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VIEWS

Synthesis, Characterization and Antimicrobial Activity of Some New 4-aryl- 8- Arylidene-5, 6-dihydro- 2- Imino-6, 6-dimethyl -4h, 7h-[3, 1] Benzothiazines

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ABSTRACT

Benzothiazines are reported to possess an array of biological properties such as antibacterial, antifungal, antihelminth, anti-inflammatory and anticonvulsant activities. Moreover, thiazine nucleus is a pharmacophore of cephalosporins that occupy a very prominent place in the field of antibiotics. A number of antibacterial drugs are in clinical use, but search for new antimicrobial agents is continuously ongoing and demanding study due to acquisition of resistance by different species, adverse effects such as alteration of gut ecology, gastric irritation, nephrotoxicity, hypoprothrombinemia, thrombocytopenia, hypersensitivity reactions etc., towards the existing molecules. In view of these observations benzothiazines and their derivatives are reviewed and a series of 4-aryl- 8- arylidene-5, 6-dihydro- 2- imino-6, 6-dimethyl -4H, 7H-[3, 1] benzothiazines (2a-o) have been synthesized in order to obtain potent antibiotics. Further these benzothiazine derivatives are characterized by their spectral data and evaluated for their biological activities.

Key Words: Synthesis, benzothiazines, biological studies.

INTRODUCTION

Synthetic potential and biological activity of benzothiazine derivatives are explored by many researchers [1-5] and reported to possess a wide spectrum of biological activities such as antibacterial [6], antifungal [7], antihelminth [8], anti-tumour [9], antiproliferative [10], anti-inflammatory [11] and anticonvulsant [12] activities. In view of these observations we synthesized 4-aryl- 8- arylidene-5, 6-dihydro- 2- imino-6, 6-dimethyl -4H, 7H-[3, 1] benzothiazines from 4, 4-dimethylcyclohexanone (Scheme-1) with an objective to get novel potent bioactive compounds. Further the newly synthesized compounds have been screened for their antibacterial activities against Gram positive and Gram negative microorganisms and fungal organisms.

Materials and Methods

Melting points of all synthesized compounds were determined in open capillary tubes on an electro thermal apparatus and are uncorrected. The progress of the reaction and purity of the compounds was checked by TLC on silica gel coated aluminium plates (Merck) as adsorbent and UV light as visualizing agent. UV Spectra were obtained on ELICO SL 244 UV Double Beam Spectrophotometer. IR spectra (KBr v_{max} cm^{-1}) were recorded on a BRUKER FTIR spectrophotometer in the range of 4000-400 cm^{-1} .^{1}H NMR spectra were recorded on a INOVA (400 MHz) NMR spectrometer using CDCl$_3$ as solvent and TMS as an internal standard (chemical shifts in δ ppm).Mass spectra were recorded on a VG Autospec MS using ESI mode positive ion trap detector.

General procedure for the synthesis of 2, 6-diarylidene-4, 4-dimethylcyclohexanone (1a-o) [13]:

A mixture of 10% sodium hydroxide, ethyl alcohol, 4,4-dimethylcyclohexanone (0.01 mol) and aromatic aldehyde (0.02mol) was stirred at 20-25ÚC for 2h. Later the reaction mixture was kept in an ice chest over night. The product was filtered, washed with ice cold water followed by ice cold ethanol, dried and recrystallized from DMF.

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General procedure for the synthesis of 4-aryl- 8-arylidene-5,6-dihydro- 2- imino-6, 6-dimethyl-4H,7H-[3,1] benzothiazines(2a-o) [14]:

A mixture of 2,6-diarylidene cyclohexanone derivative (0.01 mol), thiourea (0.015 mol) and potassium hydroxide (0.01 mol)dissolved in 10 ml of water was refluxed in isopropyl alcohol for 16-18h.Later the solvent was removed under reduced pressure and the residue obtained was treated with ice cold water, filtered dried and recrystallized from ethanol. The physical data of the compounds were given in the table 1 and table 2.

Spectral data of 4-phenyl- 8- benzylidene -5, 6-dihydro- 2- imino-6, 6-dimethyl -4H, 7H-[3, 1] benzothiazine (2a):

The compound (2a) showed characterstic IR peaks at 3262 cm^{-1} (imine NH) , 3160 cm^{-1} (cyclic NH), 1025 cm^{-1} (C-N), 1543 &1462 cm^{-1} (aromatic), 1602 cm^{-1} (C=N) and ^{1}HNMR signals at δ0.9 (s, (CH$_3$)$_2$, 6H), δ1.7 (d, CH$_2$, 2H), δ1.9 (d, CH$_2$, 2H), δ4.9 (s, -CH-S, 1H), δ8.2 (s, imine, 1H), δ6.8 (s, cyclic NH, 1H), δ7.2-7.5 (m, ArH, 10H), δ8.0 (s, benzylic H, 1H).The molecular ion peak M$^+$ was observed at 361.

Spectral data of 4-(*p*-chlorophenyl)- 8- (*p*-chlorobenzylidene) -5,6-dihydro- 2- imino-6, 6-dimethyl -4H,7H-[3, 1] benzothiazine (2b):

The compound (2b) showed characterstic IR peaks at 3259(imine NH), 3154 cm^{-1}(cyclic NH), 1007cm^{-1}(C-N),

1571&1480 cm^{-1}(aromatic), 1614cm^{-1} (C=N), and ^{1}HNMR signals at δ0.9 (s, (CH$_3$)$_2$, 6H), δ1.6 (d, CH$_2$, 2H), δ1.8 (d, CH$_2$, 2H), δ4.9 (s, -CH-S, 1H), δ6.6 (s, cyclic NH, 1H), δ7.1-7.4 (m, ArH, 8H, benzylic H, 1H), δ7.7 (s, imine H, 1H). The molecular ion peak M$^+$ was observed at 429.

Spectral data of 4-(*p*-methoxyphenyl)-8- (*p*-methoxybenzylidene) -5, 6-dihydro- 2- imino-6, 6-dimethyl -4H, 7H-[3, 1] benzothiazine (2c):

The compound (2c) showed characterstic IR peaks at 3498 cm^{-1} (imine NH), 3149 cm^{-1} (cyclic NH) , 1023 cm^{-1}(C-N), 1563 &1504 cm^{-1}(aromatic), 1601 cm^{-1}(C=N) and ^{1}HNMR signals at δ0.9 (s, (CH$_3$)$_2$, 6H), δ1.7 (d, CH$_2$, 2H), δ1.8 (d, CH$_2$, 2H), δ4.8 (s,-CH-S, 1H), δ8.2 (s, imine, 1H), δ6.7 (s, cyclic NH, 1H), δ6.9-7.3 (m, ArH, 8H), δ7.9 (s, bezylic, 1H). The molecular ion peak M$^+$ was observed at 421.

Spectral data of 4-(*p*-fluorophenyl) - 8- (*p*-fluorobenzylidene) -5, 6-dihydro- 2- imino-6, 6-dimethyl -4H, 7H-[3, 1] benzothiazine 2(g):

The compound (2g) showed characterstic IR peaks at 3371cm^{-1} (imine NH),3160 cm^{-1} (cyclic NH),1015 cm^{-1}(C-N), 1546&1494cm^{-1} (aromatic), 1597 cm^{-1}(C=N), and ^{1}HNMR signals at δ0.9 (s, (CH$_3$)$_2$, 6H), δ1.7 (d, CH$_2$, 2H), δ1.9 (d, CH$_2$, 2H), δ4.9 (s, -CH-S, 1H), δ7.7 (s, imine, 1H), δ6.6 (s, cyclic NH, 1H), δ7.2-7.6 (m, ArH, 8H, benzylic H, 1H). The molecular ion peak M$^+$ was observed at 397.

Table-1
Physical Data Of (1a-o) And (2a-o)

Compound	Ar	M.F	M.W	M.P (^{0}C)	Yield (%)
1a	Phenyl	C$_{22}$H$_{22}$O	302.4	92-94	66
1b	*p*-Chlorophenyl	C$_{22}$H$_{20}$Cl$_2$O	371.2	146-148	94
1c	*p*-Methoxyphenyl	C$_{24}$H$_{26}$O$_3$	362.4	124-126	92
1d	*m*-Nitrophenyl	C$_{22}$H$_{20}$N$_2$O$_5$	392.4	140-142	77
1e	2,5-Dimethoxyphenyl	C$_{26}$H$_{30}$O$_5$	422.5	112-113	69
1f	3,4,5-Trimethoxyphenyl	C$_{28}$H$_{34}$O$_7$	482.5	180-182	73
1g	*p*-Flurophenyl	C$_{22}$H$_{20}$F$_2$O	338.3	115-117	95
1h	*p*-Ethylphenyl	C$_{26}$H$_{30}$O	358.5	175-177	79
1i	*p*-Isopropylphenyl	C$_{28}$H$_{34}$O	386.5	193-195	90
1j	3,4-Dimethoxyphenyl	C$_{26}$H$_{30}$O$_5$	422.5	122-123	72
1k	*p*-Ethoxyphenyl	C$_{26}$H$_{30}$O$_3$	390.5	148-149	76
1l	*p*-Hydroxyphenyl	C$_{22}$H$_{22}$O$_3$	334.4	182-183	86
1m	2,4-Dihydroxyphenyl	C$_{22}$H$_{22}$O$_5$	366.4	150-152	76
1n	2-Furyl	C$_{18}$H$_{18}$O$_3$	282.3	145-146	75
1o	*p*-Dimethylaminophenyl	C$_{26}$H$_{32}$N$_2$O	388.5	120-122	78

Table-2

Physical Data Of (2a-o)

Compound	Ar	M.F	M.W	M.P (ÚC)	Yield (%)
2a	Phenyl	$C_{23}H_{24}N_2S$	360.5	148-150	70
2b	*p*-Chlorophenyl	$C_{23}H_{22}Cl_2N_2S$	429.4	284-286	84
2c	*p*-Methoxyphenyl	$C_{25}H_{28}N_2O_2S$	420.5	240-242	88
2d	*m*-Nitrophenyl	$C_{23}H_{22}N_4O_4S$	450.5	210-212	78
2e	2,5-Dimethoxyphenyl	$C_{27}H_{32}N_2O_4S$	480.6	242-246	60
2f	3,4,5-Trimethoxyphenyl	$C_{29}H_{36}N_2O_6S$	482.5	286-288	75
2g	*p*-Flurophenyl	$C_{23}H_{22}F_2N_2S$	396.4	280-281	90
2h	*p*-Ethylphenyl	$C_{27}H_{32}N_2S$	416.6	270-272	86
2i	*p*-Isopropylphenyl	$C_{29}H_{36}N_2S$	444.6	260-262	83
2j	3,4-Dimethoxyphenyl	$C_{27}H_{32}N_2O_4S$	480.6	188-189	84
2k	*p*-Ethoxyphenyl	$C_{27}H_{32}N_2O_2S$	448.6	205-206	70
2l	*p*-Hydroxyphenyl	$C_{23}H_{24}N_2O_2S$	392.5	212-213	82
2m	2,4-Dihydroxyphenyl	$C_{23}H_{24}N_2O_4S$	424.5	223-224	65
2n	2-Furyl	$C_{19}H_{20}N_2O_2S$	340.4	226-227	85
2o	*p*-Dimethylaminophenyl	$C_{27}H_{34}N_4S$	446.6	140-142	65

Spectral data of 4-(*p*-ethylphenyl)- 8- (*p*-ethylbenzylidene) -5,6-dihydro- 2- imino-6, 6-dimethyl -4H,7H-[3, 1] benzothiazine (2h):

The compound (2h) showed characterstic IR peaks at 3589 cm^{-1}(imine NH), 3176 cm^{-1}(cyclic NH), 1022 cm^{-1}(C-N), 1544&1471 cm^{-1} (aromatic), 1611cm^{-1}(C=N) and ^{1}HNMR signals at δ0.9(s, (CH$_3$)$_2$, 6H), δ1.3(s, (CH$_3$)$_2$, 6H), δ2.3(d, CH$_2$, 2H), δ2.5(d, CH$_2$, 2H), δ2.9 (q, (CH$_2$)$_2$, 4H), δ4.9 (s, -CH-S, 1H), δ6.8 (s, imine, 1H), δ6.6 (s, cyclic NH, 1H), δ7.2-7.5 (m, ArH, 10H), δ7.6 (s, bezylic, 1H).The molecular ion peak M$^+$ was observed at 417.

Spectral data of 4-(*p*-isopropylphenyl)- 8- (*p*-isopropylbenzylidene) -5,6-dihydro- 2- imino-6, 6-dimethyl -4H,7H-[3, 1] benzothiazines (2i):

The compound (2i) showed characterstic IR peaks at 3445 cm^{-1}(imine NH), 3189cm^{-1}(cyclic NH), 1017 cm^{-1}(C-N), 1544 &1465 cm^{-1}(aromatic), 1606 cm^{-1}(C=N), and ^{1}HNMR signals at δ0.9 (s, (CH$_3$)$_2$, 6H), δ 1.2 (d, (CH$_3$)$_4$, 12H), δ2.3-2.5(m,(CH)$_2$,2H), δ1.7 (d, CH$_2$, 2H), δ1.9 (d, CH$_2$, 2H), δ4.9 (s, -CH-S, 1H), δ6.8 (s, imine, 1H), δ6.6 (s, cyclic NH, 1H), δ7.2-7.5 (m, ArH, 8.0H), δ7.7 (s, benzylic, 1H).The molecular ion peak M$^+$ was observed at 445.
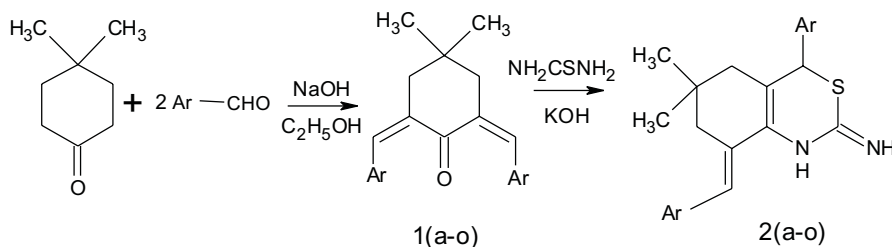
Scheme-1

Antimicrobial activity

The newly synthesized benzothiazines were screened for *in vitro* antimicrobial activity using two Gram positive organisms *viz.*, *Staphylococcus aureus* and *Bacillus subtilis*, two Gram negative organisms, *viz.*, *Escherichia coli* and *Pseudomonas aeruginosa* and two fungal organisms *viz.*, *Aspergillus niger* and *Candida albicans* by agar cup plate method [15] at 100μg. The zone of inhibition was measured in mm and the values of antibacterial and antifungal activity of (2a-o) were compared against standard references ampicillin and amphotericin B respectively. (Table-3)

Results and Discussion

The structures of new compounds prepared during the present investigation have been authentically established by their UV, IR, NMR and Mass spectral studies. In the following section the spectral studies of some selected compounds were dealt.

