to ensure constant plasma concentrations of drug, they require multiple administration, (150 mg – 450 mg ; 2-3 times daily in divided doses); which ultimately results in patient's incompliance. TH when administered as conventional tablet, it breaks down into 4-MMPPO [2 Methyl-1-(4-methyl phenyl)-propanone] and piperidine hydrochloride when enters into intestinal pH at 4 to 7. Thus the patient is exposed to an uncontrollable quantity of 4-MMPPO which causes genotoxicity. This problem is overcome by controlled release of TH in the gastric pH at 1 to 2.[6] As TH is instable in alkaline pH, extending its release in the gastric pH is desirable. Hence it is a suitable drug candidate to formulate as GRFT.

Materials

Tolperisone HCl (TH) is a gift sample received from Amanath Pharmaceuticals, Pondicherry, India. Hydroxy Propyl Methyl Cellulose (HPMC K100M), Micro crystalline cellulose (Avicel PH 101) and Hydroxy propyl cellulose (HPC EXF) are received as gift samples from Colorcon Asia Pvt Ltd, Mumbai, India. Xanthan gum, guar gum are purchased from Arihant trading Co., Bangalore. Magnesium stearate, sodium bicarbonate and talc were purchased from S.D. Fine-Chemicals Ltd., India . All the excipients used in study are of pharmaceutical grade.

Methods

Analytical method: Standard calibration curve of TH in 0.1 N HCl at the λ_{max} 260 nm using a UV-Visible spectrophotometer (Labindia UV-VIS 3000+, Labindia Analytical Instruments Pvt Ltd, India). This calibration curve was further used for drug release calculations in *in vitro* dissolution and drug content determination studies.

Drug-excipient Compatibility / FT-IR studies: In order to evaluate the integrity and compatibility of the drug with various polymers used in the study, FT-IR spectra of drug and drug-polymer (1:1) physical mixture were recorded by the Potassium Bromide pellet method using (SHIMADZU, 8400s, FT-IR Instrument, Japan.) and the comparative spectra were studied.

Preparation of TH GRFT tablets

All the formulations were prepared by direct compression method, by keeping the amount of TH constant as 150 mg per tablet. The composition of other excipients are varied as mentioned in formulation table (Table:1). In these formulations, HPMC K100M is a semi-synthetic controlled release (CR) polymer, xanthan and guar gums are natural CR polymers, HPC EXF is a solid binder for direct compression, micro crystalline cellulose (Avicel PH 101) is a directly compressible diluent, magnesium stearate is lubricant and talc is glidant. TH and all the other excipients excluding magnesium stearate and talc were co- sifted though Sieve No. # 40 (ASTM), blended uniformly in a poly bag for 10 min and lubricated with Sieve No. # 60 (ASTM) passed magnesium stearate and talc and mixed for additional 2–3 min. Tablets were compressed on a tabletting machine (Minipress by Clit, 10 stations, Chamunda Pharma Machinary Pvt. Ltd., India) fitted with a 9 mm standard flat circular punches with an average hardness of 6.0 kg/cm^2.

Pre Compression Studies: [7-11]

The directly compressible tablet blends were evaluated for pre-compression studies.

Angle of Repose (θ): was determined by funnel method, the blend was poured through the walls of a funnel, which was fixed at a position such that its lower tip was at a height of exactly 2 cm above hard surface. The blend was poured till the time when upper tip of the pile surface touched the lower tip of the funnel. The θ is calculated by the equation.

$$\theta = \tan^{-1} h / r \qquad \text{... Eq.No. (1)}$$

Where, θ = angle of repose, h = height of heap, r = radius of base of heap circle.

