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Synthesis, Characterization, *Invitro* Thrombolytic Activity and Antimitotic Activity of Some Alkyl/Halo Substituted Cyanoacetyl Hydrazone Derivatives

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Abstract

The majority of pharmaceutical products that mimic natural products with biological activity are heterocycles. Synthetic heterocyclic compounds can and do participate in chemical reactions in the human body. Moreover, all biological processes are expressed through chemical reaction. The present study describes about the synthesis and characterization and biological studies of novel alkyl/halo substituted cyanoacetyl hydrazone derivatives. The synthesized compounds characterized by FT-IR, ^{1}H-NMR and ^{13}C-NMR spectral studies. The synthesized compounds subjected to antimitotic, invitro anti diabetic activities.

This in vitro study explores the antidiabetic properties of synthesized compounds and it can be considered as a potential for the management of type-II diabetes mellitus. The synthesized compound subjected to preliminary antimitotic studies by Allium cepa root MERISTAMATIC CELLS.

Keywords: cyanoacetyl hydrazone, antidiabetic activity, acorbose, antimitotic activity, meristamatic,

1. Introduction

Heterocyclic systems having piperidine are found to possess better biological activity. They aroused great interest in the past and recent years due to their wide variety of biological properties and their presence in biologically active pharmaceutical ingredients. The emphasis on the synthesis of the above said heterocycles can be recognized owing to their presence in the molecular structure of numerous alkaloids and drugs.

Hydrazones and their derivatives constitute an important class of compounds that has found wide utility in organic synthesis. The chemistry of carbon-nitrogen double bond of hydrazone is becoming the backbone of condensation reaction in benzo-fused N-heterocycles, also it constitutes an important class of compounds for new drug development.

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Many reports are available on the conformation of various substituted 2,6-diarylpiperidin-4-ones, have elaborately discussed the conformation of 2,6-diarylpiperidin-4-ones with or without alkyl substituent at C-3 and C-3/C-5 positions[1-3]. Certain small molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally potent molecules. Recently, cyanoacetyl hydrazones have attracted great attention due to their diverse biological and pharmacological properties.

Formation of blood clot is thrombus and process is thrombosis that obstructs the flow of blood through circulatory system. Body uses platelets and fibrin to form blood clot as first step of repairing process after injury [6]. There are many drugs that are used to dissolve a clot and to treat heart attack, stroke, deep vein thrombosis and occlusion of peripheral artery such as streptokinase, S-Kinase etc [7]. Circulatory platelets are aggregated to the site of injury and become the major component for thrombus development. Thrombosis is a critical stage for arterial disease associated with myocardial infarction and stroke responsible for considerable morbidity and mortality. Moreover, for cancer patients, venous thrombosis is the second leading cause of death [8]. For treatment of these diseases, thrombolytic agents like tissue plasminogen activator (t-PA), Urokinase (UK), Streptokinase (SK) are used. In India among the thrombolytic agent, UK and SK are widely used. They have high risk of hemorrhage and severe anaphylactic reactions. Moreover, various treatments with SK is restricted due to immunogenicity [9-12]. Developing of improved recombinant variants of these drugs is disturbing due to unavailability of thrombolytic drugs [13-17].

Myocardial infarction due to arterial thromboembolism (ATE) is currently the leading causes of death under cardiovascular diseases (CVDs) in developed countries. The American Heart Disease foundation estimates more than 30% of all deaths in the world are from CVDs, thus the study highlights that a person has greater chance of dying from heart disease than cancer, AIDS, diabetes and accidents combined [18]. Indicating that ATE as the leading cause of morbidity and mortality world-wide. ATE typically forms under high shear conditions of blood flow and consists of platelets bound by small amounts of fibrin.