The compounds 1(a-o) were prepared by reaction of 4, 4-dimethylcyclohexanone with aromatic aldehydes which is an example for Claisen-Schmidt condensation. The formation of 1a from 4, 4-dimethylcyclohexanone was indicated by its UV spectrum. 4, 4-dimethylcyclohexanone exhibited λmax at 227nm.The compound 1a exhibited λ_{max} at 327.This clearly indicates that bathochromic shift was because of =CH chromophore [16].The formation of 1a from 4, 4-dimethylcyclohexanone was indicated by its IR

spectrum. 4, 4-dimethylcyclohexanone exhibited v_{max} at 1710(C=O).The compound 1a exhibited v_{max} at 1659(C=O).The appearance of a band at 1659 is mainly due to presence of the two =CH chrmophores. This clearly indicates the formation of 1a. The formation of 1a was also confirmed by its ^{1}H NMR spectrum .The presence of signals at δ 0.9 (s, (CH$_3$)$_2$, 6H), δ 1.7 (s, (CH$_2$)$_2$, 4H), δ 7.2-7.6 (m, ArH, 10H) and δ 7.9 (s, 2×methine, 2H) clearly shows the formation of **1a**.

The compounds 2(a-o) were prepared by cyclocondensation of 1(a-o) with thiourea. The formation of 2a from 1a was indicated by its UV spectrum. The λ_{max} of 1a was 327. The λ_{max} of 2a was 274. This indicate that the hypsochromic shift was attributed because of cyclocondensation. The formation of 2a from 1a was confirmed by its IR spectrum. The compound 1a exhibited v_{max} at 1659(C=O). The compound 2a exhibited v_{max} at 3345 and 3160(imine and cyclic NH). The absence of 1659 and presence of 3345 and 3160 in 2a clearly indicates its formation. The formation of 2a was confirmed by its ^{1}H NMR spectrum. The presence of signals at δ0.9 (s, (CH$_3$)$_2$, 6H), δ1.7 (d, CH$_2$, 2H), δ1.9 (d, CH$_2$, 2H), δ4.9 (s, -CH-S, 1H), δ8.2 (s, imine, 1H), δ6.8 (s, cyclic NH, 1H), δ7.2-7.5 (m, ArH, 10H), δ8.0 (s, bezylic-H, 1H) clearly shows the formation of 2a. The compounds 2b,2c,2g,2h,2i were also confirmed by their proton NMR spectra. The formation of

Table-3

Antibacterial and Antifungal Activity (2a-o)

Compound	Antibacterial activity				Antifungal activity	
	S.aureus	B.subtilis	E.coli	P.aeruginosa	A.niger	C.albicans
2a	20	19	20	17	13	13
2b	23	24	20	20	16	14
2c	24	22	20	21	14	14
2d	20	21	17	14	12	16
2e	17	18	13	10	11	13
2f	16	16	15	14	11	12
2g	27	25	24	20	16	16
2h	20	21	17	14	12	15
2i	18	17	14	10	12	13
2j	21	20	21	15	14	13
2k	19	20	18	15	12	15
2l	16	14	15	13	9	11
2m	17	18	13	10	11	13
2n	23	21	17	14	15	12
2o	18	17	17	15	11	10
Ampicillin	38	32	33	30	—-	—-
Amphotericin B	—-	—-	—-	—-	18	16

2a was also elucidated by its mass spectrum. The molecular ion peak of 2a was observed at m/e 361, which was in good agreement with the calculated molecular weight of the compound. The compounds 2a, 2b 2c 2g, 2h, and 2i were also confirmed by their mass spectra.

The compounds 2(a-o) exhibited antibacterial activity against Gram+ve and Gram–ve organisms. Among these compounds with *p*-fluorophenyl and *p*-methoxyphenyl substitutions showed maximum activity against *S.aureus*, *B.subtilis*, *E.coli*, and *P.aeruginosa*, respectively, while other compounds showed moderate to poor activity. All benzothiazines showed antifungal activity against *A.niger*, but compound with *p*-chloro substitution exhibited highest activity against *A.niger*, while other compounds showed moderate to poor activity. However none of these compounds had greater activity than the standard references, ampicillin and amphotericin B.

Acknowledgement

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Chitosan: Naturally Occurring Biopolymer for Defluroidation of Water

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ABSTRACT

Present investigation deals with removal of fluoride from drinking water using low cost naturally available biopolymer adsorbent chitosan. The effects of various physico-chemical parameters such as pH, adsorbent dose, initial fluoride concentration and the presence of interfering co-ions on adsorption of fluoride were studied. It was observed that the uptake of fluoride was higher at self pH (pH$\cong$5). The equilibrium adsorption data were fitted well for both Langmuir isotherm model and Freundlich isotherm model. It was observed that co-ions such as sodium nitrate, sodium chloride, sodium sulfate, ferrous sulfate and copper sulfate have positive effect on the uptake of fluoride from drinking water while sodium carbonate and bicarbonate has a negative effect on adsorption of fluoride. The comparison of uptake of fluoride in distilled water and field water shows slightly higher uptake of fluoride in distilled water. This may be because of latter contains different types co-ions and higher pH of the field water.

Key words: Removal of fluoride, chitosan, biopolymer, defluoridation.

Introduction

Fluoride is an essential constituent for both humans and animals depending on its concentration in drinking water. The presence of fluoride in drinking water, within permissible limits is beneficial for the production and maintenance of healthy bones and teeth, while excessive intake of fluoride causes dental or skeletal fluorosis [1-2]. Higher level of fluoride in groundwater is a world-wide problem, which includes various countries from Africa and Asia as well as USA [3]. Fluoride is one of the most abundant constituent occurring in groundwater in India and creates a major problem in safe drinking water supply. The concentration of fluoride in drinking water is as high as 30 mg l^{-1} in some places. Excess fluoride in drinking water is prevalent in 150 districts of 17 States in India [4]. According to the Department of Drinking Water Supply under Ministry of Rural Development, India, rural drinking water supply is mainly dependent on groundwater (85%). Hence, it becomes necessary to bring down the fluoride concentration within permissible limit of 1.5 mg l^{-1} according to Indian Standards. The limit varies among countries and the age of

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people exposed. World Health Organization (WHO) has set a limit range between 0.5 and 1 mg l^{-1} [5]. According to US standard it is between 0.6 and 0.9 mg l^{-1}.

Chitosan is an interesting and abundant polysaccharide, found in a wide range of organisms including bacteria and fungi, but commercially most commonly extracted from shellfish processing waste. The structure of chitosan is shown in Fig.1a. It is generally considered to be, the most abundant biopolymer in the ecosphere after cellulose which it resembles structurally. A number of publications have been reported mainly on alumina [4,8-10], calcium [6,11-13], clays [14-19] and biopolymer [20-22] however, to the best of our knowledge chitosan, has not been studied so far towards its properties for defluoridation of water. The present investigation deals with removal of fluoride from drinking water by using low cost materials such as chitosan. The effects of the various physico-chemical parameters such as pH, adsorbent dose, initial fluoride concentration and presence of co-ions on removal of fluoride were investigated. Ground water samples collected from SPSR Nellore district of Andhra Pradesh, India, were also used for fluoride removal studies. The detailed characteristics of field water are given in Table 1.
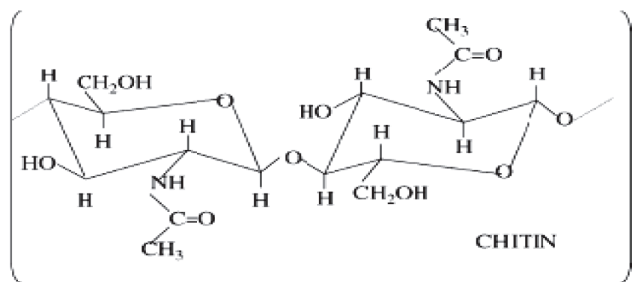
Fig. 1: Structure of chitosan unit

Experimental

Materials and Methods

Chitosan was purchased from Chemchito, Chennai. A stock solution of fluoride was prepared by dissolving sodium fluoride in distilled water and working fluoride solution of 5 mg l^{-1} was prepared from stock fluoride solution by appropriate dilution. Batch adsorption experiments were conducted to investigate the effect of various parameters. Fluoride was estimated using Ion selective electrode. The specific amount of fluoride adsorbed was calculated from:

$$q_e = (C_0 - C_e) \times \frac{V}{W} \qquad \text{............................... (1)}$$

Where q_e is the adsorbate loading (mg g^{-1}) in the solid at equilibrium; C_0, C_e are initial and equilibrium concentrations of fluoride (mg l^{-1}), respectively; V is volume of the aqueous solution and W is the mass (g) of adsorbent used in the experiments.

The effect of solution pH on fluoride removal was studied by adjusting the pH of the solution by using 0.1N HCl or 0.1N NaOH.

Result and Discussion

Characterization

XRD of chitosan and Iron loaded chitosan flakes are shown in Fig. 2a. These spectra show that chitosan exhibits a narrow high peak, at 2è = 20°, and a wide lower peak at 2è = 10°, which is a typical pattern of a chitinous material. The main IR bands of chitosan spectrum were retained on loading of iron in chitosan, especially those related to N-H vibrations of –NHCOCH$_3$ group, located at 2886, 1440 and 1369 cm^{-1} and C-O virabation at 1159 and 1014 cm^{-1} (see Fig. 2b). Surface morphology of Chitosan was studied using SEM (Fig. 1c). Chitosan have dense, firm and a rough surface without porosity

Fluoride removal using chitosan:

Chitosan can be deacetylated using NaOH where acetamide (–NHCOCH$_3$) group of chitosan, Chitosan is converted to amine (–NH$_2$) group. Fluoride removal capacity of chitosan and deacetylated chitosan has been compared and the results are given in Fig.3. It is observed that with increase in % deacetylation of chitosan fluoride removal decreases which may be due to decrease in surface area with increase in % deacetylation of chitosan.

Fig. 2: Characterization a) XRD pattern, b) FTIR spectrum, c) SEM image of Chitosan.

Fig. 3: Fluoride removal using chitin and deacetylated chitosan

Fig. 4: Effect of pH

Effect of presence of co-anions

The effect of presence of co-ions on the removal of fluoride is shown in Fig. 6. It was observed that sodium chloride, sodium sulfate, sodium nitrate, sodium bicarbonate, ferrous sulfate and 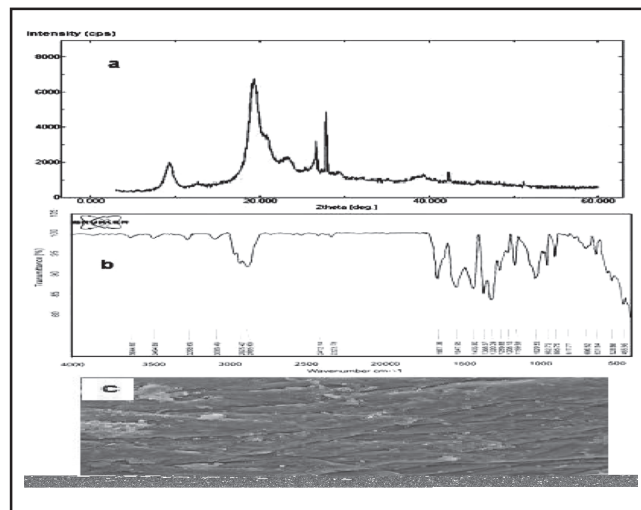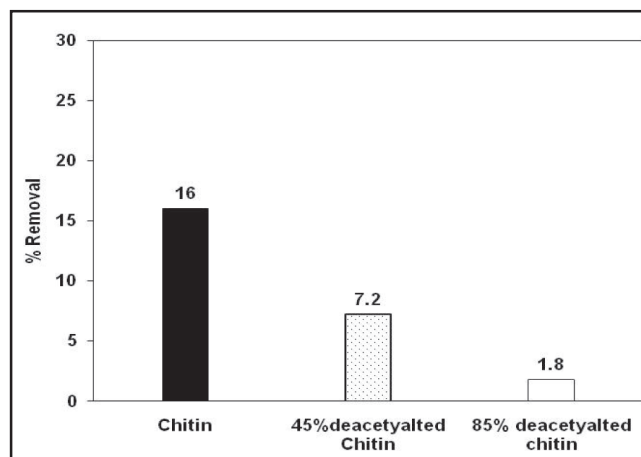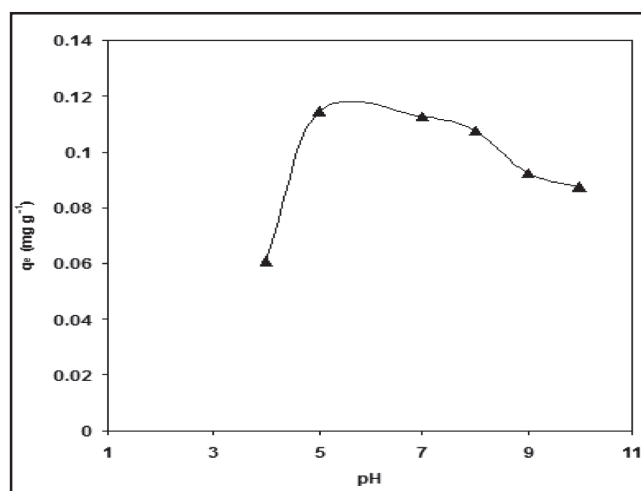copper sulfate ions shows positive effect on removal of fluoride. It was also found that sodium bicarbonate has slightly negative effect on removal of fluoride. This may be because of the change in pH as well as the competing effect of this co-ion. The pH of the fluoride solution were 7.07, 6.58 7.06, 3.28, 4.17, 9.07, 10.84, respectively for sodium chloride, sodium sulfate, sodium nitrate, ferrous sulfate, copper sulfate, sodium bicarbonate sodium and sodium carbonate while the pH of the fluoride solution was 7.29 without addition of co-ions. This indicates that addition of some of co-ions resulted in decrease in pH of fluoride solution except sodium carbonate and bicarbonate. From our experiments on effect of pH it was observed that the adsorption of fluoride was higher in the pH range of 5-7. Overall it was observed that the presence of cations and many of the anions enhance the uptake of fluoride from aqueous solution indicating fluoride specific sorption behavior.
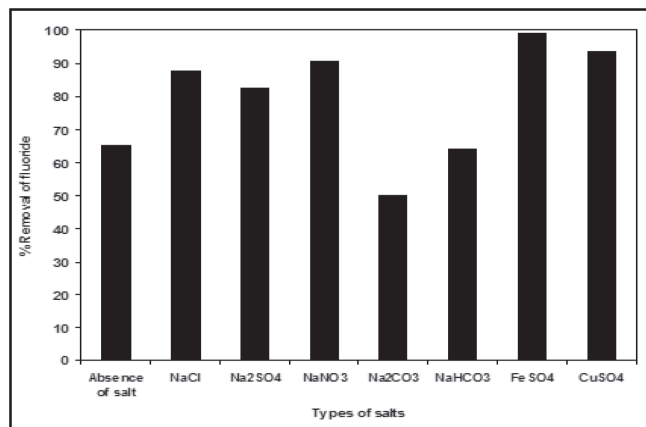