Table - 1
Formulation table of TH GRFT

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Tolperisone HCl	150	150	150	150	150	150	150	150	150	150	150
HPMC K100M	75	125	175	--	--	--	--	--	--	125	125
Xanthan gum	--	--	--	75	125	175	--	--	--	62.5	--
Guar gum	--	--	--	--	--	--	75	125	175	--	62.5
Sodium bicarbonate	50	50	50	50	50	50	50	50	50	50	50
HPC EXF	20	20	20	20	20	20	20	20	20	20	20
Microcrystalline Cellulose	195	145	95	195	145	95	195	145	95	82.5	82.5
Magnesium stearate	5	5	5	5	5	5	5	5	5	5	5
Talc	5	5	5	5	5	5	5	5	5	5	5
Total :	500	500	500	500	500	500	500	500	500	500	500

*quantities per each tablet expressed in mg, Avg. wt. of a tablet: 500 mg

Density:

Bulk density (BD): A quantity of 2 g of blend from each formulation, previously lightly shaken to break any agglomerates formed, was introduced into a 10 ml measuring cylinder and the volume is noted as bulk volume. The BD was calculated by the equation.

$$\text{Bulk density} = \text{weight of powder} / \text{Bulk volume} \quad \text{... Eq.No. (2)}$$

Tapped density (TD): After the determination of BD, the measuring cylinder was fitted to a tapped density apparatus. The tapped volume was measured by tapping the powder for 500 times. Then the tapping was done for 750 times and the tapped volume was noted (the difference between these two volumes should be less than 2%). If it is more than 2%, tapping is continued for 1250 times and was noted. The TD was calculated by the equation.

$$\text{Tapped density} = \text{Weigh of powder} / \text{Tapped volume} \quad \text{...Eq.No. (3)}$$

Carr's Index(CI): The percentage of CI is calculated by the equation.

$$\text{Carr's index} = (\text{Tapped density} - \text{Bulk density}) \times 100 / \text{Tapped density} \quad \text{...Eq.No.(4)}$$

Hausner's Ratio(HR): is a number that is correlated to the flow ability of a powder. It is calculated by the equation.

$$\text{Hausner's Ratio} = \text{Tapped density} / \text{Bulk density} \quad \text{... Eq.No.(5)}$$

The determination of micromeritics of all the formulations were carried out in triplicate, the consolidated results (mean ±SD) were tabulated in (Table.2).

Post Compression Studies: [7-11]

Shape of tablet and general appearance: was checked by magnifying lens .

Thickness of tablet: thickness of 3 tablets of each formulation was determined using a Venire calipers (Mitutoyo Corporation, Japan).

Tablet Weight Uniformity: An electronic balance (Mettler Toledo, 3-MS-S/MS-L, Switzerland) was used to accurately weigh 10 tablets of each formulation which were randomly selected and weighed.

Hardness test: To evaluate tablet hardness, 3 tablets of each formulation were tested for diametrical crushing strength using a hardness tester (Monsanto type hardness tester, MHT-20, Campbell Electronics, India.)

Friability test: The friability of the 10 tablets (n = 10) was tested by a friabilator (ERWEKA, TAR 120, Germany.), at a speed of 25 rpm for 4 minutes. The percentage friability was calculated by the equation.

$$\%\ \text{Friability} = (\text{initial wt.} - \text{wt. after friability}) \times 100/\text{initial wt.} \quad \text{...Eq.No.(6)}$$

Drug content: To evaluate the drug content uniformity, 10 tablets of each formulation were crushed; the quantity of tablet powder equivalent to 100 mg of TH was suspended in 100 ml of 0.1N HCl and ultrasonicated for 2 min to extract the TH from the tablet blend and filtered through 0.45µ Poly Tetra Fluoro Ethylene (PTFE) filter disc to filter the dispersed matter, the filtrate was suitably diluted if necessasary and its absorbance was measured by UV-Visible spectrophotometer.