ATE is the most common cause of cardioembolic events which includes myocardial infarction, ischemic stroke and Limb gangrene [19]. The treatment of acute myocardial infarction has changed during the past decade as newer approaches have become accessible, as prevention of complications has been the cornerstones for treatment. The management of ischemic heart diseases is now flanked by newer, more aggressive forms of therapy, which includes the early administration of thrombolytic drugs, highlighting clinical advantage of thrombolytic therapy for its ability to produce clot lysis, which directly restores nutritive myocardial perfusion [20]. Thrombolytic drugs like tissue plasminogen activator (t-PA), urokinase, streptokinase etc. play a crucial role in the management of ATE. The t-PA likes streptokinase and urokinase which are widely used as thrombolytic drugs have marked clinical drawback; these agents have a narrow therapeutic index and require continuous monitoring. Also, these agents have significant risk of haemorrhage, and produce anaphylactic reaction and lacks specificity. These entire therapeutic shortcomings of

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presently available streptokinase and urokinase and other t-PA indicate the need for better thrombolytic agents with clinical advantage [21].

Thrombus (blood clot) developed in the circulatory system due to failure of hemostasis causes vascular blockage and leads to serious consequences in thrombolytic diseases such as acute myocardial or cerebral infarction which may cause death[22]. Thrombolytic drugs are used to dissolve blood clots in a procedure termed thrombolysis. Alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (t-PA) are commonly used thrombolytic agents to dissolve clots. Heparin and Aspirin are only moderately efficient for acceleration of lysis and prevention of reocclusion, but are safe. Continued investigation in this area will provide new insights and promote progress towards the development of the ideal thrombolytic activity which is characterized by maximal coronary arteral thrombolysis with minimal bleeding. Selective third generation thrombolytic activity such as monoteplase, tenecteplase, reteplase etc. result in a greater angiographic potency in patients with acute myocardial infarction, although so far, mortality rates have been similar to those few drugs that have been studied in large-scale trials[23-26].

The general principles of the mechanisms of mitosis are best and most easily studied in the actively growing regions of plants such as a shoot or root apex. In *Allium cepa L*. root tip model root system of plant cells is commonly used as a test for investigating environmental pollution factors, toxicity of chemical compounds and evaluating potential anticancer properties. It has been used since 1938. It is very comfortable as it is easy to make preparations of onion roots. They contain rather homogenous meristematic cells, having only 16 chromosomes, which are very long, well visible and get stained easily. The test is a fast and inexpensive method, allowing the investigation of universal mechanisms for meristamatic plant cells and extrapolation on animal cells. The aim of this work will be investigated the antimitotic activity of synthesized compounds.

2. Materials and Methods

Chemicals were procured from E. Merck (India), S. D. Fine Chemicals (India) and reagent/solvents were used without distillation procedure. Melting points were taken in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer 157 infrared spectrometer (ν in cm–1) and NMR spectra were recorded on a Bruker spectrometer DPX-300MHz (Bruker, Germany) by using $CDCl_3$ as solvent with TMS as an internal standard. All the spectral data are consistent with the assigned structures of the desired product and the progress of the reactions was monitored on silica gel G plates using iodine vapour as visualizing agent.

Preparation of S1, S2 and S3

3-methyl-2,6-diphenylpiperidin-4-one was prepared by adopting the literature method. Condensation of 2-butanone, substituted aldehydes and ammonium acetate in warm ethanol in the ratio of 1:2:1 respectively afforded the formation of 3-methyl-2,6-diphenylpiperidin-4-ones.

Preparation of 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone

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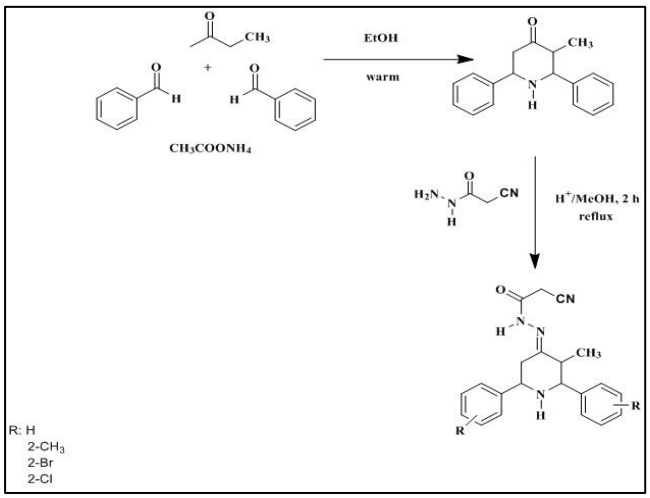
A mixture of 3-methyl-2,6-diphenylpiperidin-4-one (0.1 mol), cyanoacetic hydrazide (0.1 mol) in the presence of few drops of concentrated acetic acid in methanol was refluxed for 2 hours. After the completion of reaction, the reaction mixture was cooled to room temperature. The solid product was separated by filtration and washed with warm water and recrystallized by methanol to afford 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone.