Fig. 6: Effect of the presence of co-existing ions

Equilibrium isotherm studies

Equilibrium studies were carried out to determine the maximum fluoride removal capacity and equilibrium constant on chitosan. It was observed that adsorption capacity reaches an equilibrium value beyond which there was negligible change in the residual fluoride concentration. The distribution of fluoride between the liquid phase and the solid phase is a measure of the position of equilibrium in the adsorption process and can be expressed by the Freundlich and Langmuir equations [19, 20]. These two models are widely used, the former being purely empirical and the latter assumes that maximum adsorption occurs when the surface is covered by the adsorbate. The value of K_F is 0.195 mg g^{-1} and n is 0.849 for Freundlich isotherm. The values of Langmuir parameters, q_{max} and K are 0.275 mg g^{-1} and 0.107 l mg^{-1}, respectively. Value of $r < 1$ represents favorable adsorption. The r-value for the initial concentration of 5 mg l^{1} was found to be 0.66. The value

obtained shows that the system is favorable for adsorption of fluoride.

Comparison of fluoride uptake in distilled water and field water

The uptake of fluoride from field water samples (Marripadu) collected from Andhra Pradesh, India by using chitosan is shown in Fig. 7. This Figure also illustrates removal of fluoride from distilled water. It was observed that the percentage removal of fluoride in distilled water is slightly higher as compared to field water sample. The detailed physico-chemical characteristics of field water samples before treatment are given in Table 1. Table 1 show that field water has TDS of = 477 mg l^{-1} which is indicative of presence of other ions in addition to fluoride. This could be due to two reasons i) pH of the field water samples is alkaline and ii) it contains different types of co-ions. These ions compete with adsorption of fluoride on chitin and reduce the adsorption of fluoride. In section 3.4 it has been discussed that in the alkaline pH uptake of fluoride is low as compared to acidic pH. Therefore, overall removal of fluoride in field water is low as compared to distilled water.
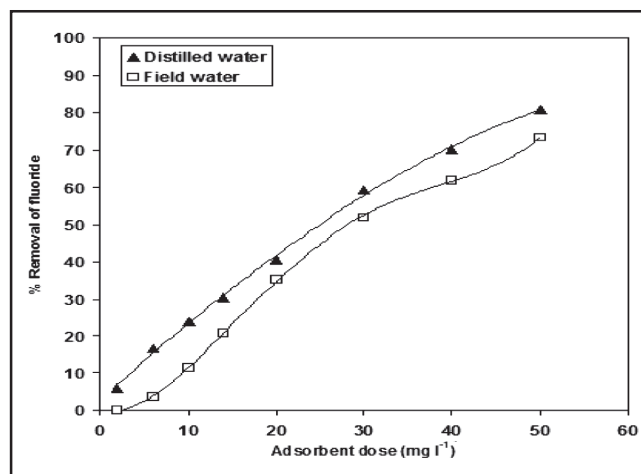
Fig. 7: Comparison of adsorption of fluoride in distilled water and field water

Conclusion

A naturally occurring bio-sorbent such as chitosan is found to be a suitable adsorbent for removal of fluoride from drinking water. The adsorption of fluoride on the surface of the adsorbent is observed to dependent mainly on the pH of the solution, initial concentration of fluoride and presence of co-anions. It was observed that in the presence of cations and most of anions (except sodium carbonate and bicarbonate) have a positive effect on the adsorption of fluoride. The adsorption of fluoride at acidic pH (pH =5) was high as compared to alkaline pH. The percentage removal of fluoride in distilled water was higher than field water; this may be because latter contains different types of ions and is having alkaline pH.

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A Study on Antimicrobial and Antioxidant Activities of Schiff's Bases Derived from New Thieno [2,3 - *d*] Pyrimidines

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ABSTRACT

Present investigation focuses on the synthesis of two series of 2-methyl-4{[(Aryl) methylene]amino}-5,6-Substituted-thieno[2,3-*d*]pyrimidine derivatives (BNB_{1-9} and BNC_{1-9}) from the condensation of aromatic aldehydes with 2-Phenyl- 5, 6-substituted thieno [2, 3-*d*] pyrimidin-4-amines. The compounds thus synthesized were evaluated for their *in vitro* antibacterial and antioxidant activities. The results of antibacterial activity revealed that compounds BNB5, BNB8, BNB9 of 5,6-dimethyl-2-phenyl[2,3-*d*]pyrimidin-4-amine series and compounds BNC8 and BNC9 of 2-phenyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidin-4-amine series found to be potent antibacterial agents. The antioxidant activity data showed that compounds BNB1, BNB4, BNC3 and BNC9 exhibited potent free radical scavenging activity.

Key words: Antioxidants, Thienopyrimidines, Schiff's base

Introduction

Pyrimidine has always been a unique interesting heterocyclic moiety for the   medicinal chemists; an exhaustive research has been done on the pyrimidines that led to the discovery and introduction of several drugs into the market. Fused pyrimidines have also been attracted a considerable interest in medicinal chemistry research due to their versatility and a broad bio active potential. Thieno pyrimidine is among those fused pyrimidines found to have a wide variety of pharmacological and biological applications. Since last four decades research has been focused on the design and synthesis of novel thienopyrimidines as medicinal agents, a large number of reports have been documented on thienopyrimidines as they found to exhibit a variety of biological activities such as antimicrobial, anti inflammatory, bronchodilatory activity, inhibition of Phospodiesterases, tyrosine kinase and VEGFR kinase[1-5]. It is evident that purine as an endogeneous scaffold plays a major biochemical role in variety of regular physiological functions such as respiration, inflammation, cell proliferation and so forth. As a bioisoster to purines, thieno[2,3-*d*] pyrimidines were also found to exhibit numerous biological activities which could be due to the interaction with various physiological elements. This observation initiated our present investigation to synthesize and to find new thieno[2,3-d]pyrimidines as potentially active medicinal agents.

In the present investigation a new series of thieno[2,3-*d*]pyrimidine Schiff's bases have been synthesized by the condensation of new 4-amino substituted thieno[2,3-*d*]pyrimidines with aromatic aldehydes and the final compounds thus obtained have been evaluated for their antimicrobial and antioxidant activity as continuation to our earlier described work depicted in **Scheme-1**

Materials and Methods

Structures of synthesized compounds were determined from their IR (Schimadzu FTIR), 1H NMR (Gemini 300 MHz) and elemental analysis (Carlo ebra 1108 elemental analyzer) was within ±0.4% of theoretical value. Melting points of all the compounds were determined in open capillaries using Toshniwal and Cintex melting point apparatus and are uncorrected. Analytical TLC was performed on Silica Gel F 254 plates (Merck) and the spots were visualized by UV or iodine vapours.

Synthesis of 2-amino 3-cyano 4, 5-substituted thiophene derivatives (B & C)

Equimolar amount (0.01mole) of sulphur, melanonitrile and a ketone (2-butanone/cyclohexanone) were taken in round bottom flask containing 20 ml of

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$R_1 = R_2 = CH_3, -(CH_2)_4-$
$R_3 = C_6H_5$

$Ar = \left[\begin{array}{l} C_6H_5, \text{ } p\text{-Cl-}C_6H_4, \text{ } p\text{-Br-}C_6H_4, \\ p\text{-N(CH}_3)_2C_6H_4, \text{ } p\text{-N(C}_2H_5)C_6H_4, \\ p,m\text{-(OCH}_3)_2\text{-}C_6H_3, \text{ } p\text{-(OCH}_3)\text{-}C_6H_4, \\ 3\text{-indolyl, 3-Thienyl} \end{array} \right]$
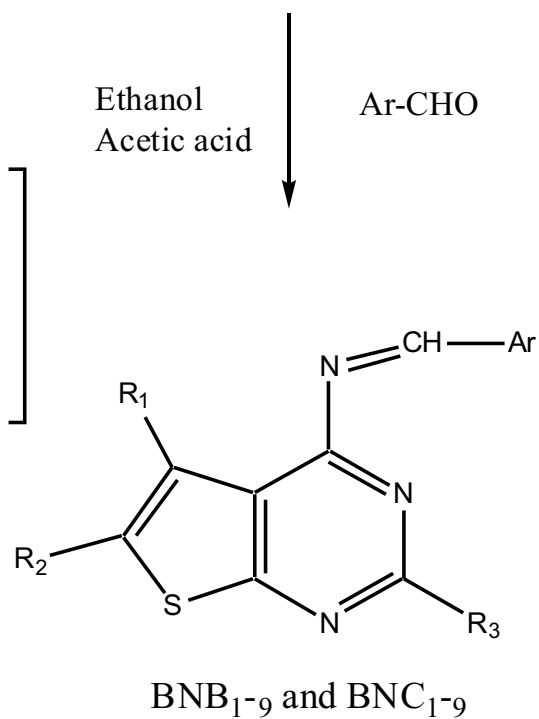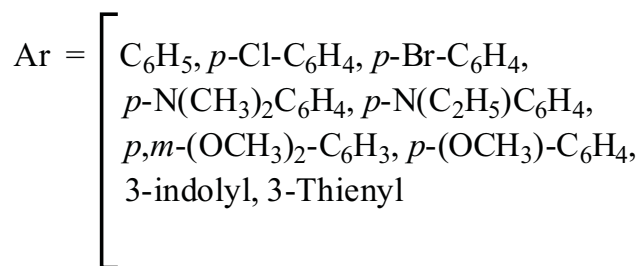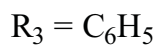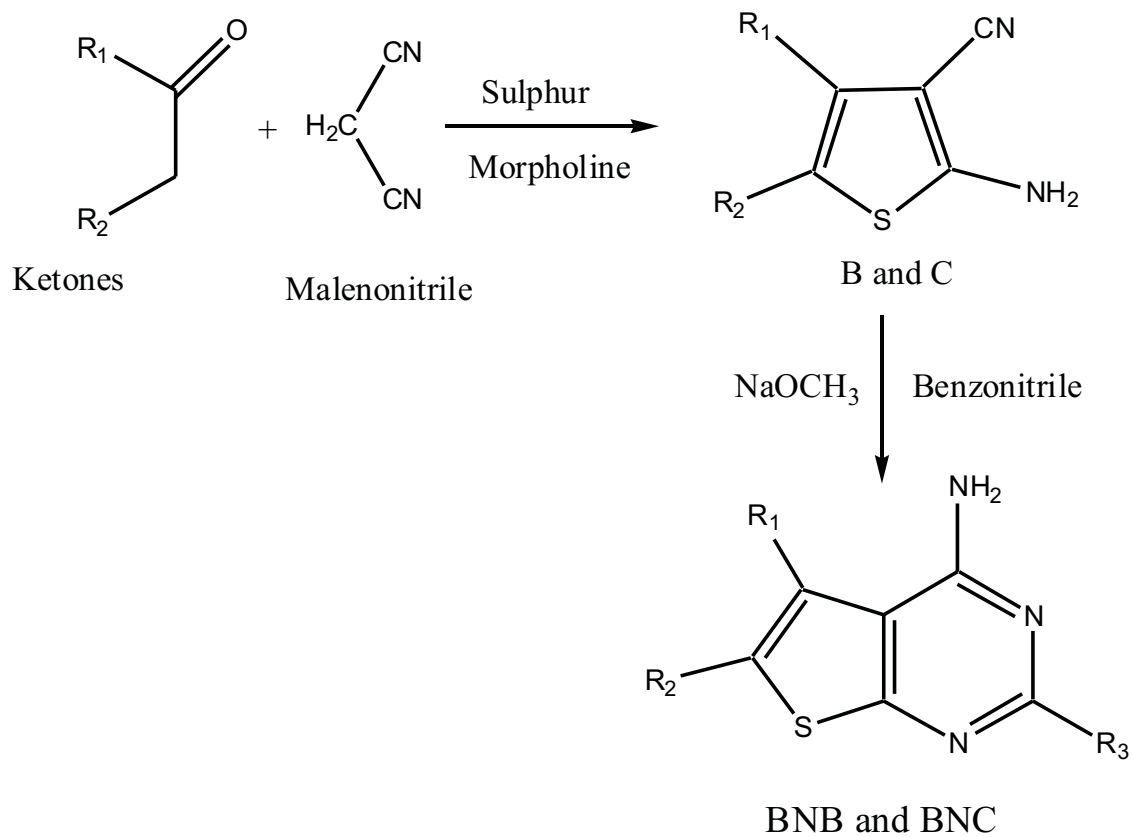
Scheme-1

ethanol. The mixture was stirred for five minutes then morpholine(0.012mole) was slowly added to the reaction mixture at 50ºC with constant stirring for 10-15 minutes. Later, the reaction mixture was allowed to stir for five hours at room temperature and left in refrigerator over night. The crystals thus formed were collected by filtration under reduced pressure and washed with cold ethanol. The compounds were further purified by recrystallization from ethanol. (Melting point: compound **B** 142-144^{0}C and compound **C** 145-147^{0}C)