Table - 2

Pre compression studies of TH GRFT

Formulation Code	(n=3)				
	Angle of repose (^{o})	Bulk density (g/cc)	Tapped density (g/cc)	Carr's Index (%)	Hausner's Ratio
F1	22.17 ± 0.15	0.515 ± 0.15	0.522 ± 0.08	13.15 ± 0.04	1.10 ± 0.07
F2	26.11 ± 0.12	0.471 ± 0.11	0.476 ± 0.12	16.23 ± 0.23	1.21 ± 0.11
F3	25.31 ± 0.23	0.505 ± 0.05	0.527 ± 0.15	14.26 ± 0.15	1.15 ± 0.11
F4	23.31 ± 0.14	0.522 ± 0.13	0.519 ± 0.02	12.36 ± 0.21	1.09 ± 0.21
F5	24.27 ± 0.22	0.496 ± 0.21	0.497 ± 0.03	17.42 ± 0.15	1.12 ± 0.08
F6	24.67 ± 0.15	0.481 ± 0.16	0.511 ± 0.14	18.09 ± 0.12	1.07 ± 0.13
F7	25.71 ± 0.13	0.515 ± 0.14	0.522 ± 0.06	13.15 ± 0.12	1.10 ± 0.07
F8	23.31 ± 0.16	0.522 ± 0.13	0.519 ± 0.02	12.36 ± 0.16	1.09 ± 0.21
F9	26.21 ± 0.11	0.496 ± 0.16	0.499 ± 0.03	17.42 ± 0.11	1.12 ± 0.08
F10	25.71 ± 0.03	0.481 ± 0.12	0.511 ± 0.14	18.09 ± 0.12	1.07 ± 0.13
F11	26.21 ± 0.20	0.496 ± 0.15	0.498 ± 0.11	17.42 ± 0.11	1.12 ± 0.08

***In vitro* buoyancy studies:**The in vitro buoyancy was characterized by floating lag time (FLT) and total floating time (TFT) and Matrix Integrity (MI) as per the method described by ,Rosa et al., 1994.[12]. The test was performed by placing the tablet in 100 ml of 0.1 N HC1 in a beaker. The time required for tablet to rise to surface of medium and duration of time the tablet constantly float on dissolution medium were noted as FLT and TFT, respectively. During the study whether the matrix was intact or disintegrating was observed for to check matrix integrity.The consolidated results of post compression and *in vitro* buoyancy studies are tabulated in (Table.3).

***In Vitro* Dissolution Study:** A dissolution test was performed for 12 h using the dissolution apparatus (Labindia Disso 2000, Labindia Analytical Instruments Pvt Ltd, India)**.** Each dissolution flask contains 900 ml of 0.1N HCl; speed of paddle was maintained at 50 rpm, the temperature was kept stable at $37^0C \pm 0.5^0C$. At every time interval, 5 ml of dissolution media was withdrawn with a pipette containing cotton filter, further the solution was filtered through 0.45µ Poly Tetra Fluoro Ethylene (PTFE) filter disc, suitably diluted if necessary and its absorbance was measured by UV-Visible spectrophotometer at 260 nm. Furthermore, 5 ml of fresh 0.1N HCL was replaced to the dissolution flask to keep the volume of dissolution medium constant. The dissolution test was repeated for 6 times to each formulation and the dissolution profiles are represented graphically in (Fig.1 & 2).

Drug release kinetics:[13-15] The drug released data of batches(F2,F3,F9,F10 & F11) were fitted with desired kinetic models such as Zero order, First order , Higuchi and Korsemeyer peppas to ascertain the drug release kinetics. The drug release from the effervescent hydrophilic matrix FDDS whether depends on drug's concentration or not was explained by zero order and first order models. Higuchi model describes the drug release is by diffusion or not. The Korsemeyer- Peppa's model explains the mechanism of drug release.