Scheme1

***In vitro* thrombolytic activity**

Thrombolytic activity determined by the method of Fatema Tabassum *et al* (2017)

Preparation of streptokinase (SK)

About 5 ml sterile distilled water was added to the commercially available lyophilized SK vial of 15, 00,000 I.U. and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis study.

Collection of blood

Whole blood was drawn from healthy human volunteers without a history of oral contraceptives or anticoagulant therapy and 1 ml of blood was transferred to the previously weighed sterile eppendorf tubes and was allowed to form clots.

Procedure

3ml venous blood drawn from own blood was distributed in four different pre-weighed eppendorf tubes and incubated at 37^0 c for 45minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight **(clot weight = weight of clot containing tube-weight of tube alone).** To each eppendorf tube containing pre-weighed clot, 100µl (100µg/ml) of sample was added and another eppendorf tube containing pre-weighed clot, 200µl (200µg/ml) of sample was added. As a negative control, 100µl of distilled water was added to the control tube. For positive control, 100µl of streptokinase (SK) was added. All the tubes were then incubated at 37^0C for 90minutes and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighted to observe the difference in weight after clot

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disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clotlysis. The equation for calculating weight of clot is given below.

Clot weight = Weight of clot filled tube –Weight of empty tube

$$\text{\% of clot lysis} = \left(\frac{\text{Weight of clot after lysis}}{\text{Weight of clot before lysis}} \times 100 \right)$$

Determination of antimitotic activity

Evaluation of antimitotic activity using Allium cepa roots

Antimitotic activity study was conducted as per the methods reported by previous workers with modifications (Grant, 1982; Fiskesjo, 1988;Shweta*et al*. 2014) [11-12].

Allium cepa bulbs:

Approximately equal size bulbs (40±10 g) of the onions (*Allium cepa* L.) were obtained from the local vegetable market at Thanjavur, Tamil Nadu, India. Any onions that were dry, moldy or have started shooting green leaves were discarded.

Growing Allium cepa meristems:

The outer scales were removed from the healthy onion bulbs leaving the root primordia intact. These bulbs were grown in dark for 48 h over 100 ml of tap water at ambient temperature until the roots have grown to approximately 3 cm. The water was changed daily during this period. The viable bulbs were then selected and used for subsequent studies.

Exposure to text samples

The bulbs with root tips grown up to 2-3 cm were removed from the water and placed on a layer of tissue paper to remove excess of water. Various concentrations of the S1,S2 and S3 were prepared i.e; 10 µg/mL, 20 µg/mL, 30 µg/mL. The bulbs were divided into four groups. The first group served as control (tap water). Second group is Allium cepa roots were dipped in the compound *S1*. Third group is Allium cepa roots were dipped in the compound *S2*. Fourth group is Allium cepa roots were dipped in the compound *S3*. Fifth group is Allium cepa roots were dipped in the Methotrexate (0.10 mg/mL) was used as a standard control. All the groups were incubated at 25±2°C for 96 h away from direct sunlight. The test samples were changed daily with fresh ones. The length of roots grown during incubation (newly appearing roots not included), root number and the mitotic index were recorded after 96 h. The % of root growth inhibition was calculated by

$$\text{\% of root growth inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

The effective concentration for 50% root length inhibition (EC_{50} value) was determined by plotting the treatment concentrations against mean root lengths as percentage of water control group.