Synthesis of 2-methyl 5, 6-substituted thieno [2,3-*d*] pyrimidin-4-amine (BNB & BNC):

2-0.001moles of 2-amino-3-cyano Thiophene, 0.001moles of Methyl nitrile along with sodium methoxide (0.002moles) were taken in dry methanol. The reaction mixture was heated under reflux for eight hours. The excess solvent was removed under vacuum and added to crushed ice. The aqueous mixture was neutralized with dilute hydrochloric acid to liberate the compound out of the solution. The compound thus precipitated was collected by filtration under vacuum, which was purified by recrystallization from alcohol. (Melting point: compound BNB 120-123^{0}C and compound BNC 125-128^{0}C)

Synthesis of Synthesis of 2-phenyl-4{[(Aryl)methylene] amino}-5, 6-Substituted-thieno[2,3-*d*]pyrimidine derivatives (BNB$_{1-9}$ and BNC$_{1-9}$):

0.001 mole of 2-phenyl 5, 6-substituted thieno [2, 3-*d*]pyrimidin-4-amines (BNB and BNC) and aromatic aldehyde were dissolved in absolute ethanol, and then catalytic amount of glacial acetic acid was added drop wise and refluxed for 17h. The reaction mixture was then cooled in ice bath and the crude product thus obtained was collected by filtration. The crude compound was further purified by recrystallization from ethanol. The physical data and elemental analysis of compounds BNB$_{1-9}$ and BNC$_{1-9}$ are presented in Table 1 and Table 2 respectively.

5,6-dimethyl-2-phenyl-*N*-[phenylmethylidene]thieno[2,3-*d*]pyrimidin-4-amine(BNB1)

IR(KBr): 2930 and 2864 cm^{-1}(-CH), 1614 cm^{-1} (C=N); ^{1}H NMR(CDCl$_3$): 8.36(s, 1H, =CH), 8.28(d, 2H, Ar-H), 7.83(s, 2H, Ar-H),7.51(m, 6H, Ar-H), 2.36(s, 3H, -CH$_3$),1.93(s, 3H, -CH$_3$).

N-[(1Z)-(4-chlorophenyl)methylidene]-5,6-dimethyl-2-phenylthieno[2,3-*d*]pyrimidin-4-amine (BNB2)

IR(KBr):2935 and 2863 cm^{-1}(-CH), 1614 cm^{-1} (C=N); ^{1}H NMR(CDCl$_3$): 8.36(s, 1H, =CH), 8.28(d, 2H, Ar-H), 7.77(d, 2H, Ar-H),7.52(m, 4H, Ar-H), 7.41(s, 1H, Ar-H) 2.36(s, 3H, -CH$_3$),1.93(s, 3H, -CH$_3$).

Table 1
Physical data of synthesized compounds BNB1-9 and BNC1-9

Compound	Molecular formula	Melting point	Yield (%)	Molecular weight
BNB1	C$_{21}$H$_{17}$N$_3$S	170-174	83	343
BNB 2	C$_{21}$H$_{16}$ClN$_3$S	117-119	81	378
BNB 3	C$_{21}$H$_{16}$BrN$_3$S	109-112	67	422
BNB 4	C$_{23}$H$_{22}$N$_4$S	160-163	74	387
BNB 5	C$_{25}$H$_{26}$N$_4$S	116-118	70	415
BNB 6	C$_{23}$H$_{21}$N$_3$O$_2$S	163-166	69	403
BNB 7	C$_{22}$H$_{19}$N$_3$OS	171-73	75	373
BNB 8	C$_{23}$H$_{18}$N$_4$S	178-179	81	382
BNB 9	C$_{19}$H$_{15}$N$_3$S$_2$	112-114	63	349
BNC1	C$_{23}$H$_{19}$N$_3$S	190-192	86	369
BNC2	C$_{23}$H$_{18}$ClN$_3$S	178-180	85	404
BNC3	C$_{23}$H$_{18}$BrN$_3$S	142-146	66	448
BNC4	C$_{25}$H$_{24}$N$_4$S	152-154	74	413
BNC5	C$_{27}$H$_{28}$N$_4$S	168-170	70	441
BNC6	C$_{25}$H$_{23}$N$_3$O$_2$S	146-148	65	430
BNC7	C$_{24}$H$_{21}$N$_3$OS	152-156	73	400
BNC8	C$_{25}$H$_{20}$N$_4$S	161-163	77	409
BNC9	C$_{19}$H$_{15}$N$_3$S$_2$	97-100	60	349

Table 2
Elemental Analysis of synthesized compounds

Compound	Substituent's			Elemental analysis % Found (calculated)		
	R_1	R_2	Ar	C	H	N
BNB1	CH_3	CH_3	-C_6H_5	73.36 (73.44)	4.91(4.99)	12.16 (12.23)
BNB 2	CH_3	CH_3	p-Cl-C_6H_4	66.66 (66.75)	4.3 (4.27)	11.1 (11.12)
BNB 3	CH_3	CH_3	p-Br-C_6H_4	59.7 (59.72)	3.78 (3.82)	9.91 (9.95)
BNB 4	CH_3	CH_3	p-N$(CH_3)_2$-C_6H_4	71.44 (71.47)	5.71 (5.74)	14.38 (14.5)
BNB 5	CH_3	CH_3	p-N$(C_2H_5)_2$-C_6H_4	72.54 (72.43)	6.36 (6.32)	13.98 (13.51)
BNB 6	CH_3	CH_3	p,m-$(OCH_3)_2$-C_6H_3	68.46 (68.46)	5.2 (5.25)	10.3 (10.41)
BNB 7	CH_3	CH_3	p-(OCH_3)-C_6H_4	70.62 (70.75)	5.14 (5.13)	11.21 (11.25)
BNB 8	CH_3	CH_3	3-indolyl	72.21 (72.22)	4.71 (4.74)	14.61 (14.65)
BNB 9	CH_3	CH_3	3-Thienyl	65.31 (65.3)	4.26 (4.33)	12 (12.02)
BNC1	-$(CH_2)_4$-		-C_6H_5	74.66 (74.77)	5.06 (5.18)	11.27 (11.37)
BNC2	-$(CH_2)_4$-		p-Cl-C_6H_4	68.88 (68.39)	4.26 (4.49)	10.39 (10.4)
BNC3	-$(CH_2)_4$-		p-Br-C_6H_4	61.56 (61.61)	4.02 (4.05)	9.26 (9.37)
BNC4	-$(CH_2)_4$-		p-N$(CH_3)_2$-C_6H_4	72.67 (72.78)	5.7 (5.86)	13.46 (13.58)
BNC5	-$(CH_2)_4$-		p-N$(C_2H_5)_2$-C_6H_4	73.54 (73.6)	6.42 (6.41)	12.69 (12.72)
BNC6	-$(CH_2)_4$-		p,m-$(OCH_3)_2$-C_6H_3	69.82 (69.91)	5.4 (5.4)	9.71 (9.78)
BNC7	-$(CH_2)_4$-		p-(OCH_3)-C_6H_4	72.1 (72.15)	5.28 (5.3)	10.51 (10.52)
BNC8	-$(CH_2)_4$-		3-indolyl	73.5 (73.5)	4.98 (4.93)	13.74 (13.71)
BNC9	-$(CH_2)_4$-		3-Thienyl	65.22 (65.3)	4.26 (4.33)	12 (12.02)

N-[(3,4-dimethoxyphenyl)methylidene]-5,6-dimethyl-2-phenylthieno[2,3-d]pyrimidin-4-amine(BNB6)

IR(KBr): 2934 and 2863 cm^{-1}(-CH), 1615 cm^{-1} (C=N); ^{1}H NMR(CDCl$_3$): 8.59(s, 1H, =CH), 8.28(d, 2H, Ar-H), 7.55(m, 3H, Ar-H), 7.4(m, 2H, Ar-H), 6.95(s, 1H, Ar-H), 3.83 (s, 6H, -OCH$_3$), 2.36(s, 3H, -CH$_3$), 1.93(s, 3H, -CH$_3$) ; MS(m/z): 403(M+)

2-phenyl-N-[phenylmethylidene]-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-amine (BNC1)

IR(KBr):2935 and 2864 cm^{-1}(-CH), 1615 cm^{-1} (C=N); ^{1}H NMR(CDCl$_3$): 8.36(s, 1H, =CH), 8.28(d, 2H, Ar-H), 7.83(s, 2H, Ar-H),7.52(m, 5H, Ar-H), 7.41(s, 1H, Ar-H), 2.83(m, 2H, -CH$_2$), 2.6(m,2H, -CH$_2$), 1.79(s, 4H, -CH$_2$) ; MS(m/z): 369(M+1)

N-[(4-bromophenyl)methylidene]-2-phenyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-amine(BNC3)

IR(KBr):2932 and 2866 cm^{-1}(-CH), 1615 cm^{-1} (C=N); ^{1}H NMR(CDCl$_3$): 8.59(s, 1H, =CH), 8.28(d, 2H, Ar-H), 7.72(s, 2H, Ar-H),7.58(d, 2H, Ar-H), 7.51-7.41(m, 3H, Ar-H), 2.83(m, 2H, -CH$_2$), 2.6(m,2H, -CH$_2$), 1.79(s, 4H, -CH$_2$); MS: 448(M+)

Antimicrobial Activity

All the synthesized compounds form BNB and BNC series were further evaluated for antibacterial activity against *Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escheresia coli* following disc diffusion method[7-9]. All the test and standard concentrations were prepared in DMSO. Streptomycin was taken as standard drug. Sterile filter paper discs were impregnated in sample or standard solutions and placed in the respective petri plates over the agar media which was previously sterilized and pre inoculated with test bacterial strains. Then plates were left for 30 min at room temperature and were further incubated at 37±2^{0}C for 24hrs. The extent of inhibition of bacterial growth was determined in terms of diameter of zone of inhibition and the results are presented as mean and standard deviation in table 3.

Antioxidant Activity

All the synthesized compounds were evaluated for antioxidant activity by studying their free radical scavenging property[10]. Stock solutions (1mM) of test and standard compounds were prepared in methanol. Further from the stock solution of each compound respective dilution were made in the range of 100nM to 1mM. To 2.8ml each dilution of test and standard compounds in separate test tube 0.2 ml

Table 3
Antibacterial activity of synthesized compounds

Compound		Diameter of zone of inhibition(mm) Mean ± SD			
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
BNB 1	500μg	13±1.3	14±2.4	13±0.8	13±1.1
	1000 μg	16±1.4	15±2.1	17±0.9	15±0.4
BNB 2	500μg	16±2.1	15±1.1	16±1.1	13±1.3
	1000 μg	18±0.9	16±0.9	17±1.2	17±1.1
BNB 3	500μg	16±3.1	13±0.8	13±1.4	13±0.9
	1000 μg	15±2.2	13±0.8	17±0.9	16±0.8
BNB 4	500μg	16±1.4	14±1.8	13±2.3	13±1.8
	1000 μg	18±1.3	17±1.7	18±1.1	14±0.1
BNB 5	500μg	16±1.0	17±0.8	13±2.2	13±2.1
	1000 μg	17±1.8	18±0.6	18±0.4	20±0.9
BNB 6	500μg	13±2.6	14±1.1	–	13±1.5
	1000 μg	18±2.3	16±1.2	17±1.0	17±1.0
BNB 7	500μg	13±1.1	14±1.4	16±1.3	13±0.9
	1000 μg	16±1.4	17±0.9	16±1.9	17±0.6
BNB 8	500μg	16±1.5	15±1.0	14±0.3	14±0.5
	1000 μg	18±1.8	19±1.0	18±1.0	19±0.4
BNB 9	500μg	16±2.1	15±1.1	14±0.8	16±0.9
	1000 μg	19±0.3	20±0.9	17±0.6	20±0.7
BNC 1	500μg	13±0.4	13±1.3	–	14±1.3
	1000 μg	16±3.4	16±0.9	17±0.6	18±1.0
BNC 2	500μg	16±2.7	15±1.1	14±0.7	16±1.2
	1000 μg	18±1.8	19±1.2	15±1.1	15±1.1
BNC 3	500μg	12±1.1	–	–	13±0.9
	1000 μg	14±0.2	14±1.1	15±0.7	16±0.8
BNC 4	500μg	13±0.3	12±1.0	12±0.6	13±0.9
	1000 μg	14±1.2	13±1.0	13±1.2	15±0.9
BNC 5	500μg	13±3.4	14±0.9	13±1.0	15±1.3
	1000 μg	16±2.1	17±0.8	16±1.0	18±1.0
BNC 6	500μg	12±2.1	14±0.3	13±0.9	15±1.0
	1000 μg	15±1.2	14±1.1	16±0.9	17±0.9
BNC 7	500μg	13±1.0	14±0.8	15±0.6	14±0.6
	1000 μg	16±3.0	16±0.7	17±1.1	18±0.5
BNC 8	500μg	16±1.3	15±0.3	16±0.9	16±0.4
	1000 μg	19±1.2	20±1.0	21±0.8	20±0.9
BNC 9	500μg	14±1.4	16±0.9	16±0.8	16±1.3
	1000 μg	18±0.5	19±0.4	20±0.3	21±0.9
Streptomycin	100 μg	23±0.3	24±0.4	21±0.2	22±0.5

of 0.05mM of DPPH solution was added and wrapped with aluminum foil. Immediately the absorbance of test and standard solutions were measured at 527nm. Percentage of free radical inhibition of test and standard compounds was determined by comparing with blank absorbance and IC_{50} values were determined. The IC_{50} values in µM are presented in table 4.