$$\textit{Zero order: } Q_t = Q_0 + K_0 t \qquad \textit{Eq.No.(7)}$$

$$\textit{First order: } Q_t = Q0\, e^{-K1t} \qquad \textit{Eq.No.(8)}$$

$$\textit{Higuchi model: } Q_t = K_H t^{1/2} \qquad \textit{Eq.No.(9)}$$

Table - 3
Post compression & *in vitro* Buoyancy studies of TH GRFT

F- F-Code	Post compression parameters					Floating characteristics		
	Avg. Wt (mg) (n=10)	Thickness (mm) (n=3)	Hardness (kg/cm^2) (n=3)	Friability (%) (n=1)	%Drug content (%) (n=10)	FLT (S) (n=3)	TFT (h) (n=3)	MI up to 12 h. (n=3)
F1	500.4 ± 0.12	5.82 ± 0.34	5.9 ± 0.26	0.59	99.98 ± 0.18	49 ± 0.51	> 8	–
F2	500.2 ± 0.22	5.91 ± 0.23	6.2 ± 0.25	0.68	100.21 ± 0.20	55 ± 0.22	> 12	+
F3	499.6 ± 0.24	5.84 ± 0.14	6.3 ± 0.21	0.58	99.67 ± 0.12	53 ± 0.63	> 12	+
F4	500.3 ± 0.31	5.88 ± 0.21	5.9 ± 0.23	0.59	100.32 ± 0.14	150 ± 0.70	> 2	–
F5	500.6 ± 0.21	5.87 ± 0.21	6.3 ± 0.13	0.62	100.65 ± 0.18	135 ± 0.83	> 2	–
F6	500.9 ± 0.23	5.34 ± 0.14	6.1 ± 0.20	0.59	99.89 ± 0.22	140 ± 0.52	> 2	–
F7	500.2 ± 0.26	5.91 ± 0.23	6.2 ± 0.25	0.68	100.21 ± 0.20	75 ± 0.24	> 2	–
F8	499.6 ± 0.18	5.84 ± 0.13	6.3 ± 0.21	0.58	99.67 ± 0.12	80 ± 0.85	> 6	–
F9	500.2 ± 0.21	5.91 ± 0.23	6.2 ± 0.25	0.68	100.21 ± 0.20	60 ± 0.32	> 12	+
F10	499.6 ± 0.16	5.84 ± 0.12	6.3 ± 0.21	0.58	99.67 ± 0.12	59 ± 0.64	> 12	+
F11	500.2 ± 0.12	5.88 ± 0.11	5.9 ± 0.23	0.59	100.32 ± 0.14	58 ± 0.71	> 12	+

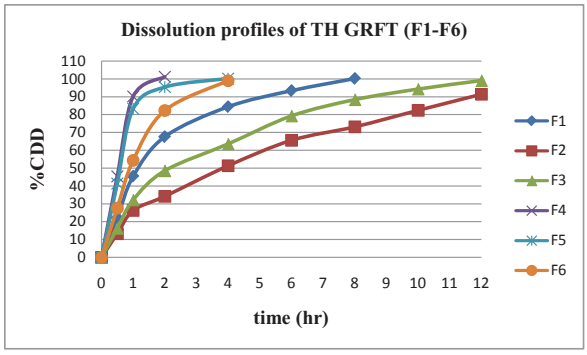

Fig.1: *In vitro* dissolution profiles of TH (F1-F6)

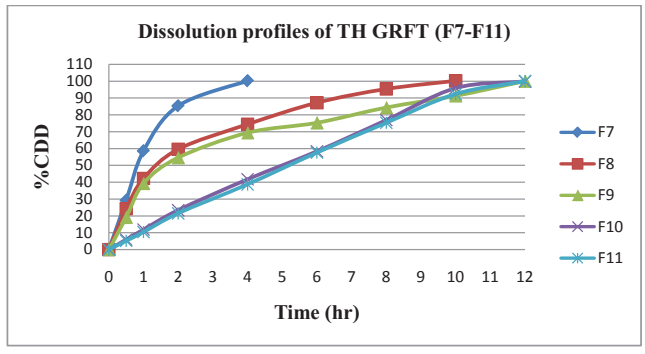

Fig.2: *In vitro* dissolution profiles of TH (F7-F11)