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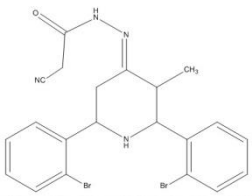
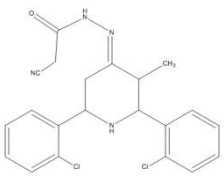
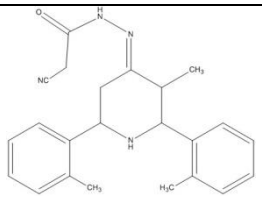
Microscopic studies and determination of mitotic index:

After 96 h, the root tips were fixed with fixing solution of acetic acid and alcohol (1:3). Squash preparations were made by staining the treated roots with acetocarmine stain (Badria et al., 2001)[13]. For each root tip, the numbers of mitotic cells and total meristematic cells were counted manually in 5-8 fields of view using high resolution (100x) bright field light microscope. The mitotic index was calculated by

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

3. Results and Discussion

Table 1. The physical data of synthesized Cyanoacetyl hydrazone derivatives

Compound	Structure	M.Formula	MW	Mp
S1		$C_{21}H_{18}Br_2N_4O$	502	174-178^oC
S2		$C_{21}H_{18}Cl_2N_4O$	412	180-182^oC
S3		$C_{23}H_{26}N_4O$	374	141-144 ^oC

3-methyl-2,6 di(bis-*o*-bromo phenyl) piperidin-4-one cyanoacetyl hydrazone (S1): Yield. 79.65%. mp. 174-178 ^oC. **FT-IR(KBr) ν_{max} (cm^{-1}):** 3099-2931 (C-H Aliphatic &Aromatic stretching), 1681 (C=O), 1567 (C=N), 2265 (C≡N), 3308-3179 (N-H). **^{13}C NMR(300 MHz, $CDCl_3$) δ ppm:** 130.10 (C-2 ipso carbon), 130.62 (C-6 ipso carbon), 128.59-129.54 (Aromatic carbons), 166.34 (C=O), 159.54 (C=N), 125.18 (C≡N), 25.11 (CH_2 carbon of cyanoacetohydrazone moiety), 65.86 (C-2), 59.64 (C-6), 39.14 (C-3), 25.23 (C-5), 12.19 (3-CH_3). **^{1}H NMR(300 MHz, $CDCl_3$), δ ppm:** 7.53- 7.31 (m,8H Aromatic protons) 10.79 (b s, 1H, N-H, Hydrazone Moiety), 2.50 (b s, 1H, N-H Piperidin moiety), 3.35 (q, 2H, CH_2 – Protons in hydrazone moiety), 0.91((d, J = 6.6Hz, 3H, 3-CH_3), 3.88 (dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 10.2Hz, 1H, H-6a), 3.35(d, $J^3_{a,a}$ = 10.2Hz, 1H, H-2a), 2.50 (dd, $J^3_{a,e}$ = 11.4Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 3.35 (dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 12Hz, 1H, H-5e), 2.51(m, 1H, H-3a Proton).

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3-methyl-2,6 di(bis-*o*-chloro phenyl) piperidin-4-one cyanoacetyl hydrazone (S3) : Yield. 80.69% . mp. 180-182ºC. **FT-IR(KBr) ν_{max} (cm^{-1}):** 3099-2933 ((C-H Aliphatic &Aromatic stretching), 1681 (C=O), 1570 (C=N), 2266 (C≡N), 3311-3183 (N-H). **^{13}C NMR(300 MHz, $CDCl_3$) δ ppm:**132.27 (C-2 ipso carbon), 133.68 (C-6 ipso carbon), 127.51-129.27 (Aromatic carbons) 165.09 (C=O), 154.98 (C=N), 115.08 (C≡N), 24.17 (CH_2 carbon of cyanoacetohydrazone moiety), 63.31 (C-2), 56.55 (C-6), 34.00 (C-3), 28.21 (C-5), 11.14 (3-CH_3). **^{1}H NMR(300 MHz, $CDCl_3$), δ ppm:** 7.42-7.32(m, 8H, Aromatic Protons), 10.09 (b s, 1H, N-H, Hydrazone Moiety), 2.24 (b s, 1H, N-H Piperidin moiety), 3.42 (q, 2H, CH_2 – Protons in hydrazone moiety), 0.971 (d, J = 6Hz, 3H, 3-CH_3) 3.73(dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 10.2Hz, 1H, H-6a), 3.3(d, $J^3_{a,a}$ = 10.2Hz, 1H, H-2a), 2.98 (dd, $J^3_{a,e}$ = 11.4Hz, $J^3_{a,a}$ = 12 Hz, 1H, H-5a), 3.4(dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 12Hz, 1H, H-5e), 2.67(m, 1H, H-3a Proton).