Results and Discussion:

The anti bacterial activity results of 5,6-dimethyl-2-phenyl[2,3-d]pyrimidin-4-amine(BNB) series and 2-phenyl-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-amine(BNC) series are presented in table 3. Thieno pyrimidines with para N,N-diethyl amino phenyl (BNB5), 3-indolyl (BNB8, BNC8) and 3-thienyl (BNB9, BNC9) substitutions exhibited potent activity against all the tested bacterial strains than the other compounds of this series. Although the antibacterial activities of tested compounds are not comparable with the standard drug Streptomycin but they exhibited moderate to potent activity at high concentrations. Compounds BNB2, BNB4, BNB6 and BNB7 were exhibited good antibacterial activity, where as compounds with phenyl (BNB1) and para bromo phenyl (BNB3) substitutions were weakly active. Compounds with phenyl (BNC1), para N,N-diethyl amino phenyl (BNC5), para 3,4-dimethoxy phenyl (BNC6) and para methoxy phenyl (BNC7) were found to show good antibacterial activity. Whereas compounds with para bromo phenyl (BNC3) and para-N,N-dimethyl amino (BNC4) were moderately active in all the tested bacterial strains.

The antioxidant data of compounds BNB$_{1-9}$ and BNC$_{1-9}$ are presented in the table 4. The results revealed that compounds of this series with para N,N dimethyl amino group (BNB4), simple phenyl group (BNB1) showed highest antioxidant activity with IC_{50} values 9 µM and 10 µM respectively. Whereas compounds with para chloro phenyl (BNB2), para bromo phenyl (BNB3), 4-methoxy phenyl(BNB7) and 3-thienyl (BNB9) groups showed moderate antioxidant activity in the range 11 to 14 µM. But compounds with para N,N diethyl amino phenyl (BNB5), 3,4-dimethoxy phenyl (BNB5) and 3-indolyl (BNB8) groups showed weak antioxidant activity.

Compounds of BNC series with 3-thienyl (BNC9) group found to be more potent followed by compound with para bromo phenyl (BNC3). Whereas compounds with phenyl (BNC1), para chloro phenyl (BNC2), para methoxy (BNC7) and and N,N-dimethylamino phenyl (BNC4) showed good antioxidant activity with IC_{50} values ranging from 12-13 µM. However compounds containing para N,N-diethylamino phenyl (BNC5), 3,4-dimethoxy phenyl (BNC6) and 3-indolyl (BNC8) groups showed weak antioxidant activity than the other compounds in the series.

Conclusion

From the present investigation it could be concluded that compounds with 3-indolyl (BNB8 and BNC8), 3-thienyl (BNB9 and BNC9) and N,N-diethyl amino phenyl (BNB5) exhibited potent antibacterial activity and compounds BNB1, BNB4 and BNC9 were showed potent antioxidant

Table 4

Antioxidant activity of synthesized compounds

Compound	IC_{50} Value (µM) (Mean ± SD)	Compound	IC_{50} Value (µM) (Mean ± SD
BNB1	10± 0.5	BNC1	13 ± 0.3
BNB 2	13± 0.3	BNC 2	12 ± 0.4
BNB 3	14± 0.2	BNC 3	11 ± 0.4
BNB 4	9± 0.4	BNC 4	14 ± 0.5
BNB 5	16± 0.4	BNC 5	17 ± 0.4
BNB 6	18± 0.3	BNC 6	17 ± 0.3
BNB 7	11± 0.1	BNC 7	13 ± 0.2
BNB 8	20± 0.1	BNC 8	18 ± 0.3
BNB 9	12± 0.4	BNC 9	10 ± 0.4
Ascorbic Acid(standard)		7.1 ± 0.1	

activity. Therefore these compounds could be considered as a new lead molecule for the development of newer class antibacterial and antioxidant agents.

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Evaluation of Hepatoprotective Activity of *Borago Officinalis* by Carbontetrachloride Induced Hepatotoxicity

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ABSTRACT

The hepatoprotective effect of ethanolic extract of leaves of *Borago Officinalis* (Boraginaceae) against carbon tetrachloride (CCl_4) induced hepatic damage were evaluated. The degree of protection was determined measuring levels of serum marker enzymes like Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatise (ALP), Total and direct bilirubin and liver weight of rat. The histopathological studies were also carried out. Silymarin was used as standard drug for comparisions. Administration of ethanolic extract of *Borago Officinalis* (50, 100 mg/kg p.o) markedly decrease CCl_4 induced elevation levels of Serum marker enzymes and liver weight in dose dependent manner. The effects of extract was compared with standard, Silymarin at 100 mg/kg dose. In ethanolic extract treated animals, the toxic effect of CCl_4 was controlled significantly by restoration of the levels of enzymes as compared to the normal and standard drug silymarin treated groups. Histology of the liver sections of the animals treated with extract showed the presence of liver cells, absence of necrosis and fatty infiltration, which further evidenced the hepato protective activity. It was concluded that Ethanolic extract of leaves of *Borago Officinalis* possesses significant hepatoprotective activity.

Key words: *Borago Officinalis*, Carbon tetrachloride, Hepato protective activity, Serum marker enzymes.

Introduction

Liver plays a major role in excretion and detoxification of many endogenous and exogenous compounds. It is one of the most important first organ to encounter ingested nutrients, drugs and environmental toxicants that enter hepatic portal blood, so regulate important metabolic function [1]. There were several diseases which can affect the liver are Wilson's disease, Hepatitis, Liver cancer and Cirrhosis, Whereas alcohol consumption alters the metabolism of the liver, Some medications have side effects that may harm liver are anticancer drugs (Eg: Methotrexate) [2]. *Borago Officinalis* (Family: Boraginaceae) or Borage also called as star flower. It grows mostly only on rubbish heaps and near dwellings. In milder climates, borage will bloom continuously for most of the year [3]. The primary chemical constituents of Borage leaves and flowers include mucilage, tannin, saponins, essential oil, alkaloid (pyrrolizidine), vitamin C, calcium and potassium. The plant reported to contain essential fatty acids, linoleic acid and gamma-linolenic acid. Traditionally the leaves of the plant *Borago Officinalis* were used as Anti-rheumatic, diuretic, Nervine, to treat jaundice, as anti inflammatory and relieves skin complaints [4,5,6]. Inspite of its reported use, there are no systematic clinical experimental studies have been carried out to posses the hepato protective activities of this species. Hence an effort has been made to evaluate the hepato protective effect of the ethanolic extract of leaves against CCl_4 induced liver damage in rats.

Methods

Preparation of the extract

The leaves of *Borago Officinalis* was collected from local area of Hyderabad and authenticated by Botanist Dr. K.Madhava Chetty, S.V University, Tirupathi. The leaves were shade dried and powdered and extracted with 95 % ethanol for 48 hrs in soxhlet apparatus. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator. The extract was subjected to qualitative phytochemical screening for the identification of phytoconstituents.

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Animals

Albino rats (160-180 g) of either sex were used and were maintained at standard housing conditions. The animals were fed with standard rodent diet and provided water *ad libitum* during the experiment. The study was permitted by the Institutional Animal Ethical Committee (IEAC) approved the use of animals for the present study, Ethical clearance number: IEAC/SUCP/03/2009.

Hepato Protective Activity

The animals were divided into 5 groups of 6 animals each

Group A served as normal control and received subcutaneous administration of liquid paraffin (L.P) only 3 ml/kg on alternate days for one week. All other groups **B, C, D** and **E** received Carbon tetrachloride CCl_4 (1ml/Kg) subcutaneously in the lower abdomen in a suspension of L.P in the ratio 1:2 v/v on alternate days for week.

Group B Animals were maintained as Carbon tetrachloride group.

Group C Animals were treated with Silymarin 100 mg/kg [7] orally for 7 days.

Group D and **E** Animals were treated with Ethanolic extract of *Borago Officinalis* 50 and 100 mg/kg orally respectively for 7 days.

After drug treatment all the animals were sacrificed, blood was collected by puncturing the retro orbital plexus and was allowed to clot for 45 min at room temperature, serum was collected by centrifugation at 2500 rpm for 15 min, used for estimation of various bio-chemical parameters [8, 9] .

Assessment of Liver function

Bio-chemical parameters such as Serum Glutamate Oxalaocetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) [10], Alkaline Phosphatase (ALP) [11] and Serum Bilirubin [12] were determined.

Statistical Analysis: Results are expressed as mean ± SEM. Statistical analysis was performed with one-way ANOVA using Tukeys test.

Results

The results obtained from various parameters are summarized in the tables given below.

The values are expressed as Mean±SEM, n = 6 in each group. Significance at $^a p<0.001$, $^b p<0.01$, $^* p<0.05$ when compared to control. Significance at $^c p<0.001$, $^d p<0.01$ when compared to stress control. Statistical test employed is ANOVA followed by Tukeys test.

Table - 2

Effect of Ethanolic extract of *Borago Officinalis* (BO) on Liver weight in Carbon tetrachloride induced Hepatotoxicity in Rats

Groups	Liver Weight (gm)
A-Control	6.02 ±0.14
B-CCl_4	10.26 ±0.02^b
C- CCl_4+ Silymarin	5.85 ±0.26^a
D- CCl_4+50 mg/kg of BO Extract	7.01 ±0.05
E- CCl_4+ 100 mg/kg of BO Extract	7.22 ±0.01^a

Values are expressed as Mean±SEM (n=6) $^a p<0.01$, $^b p<0.001$ compared to control group.

Table - 1

Effect of Ethanolic extract of *Borago Officinalis* (BO) on Carbon tetrachloride induced Hepatotoxicity in Rats

Group	SGPT (IU/L)	SGOT (IU/L)	ALKP (KA Units)	BILIRUBIN TOTAL (mg %)	BILIRUBIN DIREC (mg %)
A-Control	46.76±3.252	49.23±4.27	30.05±3.17	0.98±0.11	0.19±0.02
B-CCl_4	120.47±5.07^b	130.8±2.862^a	74.61±1.9^a	2.80±0.1^a	0.38±0.02^a
C- CCl_4+ Silymarin	72.5±3.88^{ac}	85.16±5.07^{ac}	48.26±3.78^{ac}	1.3±0.09^c	0.28±0.01*
D- CCl_4+ 50 mg/kg of BO Extract	118.1±3.6^a	127.8±3.93^a	76.2±1.29^a	2.06±0.09^a	0.35±0.02^a
E- CCl_4+ 100 mg/kg of BO Extract	107.02±4.86^a	113.9±5.4^a	65.63±2.57^a	1.74±0.06^{bc}	0.32±0.02^b
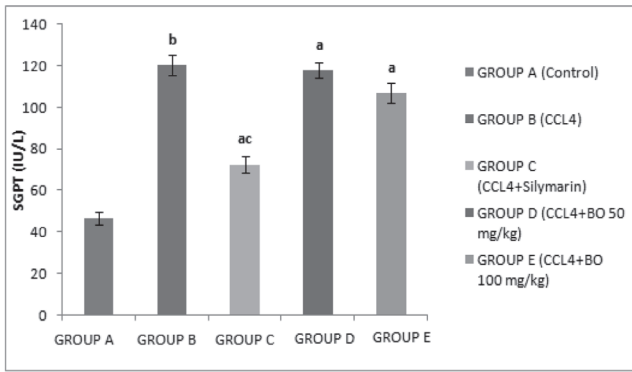
Fig. 1: Effect of *Borago Officinalis* on SGPT levels in CCl₄ induced hepatotoxicity in rats
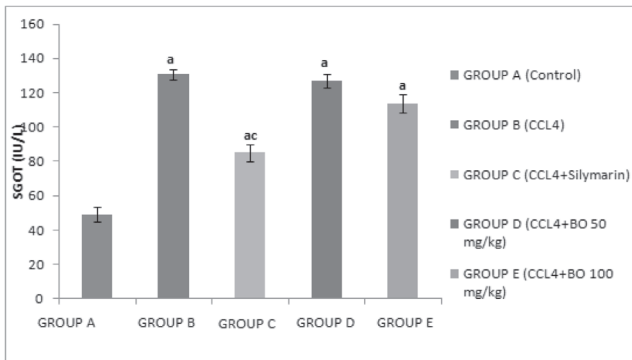
Fig. 2: Effect of *Borago Officinalis* on SGOT levels in CCl₄ induced hepatotoxicity in rats
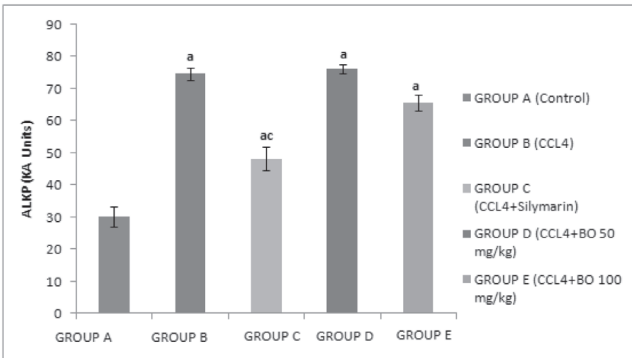
Fig. 3: Effect of *Borago Officinalis* on ALKP levels in CCl₄ induced hepatotoxicity in rats
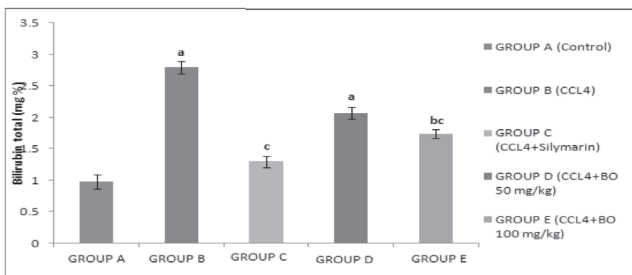
Fig. 4: Effect of *Borago Officinalis* on Total Bilirubin levels in CCl₄ induced hepatotoxicity in rats
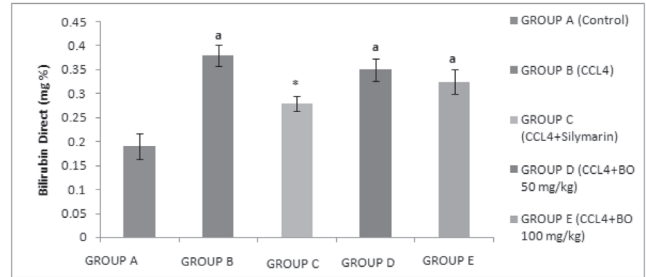
Fig. 5: Effect of *Borago Officinalis* on Direct Bilirubin levels in CCl₄ induced hepatotoxicity in rat