Korsemeyer peppas model:

$$Mt/M\alpha = K\,t^{n} \qquad \textit{Eq.No.(10)}$$

Where *Qt* is the amount of drug dissolved in time *t, Q0* is the initial amount of drug in the solution, Mt/Mα is the fraction of drug release at time t and n is diffusion exponent. *K0, K1,* K_H and k refer to the rate constants of respective kinetic models (11-14). Drug release mechanisms for cylindrical shape according Korsmeyer - Peppas model, depending on the diffusion exponent (n) are mentioned in (Table.4). The consolidated drug release kinetic parameters of selected TH GRFTs were tabulated in (Table.5).

Comparative FT-IR & DSC studies of pure TH & optimized formulation (F11)

FT-IR studies: In order to evaluate physical & chemical stability of the drug in the optimized formulation (F11), FT-IR spectra of drug and blend of formulation (F11) were recorded by the potassium bromide pellet method using (SHIMADZU, 8400s, FT-IR Instrument, Japan.) and the comparative spectra were demonstrated in (Fig.3)

Differential scanning calorimetry (DSC) studies: DSC scans of pure drug (TH) and the optimized formulation (F4) were performed; using an automatic Thermal Analyzer (DSC 60, Shimadzu, Japan). Sealed and perforated Aluminum pans were used in the experiments. Temperature calibrations were performed using Indium as standard. An empty pan sealed in the same way as the sample was used as a reference. The entire samples were run at a scanning rate of 10°C / min from 50-300°C. The DSC- Thermo grams of pure drug (TH) and optimized formulation (F11) were shown in (Fig. 4 and 5) respectively.

Accelerated stability studies: of the optimized formulation (F11) in final pack up to for 3 months were carried out according to International Conference on Harmonization (ICH) guidelines.[16] 20 tablets were packed, properly labelled and sealed in 10 CC HDPE containers and placed in a humidity chamber (NSW-175, Narang Scientific work, India) maintained at 45°C ± 2°C and 75% RH. At the end of every month the, samples were withdrawn and evaluated for post compression studies. The consolidated results of

Table - 4

Drug release mechanisms for cylindrical shape in Korsmeyer - Peppas model:

Diffusion Exponent (n)	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45 < n < 0.89	Non-Fickian diffusion
0.89	Case II transport
n > 0.89	Super Case II transport

Table - 5

Release kinetics of TH GRFT whose release was extended up to 12 hrs

Kinetic Model	Kinetic parameter	F2	F3	F9	F10	F11
Zero order	r^2	0.865	0.930	0.819	0.980	0.988
First order	r^2	0.977	0.976	0.869	0.715	0.608
Higuchi	r^2	0.988	0.998	0.973	0.920	0.910
Krosmeyer-Peppas	r^2	0.980	0.990	0.998	0.998	0.998
	n	0.483	0.520	0.466	0.886	0.945

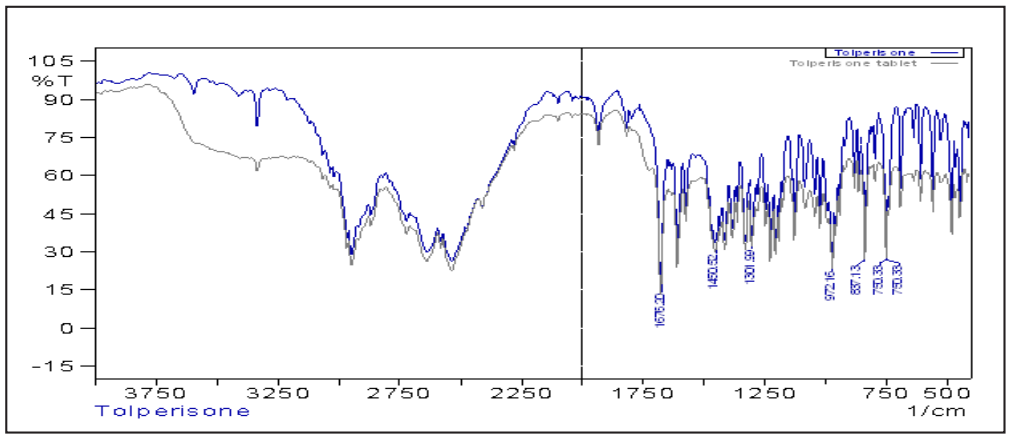

Fig.3: FT-IR Spectrographs of A) Pure TH & B) Formulation (F11)