3-methyl-2,6 di(bis-*o*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone (S3) : Yield. 82.6%. mp. 141-144 ºC. **FT-IR(KBr) ν_{max} (cm^{-1}):** 3025-2852 (C-H Aliphatic & Aromatic stretching), 1674 (C=O), 1568 (C=N), 2267 (C≡N), 3440-3184 (N-H). **^{13}C NMR(300 MHz, $CDCl_3$) δ ppm:**139.98 (C-2 ipso carbon), 140.49(C-6 ipso carbon), 126.49-129.77 (Aromatic carbons), 164.48 (C=O), 158.05 (C=N), 114.35 (C≡N), 24.16 (CH_2 carbon of cyanoacetohydrazone moiety), 76.57 (C-2), 56.12 (C-6), 44.89 (C-3), 34.56 (C-5), 11.15 (3-CH_3) 19.20 (*o*-CH_3). **^{1}H NMR(300 MHz, $CDCl_3$), δ ppm :** 7.32-7.13 (m, 8H, Aromatic Protons), 10.09 (b s, 1H, N-H, Hydrazone Moiety), 2.09 (b s, 1H, N-H Piperidin moiety), 3.50 (q, 2H, CH_2 –Protons in hydrazone moiety), 0.92 (d, J = 6Hz, 3H, 3-CH_3), 3.89 (dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 10.2Hz, 1H, H-6a), 3.11 (d, $J^3_{a,a}$ = 10.2Hz, 1H, H-2a), 2.39 (dd, $J^3_{a,e}$ = 11.4Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 3.07(dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 12Hz, 1H, H-5e), 2.57 (m, 1H, H-3a Proton), 2.33 (s, 3H, *o*-CH_3 protons).

***In vitro* thrombolytic activity of synthesized compounds**

Thrombolytic therapy, also known as clot busting drug, is a breakthrough treatment which has saved untold lives. It has been used in the clinical area to treat venous and arterial thromboembolic complaints which are a foremost cause of death.

The *in-vitro* thrombolytic activity of the synthesized compounds was determined by clot lysis study. The activity of the compounds was determined by comparison with the thrombolytic activity of Streptokinase. The test compound was measured for the decrease in clot weight at different concentrations.

The *in-vitro* thrombolytic activity of the N-acetyl cyanoacetyl hydrazone derivatives were determined by clot lysis study. The activity of the compounds was determined by comparison with the thrombolytic activity of Streptokinase. The test compounds were measured for the decrease in clot weight at different concentrations 100 and 200 µl, respectively, streptokinase (30,000 IU) was employed as positive control and distilled water as negative control. The results were plotted conc. vs percentage clot lysis were obtained by regression analysis.

The results of the *in vitro* thrombolytic activity were encouraging and the tested compounds exhibited substantial aggregation inhibition. All the seven tested N-acetyl cyanoacetyl hydrazone derivatives exhibited substantial clot lysis, with percentage value

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ranging from 40.5 to 78.45% in comparison to 84.57 % clot lysis exhibited by the reference standard streptokinase (30,000 IU).

In the present study, thrombolytic activity analysis of compound S1 (100µl and 200µl) showed removal of clot by 40.57% and 69.19%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clotlysis.

The thrombolytic activity analysis of compound S2 (100µl and 200µl) showed removal of clot by 46.59% and 74.93%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clotlysis.

The thrombolytic activity analysis of compound S3 (100µl and 200µl) showed removal of clot by 47.62% and 76.21%, respectively, with that of positive Control streptokinase (SK) of 84.57% and negative control of 26.74% clotlysis.

The clot lysis at 100 µl 200 µl of compound S3 was 47.85%, in 37^0c at 45 min respectively while standard shows 84.57%. The highest dose as 200 µl of compound has significant activity and near to the standard.