The values are expressed as Mean±SEM, n = 6 in each group. Significance at [a]$p<0.001$, [b]$p<0.01$, *$p<0.05$ when compared to control. Significance at [c]$p<0.001$, when compared to stress control. Statistical test employed is ANOVA followed by Tukeys test.

Fig.6 : Effect of Ethanolic extract of PV and BO on CCl₄ induced Histopatholgical changes in rat liver
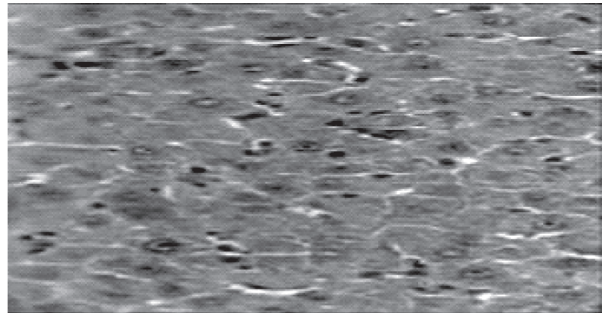
Fig.6a: Histopathological liver section of Normal Control rat: Liver section showing normal hepatic cells
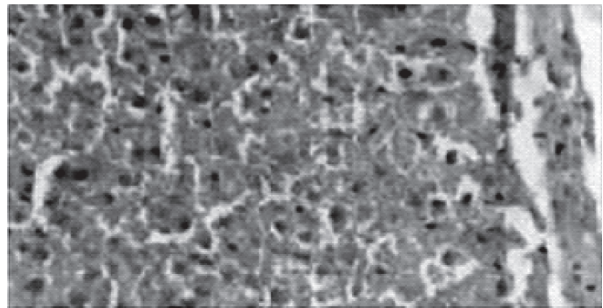
Fig.6b: Histopathological liver section of CCl₄ treated rat: Liver section showing focal necrosis, Fatty degeneration and vacuolization
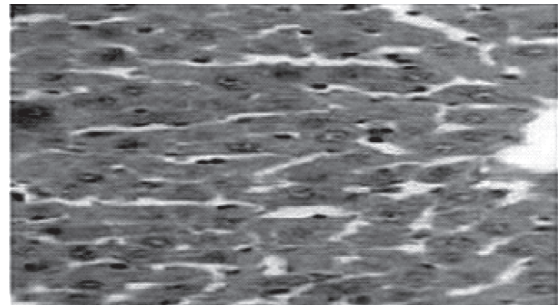
Fig.6c: Histopathological liver section of Silymarin treated rat: Liver section showing normal hepatocytes and their lobular architecture was normal
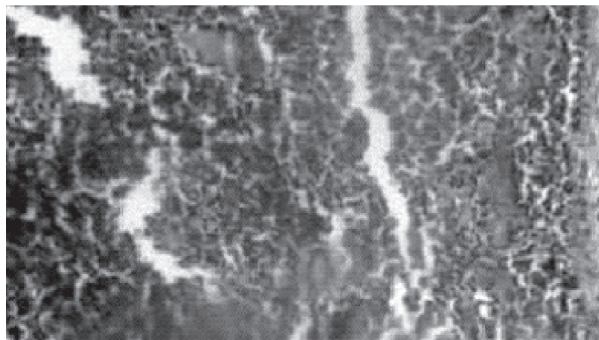
Fig.6d: Histopathological liver section of BO extract 100 mg/ kg treated rat: Liver section showing typical lobular arrangement and few hepatocytes shows steatotic accumulation

Phytochemical Screening

The preliminary phytochemical tests indicate that the following Chemical constituents alkaloids, sugars, saponins, tannins, phenols and flavanoids are found to be present in the ethanolic extract of leaves of *Borago Officinalis*.

Effect of *Borago Officinalis* extract on Biochemical parameters and liver weight

Increased levels of SGPT, SGOT, ALP, Total and Direct bilirubin and liver weight were observed in CCl_4 treated group. The treatment with *Borago Officinalis* decreases the elevated manner levels of biomarker enzymes of liver to the near levels in a dose dependent manner. The changes in biochemical markers are shown in Table 1 compared to standard drug Silymarin 100 mg/kg.

Histopathological Observation

Histopathological study of liver from **Group A** animals showed a normal hepatic cells **(Fig 6a)**. In CCl_4 treated group, severe hepatotoxicity was evidenced by profound central lobular fatty degeneration, focal necrosis and vacuolization **(Fig 6b)**. In **Group E** animals, the liver exhibited an typical lobular arrangement, few hepatic cells shows fat accumulation, represents moderate protection in CCl_4 induced liver damage **(Fig 6e)**. In **Group C** animals showed significant protection to considerable extent as evident from the formation of normal hepatocytes and their lobular architecture was normal.

Discussion

The CCl_4 has been used as hepatotoxin to induce hepatotoxicity in experimentals. This toxic chemical caused per oxidative degradation in the adipose tissue resulting in fatty infiltration of hepatocytes. The increase in the levels of serum bilirubin reflects the depth of jaundice and increase in transaminases and alkaline phosphatase was the clear indication of cellular leakage and loss of functional integrity of the cell membrane. The changes associated with CCl_4 induced liver damage are similar to that of acute viral hepatitis.

The CCl_4 is biotransformed by the cytochrome P_{450} system to produce the trichloromethyl free radical, which inturn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca^{2+} homeostasis and finally result in cell death [13]. Estimating the activities of serum marker enzymes, like SGOT, SGPT, ALKP, Direct and Total bilirubin can make assessment of liver function. When liver plasma membrane is damaged, a variety of enzymes normally located in the cytosol were released into the blood stream. Their estimation in the serum was a useful quantitative marker of the extent and type of liver damage. Hepatocellular necrosis leads to very high level of biomarker enzymes and bilirubin released from liver in the blood. Reduction in the level of SGOT, SGPT enzymes towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. Suppression of increased ALP activity with concurrent depletion of raised bilirubin level suggests the stability of the biliary dysfunction in rat liver [14]. The ethanolic extract of *Borago Officinalis* decreases the CCl_4 induced elevated enzyme levels in dose dependent manner. It represents the ethanolic extract at 100 mg/kg showed the protection of structural integrity of hepatocyte cell membrane and helps in the regeneration of damaged liver cells. The effectiveness of the normal functioning condition of the liver was indicated by the decreased levels of serum bilirubin. The results suggest that leaves of *Borago Officinalis* prevent the formation of fatty liver comparable to Silymarin used as hepatoprotective agent. A complete histopathological study of liver from different groups further corroborated the hepatoprotective efficacy of *Borago Officinalis.* Further work is in progress to isolate and purify the active principle involved in hepatoprotective activity.

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Optimization of Fermentation Conditions for the Production of Superoxide Dismutase from *Saccharomyces mellis*

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ABSTRACT

This work is sought to optimize fermentative process for the microbial production of superoxide dismutase (SOD), to overcome extraction from animal tissues. Five wild type yeast strains were screened for SOD productivity on various carbon sources such as Dextrose, Maltose, Lactose and Sucrose at 30^0C with p^H 6.0.

S mellis showed the highest SOD activity 59.0 I U Mg-1 when Sucrose was the carbon source and 55.0 U Mg-1, when Maltose was used as Carbon Source. Parameters such as p^H, temperature and various carbon sources were studied during fermentative cycles. Growth and productivity on different carbon sources were compared specific activity was higher on sucrose than on Dextrose or Maltose or Lactose. Similarly highest Biomass yield was achieved on Sucrose. Therefore it may be the best substrate for SOD production from *S.mellis*.

Introduction

Reactive oxygen species such as Superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and Hydroxyl radicals (OH^-) are toxic by products of oxidative metabolism, since they are produced by the respiratory chain, by H_2O_2 generating oxidases or during stress conditions exposure to ionizing radiations or pro-oxidants such as H_2O_2, paraquat or menadione is also a source of ROS and leads to oxidative stress. Since ROS exert cytotoxic and mutagenic effects by peroxidation of the membrane fatty acids, protein oxidation and DNA damage, all aerobic and aerotolerant organisms have evolved defense mechanisms to prevent or repairs ROS mediated oxidative damages. Detoxification of ROS depends upon low molecular weight antioxidants and Enzymes, such as superoxide dismutase (SOD), catalase (CAT), Glutathione Reductase(GR) and several peroxidases. SODs are metalloenzymes that detoxify superoxide radicals by conversion to hydrogen peroxide radicals and oxygen. A wide range of therapeutic applications of SOD have been described. These include prevention of oncogenesis and tumor promotion [19], protection of tissues following infective ischemic, traumatic of burn injuries (1,7,23-25) and treatment of inflammatory diseases and arthritis [1,6,26], in addition to clinical purposes, SOD have been increasingly used in the last decade for the production of antioxidant cosmetics and dietary supplements such as capsules, tablets, milk, beer and beverages.

Since SOD is now a days produced by extraction from animal tissues, mostly bovine liver or erythrocytes, a microbial production process could represent a significant improvement in terms of yields, costs, purity and product safety. Some efforts had been already made forward production of SOD with filamentous fungi (1,1) but large scale biotechnological processes have never been followed industrially. The utilization of GRAS [Generally regarded as safe] yeasts would prove very attractive, for industrial-scale fermentative production of food or pharmaceutical grade SOD.

Like most other eukaryotes, yeasts produce 2 SODs that protect mitochondrial and cytosolic constituents from oxidation the copper and zinc containing SOD (Cu-ZnSOD) is encoded by SOD1 and accounts for up to 90% of the total SOD. CuZn-SOD is primarily located in the cytosol, though a minor amount has been found in the mitochondrial intermembrane space [18,22,17]. The manganese containing enzyme [Mn-SOD] encoded by the gene SOD2, is located in the mitochondrial matrix where it operates as the major superoxide scavenger, accounting for 5-15% of the total SOD(15,17).

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The regulation of oxidative stress defences in yeasts has been deeply studied and many factors affecting the expression of SOD1 and SOD2 genes have been characterized. Respiratory metabolism, glucose depletion and oxidative or osmotic stress were found to be involved in SOD increase [5,9-13,17,20-22]. Nevertheless, yeasts have not yet been proposed for the industrial production of SOD. The aim of this work is the development of a fermentative process for the production of SOD by yeasts.

A preliminary screening of 5 wild type yeasts strains sought to identify the yeast with the highest SOD activity to be used for the process optimization. Specific activity was studied through fermentative experiments in 3 lit bioreactor with 1 liter working volume. Data concerning yield improvements in pilot scale bioreactor are reported.

Materials and Methods

Yeast strains and maintainance

Yeast strains were obtained from NCIM, pune. baker's yeast were obtained from commercial suppliers NICE chemicals, India yeast cultures were maintained on YPD agar slants.

Chemicals and Media

All chemicals were obtained from SD fine chemicals (Mumbai,India) Microbiological products were purchased from Ginie India Ltd. Complex YPD medium contained; Dextrose $20gl^{-1}$, yeast extract $10gl^{-1}$; Bactopeptone,$20gl^{-1}$; Autoclaved for 30min at 110^{0}C. Other 3 sets of YPD was prepared in which Dextrose was replaced with Maltose, Sucrose and Lactose at the concentration of $20gl^{-1}$. The carbon source was autoclaved separately and added into the cultural medium.

Shake Flask Cultivation

Batch cultures were carried out in 500ml Erlenmeyer flasks with 100ml sterile medium flasks were inoculated with 10% V/V exponential phase cultures of 24 hours age were incubated at 30^{0}C for 36 hours in an orbital shaking incubator at p^{H} 5.5 and 6.0 at 180 rpm, then broths were harvested for biomass measurement and SOD analysis.

Bioreactor cultivation

Batch and Bioreactor experiments were performed in a Eastbio GUCS-3 bioreactor (SIEMENS PLC,India). The fermentor was stirred by 2 rushton turbines and was sparged using compressed air via a ring shaped sparger at the base of the vessel. The bioreactor was equipped with Inpro 6800 and Inpro 3030 (SIEMENS PLC) for the continuous measurement of p^{H} foaming was controlled by the automatic addition of PPG 2000 (Cyanamid, Catania, Italy). Temperature was kept to 30^{0}C.