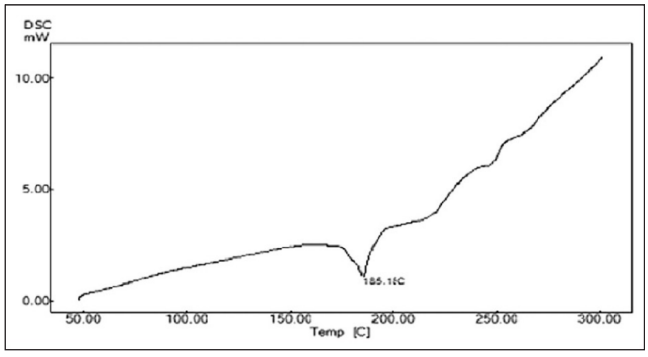

Fig. 4: DSC thermo gram of pure drug (TH):

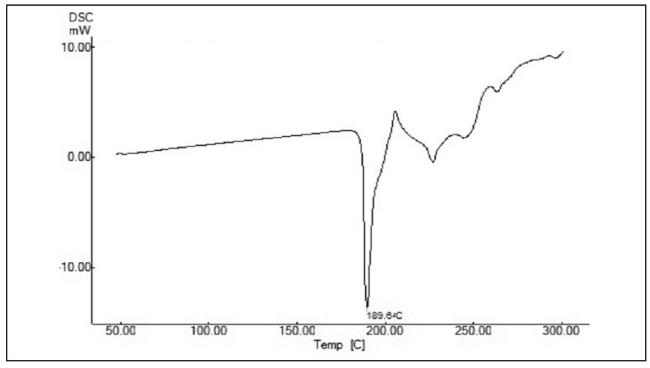

Fig.5: DSC thermo gram of optimized formulation (F11)

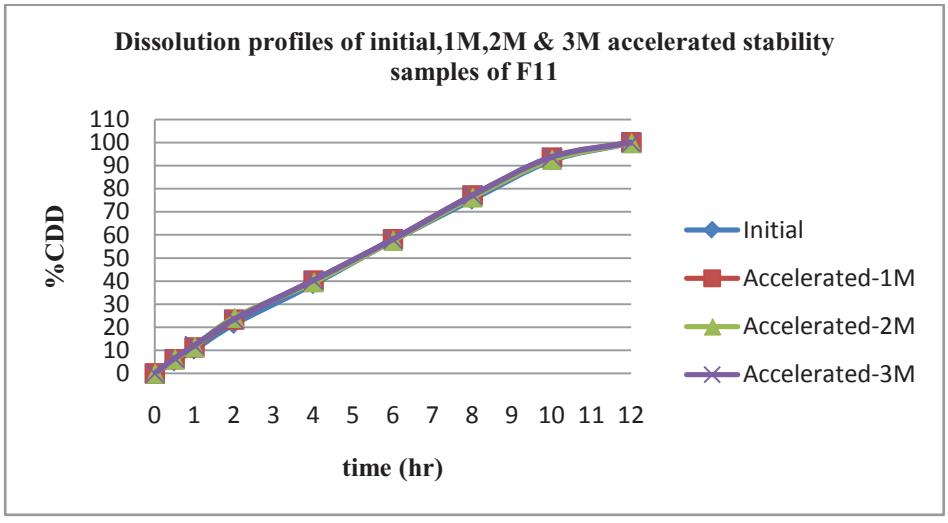

Fig.6: *In vitro* dissolution profiles of Accelerated stability samples of TH GRFT for optimized formulation (F11)

accelerated stability studies data for optimized formulation, F11 are tabulated in (Table.8). *In vitro* dissolution profiles of accelerated stability samples were shown in (Fig.6).