Table 2. Thrombolytic activity of Synthesized compounds

Samples	% of clot lysis			
	Control	100 (µg/ml)	200 (µg/ml)	Standard
S1	26.74 ± 1.87	40.57 ± 2.83	69.19 ± 4.84	84.57 ± 5.91
S2	26.74 ± 1.87	46.59 ± 3.26	74.93 ± 5.24	84.57 ± 5.91
S3	26.74 ± 1.87	47.62 ± 3.33	76.21 ± 5.33	84.57 ± 5.91

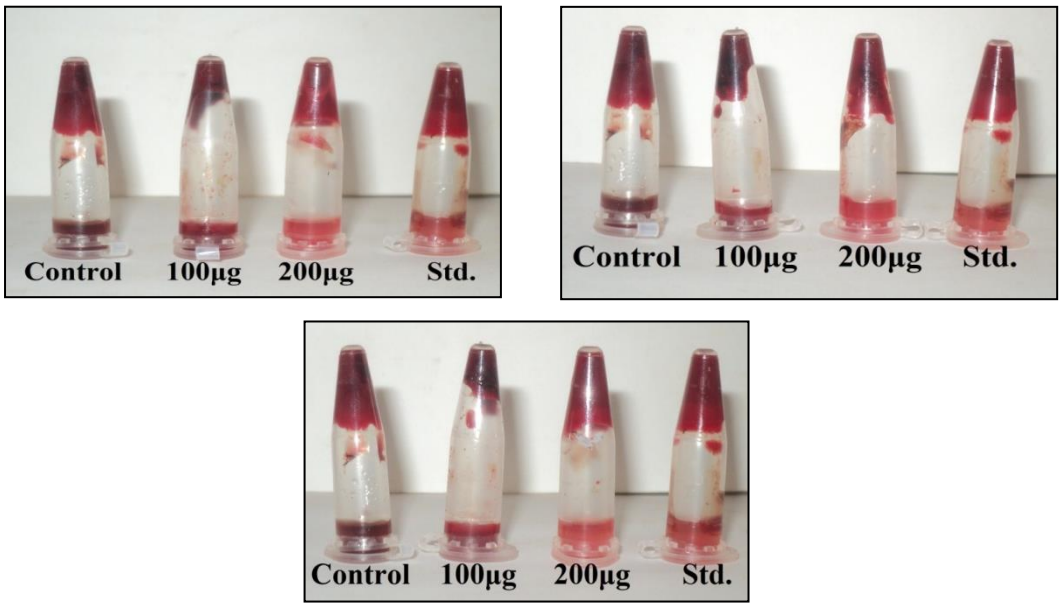

Plate.1: Thrombolytic activity test photos

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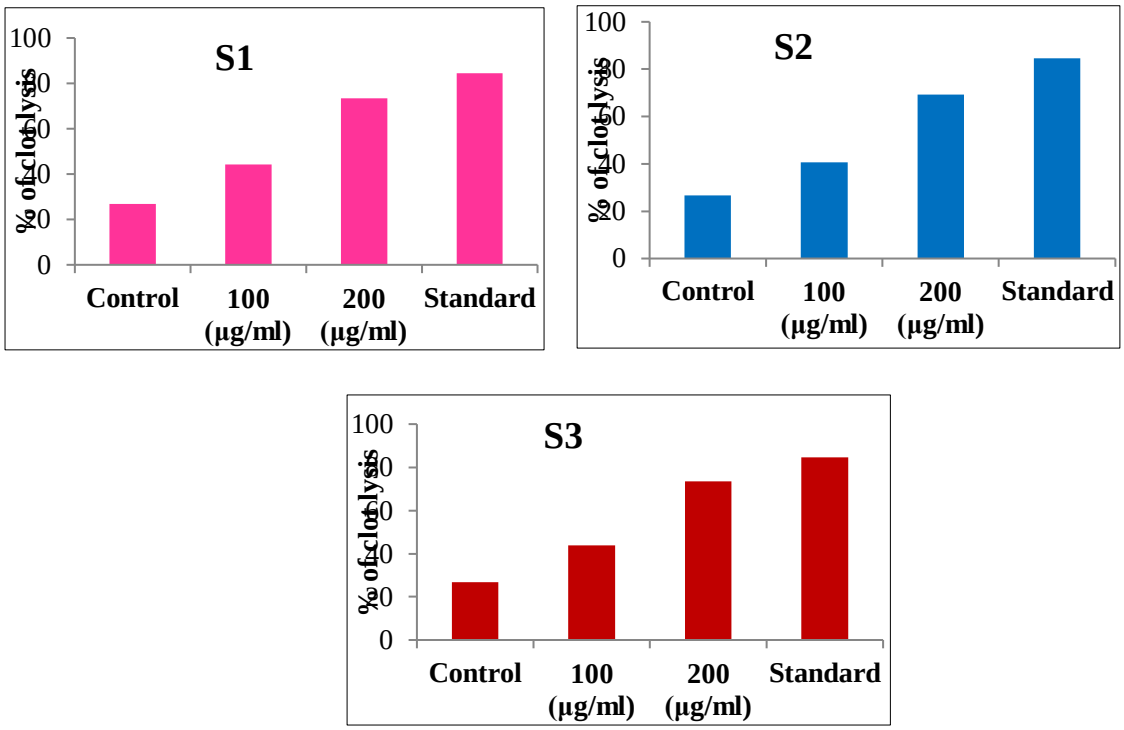

Plate.2: Thrombolytic activity graph

Antimitotic activity of compounds (S1, S2 and S3) using Allium cepa root meristamatic cells

The antimitotic activity was screened using Allium cepa root meristamatic cells which have been used extensively in screening of drugs with antimitotic activity. The inhibitory effect of compounds(S1,S2 and S3) were evaluated on the growth and mitotic activity of Allium cepa root meristems and the effect was compared with standard anticancer drug methotrexate. A progressive increase in average mean root length (8.10mm), average mean root numbers (7) and mitotic index (87.50%) observed in control group after 96 hrs of experimental period

The compound (S1) and methotrexate produced root decay and decreased the root length and root number significantly at 96 h as compared to control. The average mean root length at 10, 20,and 30µg/mL of compound (S1) was 6.20mm, 5.00mm, 3.4mm at 96 hr respectively while standard shows 2.60mm. The average mean root numbers at 10, 20, and 30µg/mL of compound (S1) was 6, 5 and 3 at 96 hr respectively while standard shows 3 numbers. The mitotic index at 10, 20, and 30 µg/mL of compound (S1) was 74.62, 60.64 and 49.62 at 96 hr respectively while standard shows 36.76%.