A 1.0 Lit working volume was used for batch cultures. Exponential phase seed cultures grown in the same medium inoculated (10% V/V) the bioreactor p^{H} was controlled at 6.3 by automatic titration with 4M NaOH. Stirring was kept to 500rpm, air flow to 1.0VVM (Volume/Volume/Minute) and pressure to 0.3 bar. When a dot cascade regulation was applied, stirring and air flow were automatically adjusted in the range of 200-900rpm and 0.5-2.0VVM.

Preparation of cell free extracts

Biomass was harvested by centrifugation (5000 X g for 10min at 4^{0}C), washed twice with PP buffer (potassium phosphate buffer) p^{H} 5.5 and p^{H} 6.0 50mm; EDTA, 0.1mM) and re suspended in the same buffer 1:1 W/V. Cells were disrupted for 30 min at 4^{0}C by 0.3mm glass beads in a vibration homogenizer at 1800 rpm. whole cells and debris were removed by centrifugation at 13000xg for 15min at 4^{0}C and the supernatant was dialysed for 16h against PP buffer. Protein concentration was assyed according to lowry (14) using bovine serum albumin as standard.

Enzyme activity

SOD activity was assayed by measuring the inhibition of Epinephrine autoxidation, as described by misra and Fridovich (16). Bovine SOD (Sigma aldrich) was used as standard for the calibration curve in the range between 0 and $10Uml^{-1}$. One unit is defined as the amount of SOD that inhibits by 50% the reduction of nitro blue tetrazolium (3). Specific activity of cell free extracts was expressed as enzymatic units per mg of protein. Cu Zn SOD was inactivated by adding 30mm KCN into the reaction mixture in order to discriminate Mn-SOD activity. Cu Zn SOD activity was calculated as difference between total and Mn-SOD acivities.

Screening of Yeats

SOD production was analyzed for 5 wild type yeast strains, after 36 h shake flask cultivation on YPD medium. SOD specific activities are reported in Table 1. The highest activities were measured in the cell free extracts of *S mellis* ($59.0U$ mg^{-1}, 55.0 u $mg^{-1)}$), followed by *S ludwiggi* (47.0 u mg^{-1}, 41.0 u mg^{-1}), *K lactis* (13.0 u mg^{-1}, 10.0 u mg^{-1}), Baker's yeast (11.0 u mg^{-1}, 7.0 u mg^{-1}) and *P fermentans* (5.0 u mg^{-1}, 3.5 u mg^{-1}).

Batch Cultures of *S mellis*

Biomass concentration and SOD specific activity were monitored during p^{H} regulated batch fermentation of *S mellis* in order to study growth and production Kinetics. The cultures grew on YPD where in one Set Dextrose was replaced with Sucrose and P^{H} adjusted to 6.0 and in another set Dextrose is replaced with Maltose at p^{H} 6.0. A peak in SOD specific activity was observed during the early exponential phase, increased again. The maximum Specific activity 50.0 g u mg^{-1} and 55.0 u mg^{-1} was observed.

Table - 1

SOD specific activity of 5 wild type yeast strains measured on cellular extracts after a 24 h growth in YPD medium containing 10 g L^{-1} Dextrose, Sucrose, Maltose and Lactose

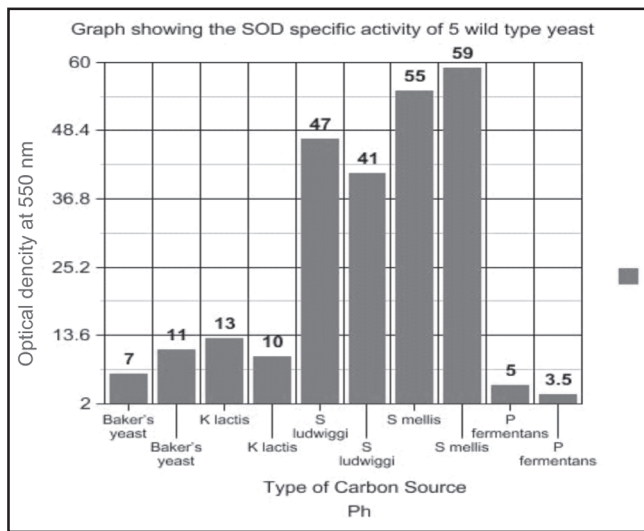| Yeast Strain	Carbon Source	pH	SOD u mg^{-1}
Baker's yeast	Maltose	5.5	7.0
Baker's yeast	Dextrose	5.5	11.0
K lactis	Sucrose	6.0	13.0
K lactis	Lactose	6.0	10.0
S ludwiggi	Sucrose	5.5	47.0
S ludwiggi	Dextrose	5.5	41.0
S mellis	Maltose	6.0	55.0
S mellis	Sucrose	6.0	59.0
P fermentans	Sucrose	6.0	5.0
P fermentans	Dextrose	6.0	3.5

Fig.1 : Graph showing the SOD specific activity of 5 wild type yeast strains

The Results are mean values from three separate experiments (standard Deviation always less than 10%).

After 12 h, at the transition between exponential and stationary phases, achieving $140 \times 10^3 uL^{-1}$, corresponding to $11 \times 103 u(Lh)^{-1}$ as the maximum volumetric productivity. The trend of specific activity was measured after 20 h. In both Sucrose and Maltose carbon sources SOD specific activity was maximum with S mellis. The initial peak in SOD activity decreased during the exponential phase. Characterization of SOD activity of *S mellis* demonstrated that cuzn-SOD accounted for more than 9.5% of total activity, independent of the growth phase and the culture medium.

Effects of carbon source

Shake Flask Cultures of *S mellis, S ludwiggi, P fermentancc, K lactis* and Baker's yeast were carried out using Dextrose, Maltose, Sucrose, Lactose to monitor Biomass concentration and SOD specific activity throughout exponential and stationary phases. Significant differences were observed in biomass yield coefficients. A maximum of 1.08 (O.D) Biomass yield was obtained on Sucrose for S mellis, similarly SOD Specific activity was also maximum, which is found to be 59.0 u mg^{-1}. Compared to all the other carbon sources, Sucrose gave the highest Biomass yield and maximum SOD specific activity.

The next highest maximum Biomass productivity was seen on Maltose 1.01 (OD) for Baker's yeast but SOD specific activity was significantly lower with Baker's yeast, both on Maltose and Dextrose. Biomass productivity was moderately good with *S ludwiggi, K lactis P fermentans* such as 0.80, 0.91, 1.02, respectively. SOD specific activity is considerably lower with these yeast stains namely 47.0, 13.0, 5.0 respectively.

Discussion

SOD detoxifies Superoxide radicals origination from respiratory metabolism and oxidative stress conditions. Hence, SOD from bovine liver and Red Blood cells is increasingly used for pharmaceutical applications and as a component of antioxidant dietary supplements. This work is aimed to develop a fermentative process for the production of SOD, in order to overcome extraction from animal cells & tissues. Data pointed out the parameters that affect SOD production in *S mellis,* Baker's yeast, *P fermentans, S ludwiggi, K lactis* and represent a contribution toward application of yeasts for the biotechnological production of SOD. 5 wild type yeast strains were preliminarily screened for SOD production. *S mellis* yields the highest SOD activity (59.0 u mg^{-1}) and was used for the process development.

Most information on SOD production in yeasts came from studies on *S cerevisiae*, stating that it was generally associated to the respiratory metabolism, as a consequence of ROS released in the mitochondrial electron transfer chain. In fact, the synthesis of CuZn-SOD and Mn-SOD was repressed during exponential growth of aerobic batch cultures of *S cerevisiae*, growing mainly by fermentation [5,17]. Enzyme activities increased during the diauxic shift, when respiratory adaptation, occurred between Ethanol production and consumption, and at the stationary phase, when all energy sources were depleted. Oxygen limitation also decreased the translation of both SOD 1, SOD 2 genes and resulted in low activities during the exponential phase in *S cerevisiae* (g). In stationary phase, *S cerevisiae* mitochondria exhibited a burst of ROS production, and SOD were involved in protecting against aging process and stationary phase death [13, 22]. Moreover, past

translational changes were involved in increasing SOD activity at the shift between fermentative and respiratory phases, since O_2 or Superoxide were required for activation of CuZn –SOD apoprotein by ccs, the Copper chaperone for SOD 1 in *S cerevisiae* (4,8).

During batch fermentations of *S mellis* high specific activities were observed at the early exponential phase and at the onset into stationary phase. The sudden increase of SOD, as a response to the enhanced oxygen availability, could be consistent with a mechanism of post translational activation of an existing apo-pool of CuZn –SOD [4].

S mellis

Growth and productivity curves on YPD with Maltose, Dextrose and YPD with Sucrose at pH 6.0 were compared. Both specific activity and volumetric productivity were 5 fold higher on Sucrose, later followed by Maltose when compared to Dextrose. Hence Sucrose was used for further process optimization.

S ludwiggi

Mean while Biomass production and SOD specific activity values were compared on YPD with Dextrose, Sucrose at p^H 5.5 at 30^0c with this case also Sucrose is found to give good Biomass yield where as SOD Specific activity was quite less when compared to *S mellis* but it was Noticed that SOD Specific activity was increasing with increasing Biomass productivity. In fact similar process was observed in *S mellis* too.

Baker's yeast, K lactis, P fermentans

Biomass yield of Baker's yeast, *K lactis, p ferments* was higher on Maltose, Sucrose and Destrose respectively. But SOD Specific activity was significantly lesser in all these 3 wild type yeast strains with these 3 wild type yeast strains, SOD productivity was not directly proportional to the Biomass yield.

Whereas with *S mellis* and *S ludwiggi*

SOD specific activity was directly proportional to the Biomass yield. Among *S mellis* and *S ludwiggi*, the later (*S mellis*) was found to be the yeast type which gives Maximum SOD yield on Sucrose and later followed by Maltose with p^H 6.0 at 30^0c. This could be recommended for Industrial scale SOD production.

A comparison on different carbon sources was established. The substitution of glucose with Lactose, Maltose, Sucrose and Fructose as the sole source of Carbon requirement caused the maximum SOD specific activity to increase. Glucose being the most common carbon source in all Microbiological experiments, was expected to give highest biomass yield and SOD Specific activity. But substantially Sucrose later followed by Maltose was found to give maximum Biomass yield and also SOD specific activity in all 5 wild type yeast strains. It was found that

least Biomass yield and SOD productivity was given by Fructose – yeast extract – Peptone media. Sucrose is the best substrate to be used in a SOD producing process.

The results of this study provide a better understanding of the parameters which affect SOD production in *S mellis*, *S ludwiggi*, Baker's yeast, *K lactis* and *P fermentans.*

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Rheological Studies of Poly (Ethylene Glycol) in Water, Aqueous Dimethyl Sulphoxide and Dimethyl Sulphoxide

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ABSTRACT

The rheological studies, the viscosity, the relative viscosity, reduced viscosity and intrinsic viscosity of poly (ethylene glycol) with molecular weight 6000 in pure H_2O, H_2O + DMSO (5O%, V/V) and pure DMSO at various concentrations and temperatures, 30, 35, 40, 45^0 C are measured. The results have been used to calculate the Huggin's constant 'K' and Hydrodynamic Volume 'Ve'.

Key words:- Viscosity, Poly (ethylene glycol), relative viscosity, reduced viscosity, intrinsic viscosity, Huggin's constant, Hydrodynamic volume.

Introduction

The dependence of the viscosity of polymer solutions on concentration and molecular weight is generally important for processing and applications of polymers (1). However, dilute solutions characterization should be an integral part of any programme for the development of new polymers or applications and for modification of existing ones (2). Application of polymers as drag-reducing additives is normally done in the dilute solution state.

This paper reports the findings of an experimental investigation on the flow time changes occurring during viscometric measurements of different concentrations of dilute solutions of PEG at different temperatures in water, aqueous DMSO and in pure DMSO.

Staudinger, during the early 1930s, used viscosity as a measure of the molecular weight of the polymer to postulate his hypothesis about the long chain nature of polymer molecules, the proposed the relationship.

$$\eta Sp = K_S CM \qquad \dots (1)$$

Where K_S is a constant for a given polymer/solvent temperature, C is the concentration, M is molecular weight, and ηSp the specific viscosity denoting the increase of viscosity of a polymer solution over that of the pure solvent according to the relation

$$\eta s_p = \frac{\eta - \eta_o}{\eta} \qquad \dots (2)$$

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Where

η = viscosity of the polymer solution

$\eta_{0 =}$ viscosity of the solvent.

From the equation (1) it is clear time that the specific viscosity is dependent on the concentration.

In order to quantify a viscosity function of a polymer in a solvent, which will be independent of the concentration, the limiting value of reduced viscosity (ηS_p/C) or that of the inherent viscosity (1n ηr/C) at infinite dilution is chosen and termed intrinsic viscosity of limiting viscosity number [η]:

$$(\eta Sp/C) \ c \rightarrow c = [\eta] = (1n \ \eta V/C) \ c \rightarrow o \ \dots (3)$$

The term [η] has been related to the two viscosity functions through the following two equations by Huggins and Kraemer, respectively:

$$\frac{\eta Sp}{C} = [\eta] + K^1 [\eta]^2 C \qquad \dots (4)$$

$$\ln \eta r/c = [\eta] - K^{11} [\eta]^2 C \qquad \dots (5)$$

Where $K^1 + K^{11}$ has been shown to be 0.5.are constants for a given polymer/solvent/temperature system. For many linear flexible polymer systems, K often indicates the measure of the solvent power. The poor the solvent, the higher the values of K^1 .The value of $K^1 + K^{11}$ has been shown to be 0.5.

$$\frac{\eta Sp}{C[\eta]} = Exp [K^1 [\eta] C/1-bc] \qquad \dots (6)$$

Where b is a constant. The values of K^1 and $[\eta]$ for a polymer-solvent system can be found out using equation (4).