Results & Discussion

Analytical Method: A spectrophotometric method for estimation of TH, based on the measurement of absorbance at 260 nm in 0.1N HCl, gives a straight line with a regression (r^2) of 0.999.

Drug-excipient Compatibility Study: The FTIR spectra of drug- polymer (1:1) blends were compared with that of the Pure TH. There are no significant shifts or reduction in intensity of the FTIR bands was observed. Hence there was

no compatibility problem between the drug and polymers used.

Pre Compression studies: Pre compression studies on directly compressible blends of all formulations, reveals that the angle of repose was found between 22˚.17'to 26˚.21', bulk density between 0.471 to 0.522 gm/cm^3, tap density between 0.476 to 0.527 gm/cm^3, Carr's index between 12.36 to 18.09% and Hausner's Ratio between 1.07 to 1.21. The micromeritic studies indicate better flow and compression characteristics of all formulations. (Table: 2)

Post Compression studies: The avg. wt. of tablets of all the formulations was found to be 500.9 ± 0.3 mg. The average thickness of tablets was found to be 5.91 ± 0.23 mm. The average hardness of the tablets was 6.3 ± 0.13 Kg/cm^2, indicating satisfactory mechanical strength. The average % wt. loss in the friability test ranges from 0.59 to 0.68%, which indicates good mechanical resistance of the tablets. Tablets of all the prepared batches contain TH within 100.65 ± 0.18% of the labelled content, indicating content uniformity of the prepared formulations. (Table: 3).

***In vitro* buoyancy studies:** shows the order of FLT is: XG > GG > (HPMC K100M +XG) > (HPMC K100M + GG) > HPMC K100M. HPMC K100M being more hydrophilic swells rapidly forms a buoyant matrix, which has lesser FLT when compared to others. MI & TFT up to 12 hrs were not maintained by the matrices formed by XG at all concentrations. But they were maintained at higher concentration of XG, all concentrations of HPMC K100M and in the combination of (HPMC K100M with XG & GG). Maintenance of buoyancy also mainly depends upon the concentration of effervescent (sodium bicarbonate), which is maintained constant 10% w/w in all the formulations. (Table: 3)

In vitro* dissolution studies*: results are represented graphically in (Fig.1 & 2) indicates that the release rate retards as the concentration of polymers (HPMC K100M, XG & GG) increased. At higher polymer concentrations, the viscosity of the gel matrix is increased which results in a decrease in the effective diffusion coefficient of the drug. [17] This further suggests that, drug: polymer ratio is important factor affecting the rate of drug release from the matrix formulations. Other factors that may contribute to differences in drug release profiles include: differences in water penetration rate, water absorption capacity and polymer swelling.[18] The pH independent, zero order release profile of hydrophilic drugs like TH can be attained from the matrix systems, by combining the synthetic polymer HPMC K100M with natural polymers like XG and GG than HPMC alone.[19] The combined matrix when exposed to gastric fluids, the HPMC hydrates to form a gel layer at the surface of the tablet while the natural gums (XG and GG), due to lesser hydration rate than HPMC remains insoluble. The resulting matrix acts as a barrier to diffusion of the freely soluble drugs and extends drug release. [19] The proportion of HPMC K100M: Natural gums had significant effect on extending the release profiles of drug, which has to be optimized.[19] Formulation (F11) contains (25% HPMC K100M and 12.5% GG) and extends the release of TH up to 12 h with a zero order kinetic profile, FLT of 58 s, TFT and a better MI up to 12 h. (Fig.1 & 2)