The compound (S2) and methotrexate produced root decay and decreased the root length and root number significantly at 96 h as compared to control. The average mean root length at 10, 20,and 30µg/mL of compound (S2) was 5.70mm, 3.70mm and 2.90mm at 96 hr respectively while standard shows 2.60mm. The average mean root numbers at 10, 20, and 30µg/mL of compound (S2) was 6, 5 and 3 at 96 hr respectively while standard shows 3

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numbers. The mitotic index at 10, 20, and 30 µg/mL of compound (S2) was 75.51 , 64.62 and 40.53% at 96 hr respectively while standard shows 36.76%.

The compound (S3) and methotrexate produced root decay and decreased the root length and root number significantly at 96 h as compared to control. The average mean root length at 10, 20,and 30µg/mL of compound (S3) was 6.20mm, 4.00mm and 2.40mm at 96 hr respectively while standard shows 2.60mm. The average mean root numbers at 10, 20, and 30µg/mL of compound (S3) was 6, 5 and 4 at 96 hr respectively while standard shows 3 numbers. The mitotic index at 10, 20, and 30 µg/mL of compound (S3) was 76.92 , 67.70 and 51.72% at 96 hr respectively while standard shows 36.76%.

The water control shows normal growth with greater root length and numbers. Treatment with different concentrations (10,20, and 30 µg/mL) of compounds (S1,S2 and S3) show decreased the growth gradually in dose dependent manner. The highest dose as 30µg/mL of compound (S1) has significant activity in root length, number and mitotic index and near to the standard.