Drug release kinetics

The drug release kinetics of optimized formulation (F11) fitted best to the Zero-order kinetics ($r^2 = 0.988$). The regression coefficient value of Higuchi model is ($r^2 = 0.910$); suggesting that the drug release process is predominantly by diffusion (as $r^2 > 0.9$). The diffusion exponent value for the Korsmeyer-Peppas model; in cylindrical shape is (n=0.945), suggested the mechanism of the drug release is Super Case II transport (as $0.45 < n < 0.89$). (Table.5)

Comparative FT-IR & DSC studies of pure TH & optimized formulation (F11):

FT-IR studies: in the FT-IR spectra of pure TH, following functional groups are observed at the corresponding frequencies : C=O amide : 1676.20 cm^{-1} , C-N stretch : 1450.52 cm^{-1}, C-N stretch: 1327.07 cm^{-1} , Alkenes C-H bending: 972.16 cm^{-1} , Alkenes C-H bending: 837.13 cm^{-1} and Aromatic C-H bending: 750.33 cm^{-1}.FTIR spectra of (F11), shows the same functional groups at the corresponding frequencies as that of pure drug. Thus, indicates no significant chemical interaction and change in functional groups of TH occurred in the optimized formulation (F11) and the drug is compatible with the polymers and excipients used in the study. (Fig.3)

DSC Studies: DSC Thermo grams in Fig. 4 and 5 is pure drug and optimized formulation F11 respectively, reveals that the melting point of TH is 186.16^{0}C and that of TH in the formulation F11 is 189.6^{0}C. As there is no much difference in the melting points, it indicates that the drug is in same state even in the optimized formulation (F11) without interacting with the polymers and excipients.

Accelerated stability studies: as there were no significant differences in post compression, floating characteristics (FLT, TFT & Matrix integrity) and *in vitro* dissolution profiles, formulation F11 passes the test for stability (Table.6 & Fig.6).

Conclusion

In the view of above findings, effect of combination of semi-synthetic polymer (HPMC K100M) with natural gums (XG & GG) in extending the release of TH from its GRFT is better understood. The formulation F11 (25% HPMC K100M and 12.5% GG) is the optimized formulation. It was further concluded that the optimization of the proportion of HPMC K100M: natural gums, had significant effect on extending the release profiles of TH. Among the two natural gums, GG in combination with HPMC K100M in the ratio 1:2 respectively forms a better matrix for the extending the release of TH in gastric pH for 12 hrs. A matrix design of this kind can serve as an alternative strategy for extending the release of other BCS class I drugs and their salts, which are having shorter half-life ($t_{1/2} < 5$ hrs).

Table - 6
Accelerated stability data for Optimized formulation (F11) of TH GRFT

Time Interval	Post compression parameters					Floating characteristics		
	Avg. Wt (mg) (n=10)	Thickness (mm) (n=3)	Hardness (kg/cm^2) (n=3)	Friability (%) (n=1)	%Drug content (%) (n=10)	FLT (S) (n=3)	TFT (h) (n=3)	MI up to 12 h. (n=3)
Initial	500.2 ± 0.12	5.88 ± 0.11	5.9 ± 0.23	0.59	100.32 ± 0.14	58 ± 0.71	> 12	+
1 month	501.1 ± 0.11	5.88 ± 0.21	5.8 ± 0.12	0.61	100.12 ± 0.08	60 ± 0.09	> 12	+
2 month	501.2 ± 0.21	5.88 ± 0.22	5.9 ± 0.13	0.64	100.24 ± 0.21	63 ± 0.11	> 12	+
3 month	501.2 ± 0.13	5.88 ± 0.12	5.9 ± 0.21	0.66	99.64 ± 0.11	64 ± 0.12	> 12	+

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Conflict Of Interest

Authors have declared no conflict of interest to declare.

Further the optimized formulation (F11) must be investigated for *in vivo* floating studies by X-ray radiographic studies and pharmacokinetic studies in rabbit model.

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