The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and extends a few mm after that. Cell of this region undergo repeated divisions. The fate of cell division is higher in this region compare to that of the other tissues. This region is called the meristamatic region. This division is similar to the above mentioned cancer division in humans. Hence, these meristamatic cells can be used for preliminary screening of drugs with anticancer activity[14-17].

Cytotoxicity at all concentrations test extract were evidenced by evaluating macroscopic parameters, i.e., reduction in root number and root length both of which were indicative of root growth inhibition. In the present study mitotic index of different concentrations of extract clearly indicates the efficiency in the inhibition of growth of cancer cells either by affecting microtubules or encouraging microtubule formation, and thus stopping the microtubules from being broken down. This makes the cells become so clogged with microtubules that they cannot continue to grow and divide. The rate of tumor growth is dependent upon a balance between the rates of proliferation and apoptosis.

Table: 3 Effect of Compounds (S1,S2 and S3) on Root length, Root number and Mitotic Index of Allium cepa roots

Group	S1			S2			S3		
	Root Growth	Root Numbers	MI(%)	Root Growth (mm)	Root Numbers	MI (%)	Root Growth (mm)	Root Numbers	MI (%)
Water	8.1	7	87.5	8.1	7	87.5	8.1	7	87.5
10 µg/ml	6.2	6	74.62	5.7	6	75.51	6.2	6	76.92
20 µg/ml	5.0	5	60.64	3.7	5	64.62	4.0	5	67.70
30 µg/ml	3.4	3	49.62	2.9	3	40.53	2.4	4	51.72
Std Methotrexat (0.1mg/ml)	2.6	3	36.76	2.6	3	36.76	2.6	3	36.76

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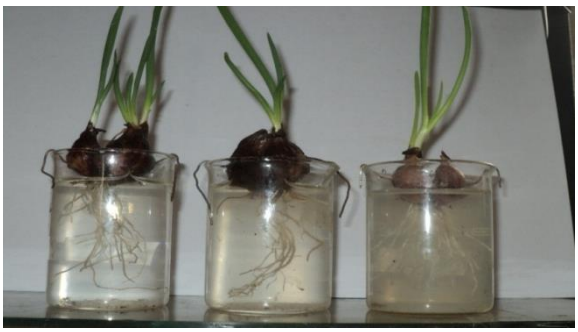

Fig.2 Various Concentrations of Compound 10µg/ml, 20µg/ml, 30µg/ml

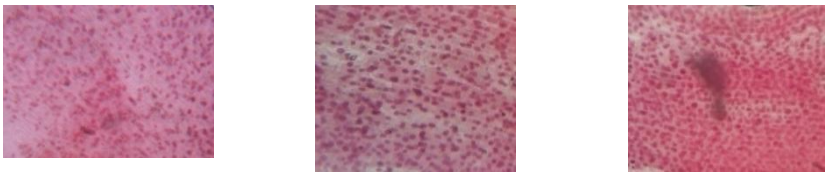

Fig.3 Photomicrograph of Compounds (S1,S2,S3) on mitotic Index of Allium cepa.

6. Conclusions

Synthesized a series of new Cyanoacetyl hydrazone derivatives obtained with good yield. All the compounds were characterized by using IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

The result from the study showed that the synthesized compounds have excellent thrombolytic activity that was comparable to the activity of Streptokinase. As from the research findings of the under taken in vitro clotlysis study, we demonstrated that the compounds showed mainly moderate thrombolytic activity. Our findings support the reported therapeutic use of this compound as clot lysis or thrombolytic agent in the Indian system of medicine. This is only a preliminary study and the synthesized compounds should be thoroughly investigated pharmacologically to exploit their medicinal and pharmaceutical potential.

The antimitotic activity, among the three compounds, S2 has greated activity than S1 and S3. Maximum numbers of non dividing cells were observed. As a result of this cells arrest in mitosis and eventually die by apoptosis. Our findings support the reported therapeutic use of these compounds as thrombolytic agent and anticancer (antimitotic) agent in the Indian system of medicine.

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