Viscosity as a function of molecular weight and concentration can be expressed by the equation.

$$\eta = K^* \, [C^\alpha / \beta_M]^{3,4} \qquad \qquad …(7)$$

Where K^* is a characteristic constant for a polymer-solvent system and is a temperature dependent. Its value is roughly in the order of 2.0×10^{-15} when C is expressed in g/ml and η in poise. The value of α/β ranges from 1.38 to 1.85 with an average value of 1.5.

Experimental Methods

Poly (ethylene glycol) with molecular weight 6000 supplied by BDH Chemical Ltd. (Poole, England) was used. Dimethyl sulphoxide (Merck, Proanalysis) was used after necessary purification (3).

All solutions were prepared on a weight basis to an accuracy of 0.0005 g, using water from a high efficiency dilution system, i.e. triple distilled water, pure water, water + DMSO (50%, V/V) and pure DMSO were used as solvents. In the present investigation, the densities of the solutions were determined using pycnometer. The experiment was repeated till reproducible results with an accuracy of two in 10^4 parts was obtained for water and DMSO.

Viscosity measurements were made with an Ostwald's type viscometer by measuring flow times for the above solutions. The reported viscosity data was reproducible within $+ 0.3\%$. Measurements were repeated until differing only by $+ 0.03$ were obtained. No kinetic energy corrections were made because the flow times of the pure solvents were at least 100 sec. The bath temperature was controlled to $+ 0.05^0C$.

RESULTS AND DISCUSSIONS

The viscosities, relative viscosities and reduced viscosities of PEG (Mol. Wt. 6000) in H_2O, H_2O + DMSO 950%, V/V) and pure DMSO were determined at various concentrations and temperatures 30, 35, 40 and 45^0C respectively.

Figs. 1-3 illustrate the variation of viscosity with concentration of the polymer in HH_2O, H_2O + DMSO (50%, V/V) and DMSO at the above mentioned temperature respectively. The viscosity shows linear variation in the lower range of concentration (C < 0.002 M) of PEG in the three solvents.

When the concentration of PEG is higher, the linearity is not maintained may be because of the difference between the absolute viscosity of the solvent and PEG is very large. So at higher concentrations of PEG, the viscosity of solution
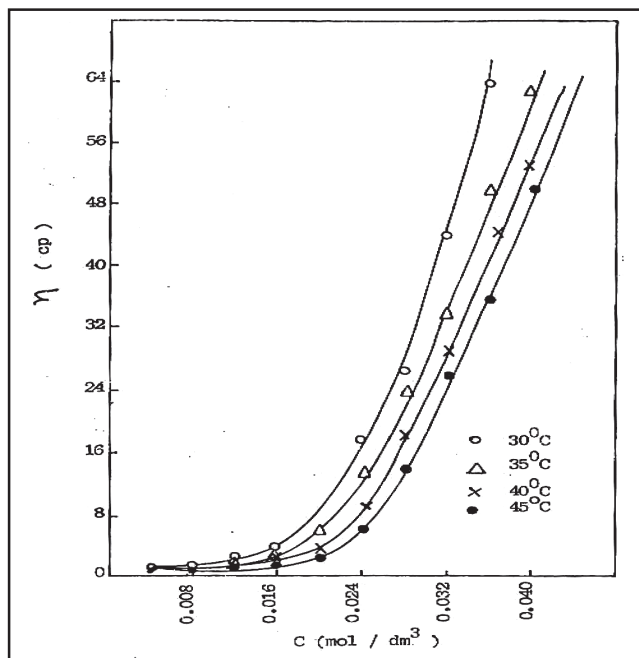
Fig.1 : Variation of Viscosity (η) with concentration (C) for PEG in H_2O.

Fig.2 : Variation of Viscosity (η) with concentration (C) for PEG in H_2O + DMSO (50%, v/v) Mixture.

rises sharply. The viscosity of the solution is high due to the increase in hydrogen bonding of the solvents. The hydrogen bonding capacity between solute and solvents is in the following order:

PEG–Water>PEG–(Water + DMSO)>PEG-DMSO

Because of this reason, the observed viscosity values are also in the same order. Hence it is expected that the hydrogen bonding between polymer and the solvent is responsible for the increase in viscosity by causing the

Fig.3 : Variation of Viscosity (η) with concentration (C) for PEG in DMSO.

increase in radius of gyration over riding other solvent effects. A similar observation was made by Bagchi *et al* (4). From the ultrasonic and viscosity studies of ISROPOLYOL in various solvents. In the present study it is clear that in each system the viscosity decreases with increase in temperature. Figs. 4-6 illustrates the change of relative viscosities (efflux times of solutions/efflux times of the pure solvent) for solutions of PEG as a function of polymer concentration, C mol/dm^3 in H$_2$O, H$_2$O+ DMSO and pure DMSO at 30, 35, 40, 45^{0}C respectively.

The intrinsic viscosity [η] and Huggin's constant K, were obtained from the following equation (5).

$$\eta Sp/C = [\eta] + K [\eta]^2 C \qquad \ldots(8)$$

Where ηSp is the specific viscosity (the relative increment solution viscosity over that of the solvent) ηSp/

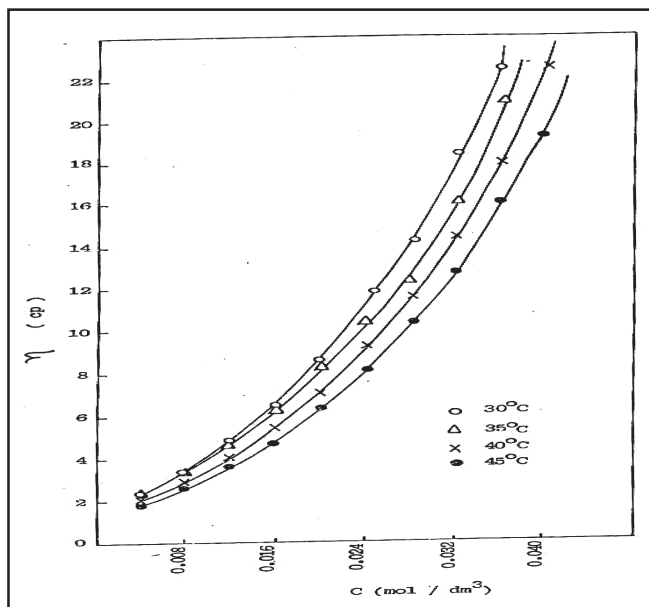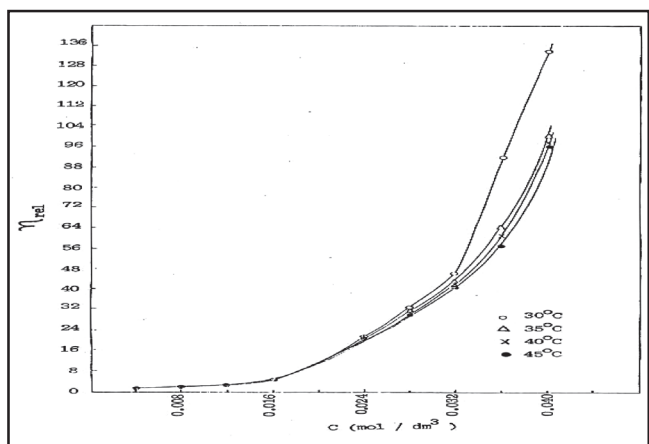**Fig.4 : Variation of relative Viscosity (η$_{rel}$) with concentration (C) for PEG in H$_2$O.**
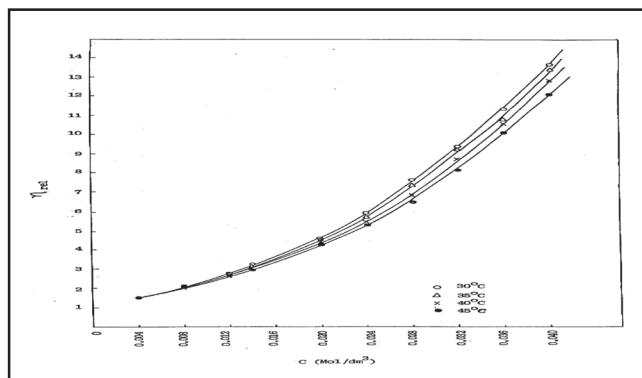
Fig.5 : Variation of relative Viscosity (η$_{rel}$) with concentration (C) for PEG in H$_2$O + DMSO (50%, v/v) Mixture.
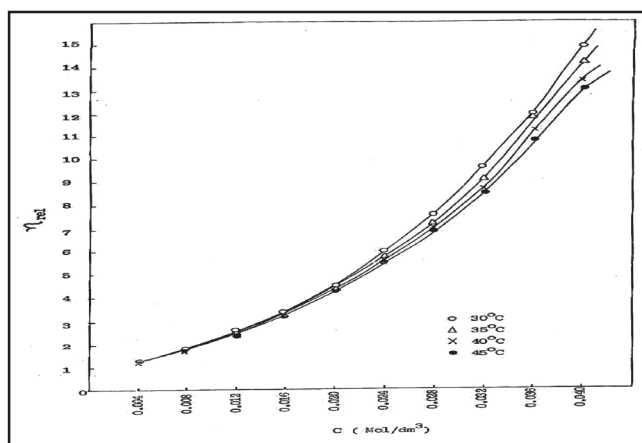
Fig.6 : Variation of relative Viscosity (η$_{rel}$) with concentration (C) for PEG in DMSO.

C, the reduced viscosity [η], the intrinsic viscosity and C, the polymer concentration.

From Figs. 4-6, it is clear that the relative viscosity of polymer solution increases with concentration of polymer in all the three systems at different temperatures and the relative viscosities of polymer solution decreases with increasing temperature.

From Figs. 4-6, it can be concluded that the viscosity of polymer solutions vary in the following order for the systems studied at any temperature.

H$_2$O > H$_2$O + DMSO > DMSO

The intrinsic viscosities were obtained by extrapolating plots ηSp/C versus C to infinite dilution (a typical plot is shown in Fig.7), while Huggins constant K was calculated from the slope of the plots (Table 1).

Intrinsic viscosity data was used to calculate the equivalent hydrodynamic volume Ve, a measure of the size of the polymer molecule at infinite dilution where polymer coils behave essentially like hard spheres and do not interpenetrate (6,7). The following equation was used to obtain Hydrodynamic volume, Ve;

Table :1

Intrinsic viscosity [η] , Huggins Constant 'K', and Equivalent Hydrodynamic Volume 'Ve' of PEG in water, water + DMSO (50%, v/v) and DMSO at different temperatures.

System	Temperature ^oC	Intrinsic viscosity [η]	K	Hydrodynamic Volume, Ve x 10^{19} (Cm^3/molecule)
PEG in water	30	100.0	0.67	4.0
	35	120.0	0.48	4.8
	40	140.0	0.26	5.6
	45	160.0	0.14	6.4
PEG in water + DMSO (50%,v/v)	30	83.0	0.77	3.3
	35	95.0	0.53	3.8
	40	102.5	0.31	4.1
	45	107.5	0.28	4.3
PEG in DMSO	30	30.0	8.68	1.2
	35	37.5	5.17	1.5
	40	40.0	3.96	1.6
	45	50.0	2.50	2.0

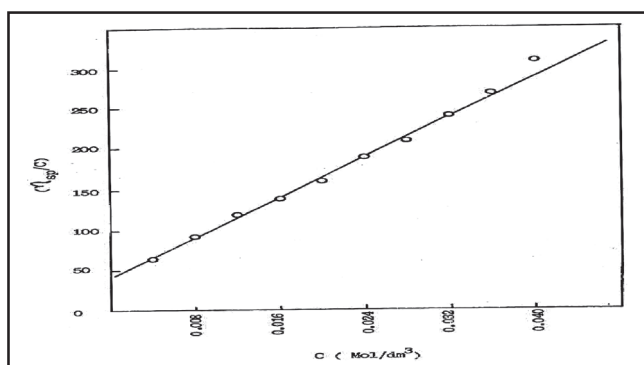**Fig.7 : Variation of reduced Viscosity (η_{sp}/C) with concentration (C) for PEG in DMSO at 40°C.**

$$Ve = M [\eta] \text{ í } N \qquad …(9)$$

Where M is the molecular weight, N, Avogadro's number and í, a shape factor for spheres (8), í is equal to 2.5.

Conclusion

Equivalent hydrodynamic volumes for the PEG in H_2O, H_2O + DMSO and DMSO were given in Table 1. The values increase with increasing temperature. The larger the value obtained for PEG in H_2O compared to H_2O+DMSO and DMSO indicate that water is a good solvent compared to DMSO.

The results of numerous theoretical works show that the intrinsic viscosity-temperature relation can be very complex (9). Reasons for the viscosity variation with increasing temperature can be different viz. ordering or (segments) and solvent molecules, dissolutions of macro molecules (10), conformational changes (11) and helix-coil transactions (12).

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Evaluation of Analgesic and Antidepressant Effects of Ethanolic Extract of Sesbania Grandiflora Flowers

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ABSTRACT

In this work, the antidepressant and analgesic effects of ethanolic extract of *Sesbania grandiflora* flowers were evaluated in mice. The preliminary phytochemical screening of the flowers revealed the presence of saponins, terpenoids, tannins, flavonoids, glycosides and sugars. The immobility time of mice subjected to the forced swimming test. The peripheral analgesic activity was evaluated using acetic acid induced writhing test. The study was carried out using dose (250 mg/kg, p.o.) of the extract. Indomethacin (20mg/kg, i.p.) is used as the standard for peripheral acting analgesic activity. The statistical analysis was carried out using one way ANOVA followed by Dunnet's test. P value less than 0.5 were considered significant. The ethanolic extract of *Sesbania grandiflora* significantly ($p<0.05$) reduced writhing reaction induced by acetic acid. The ethanolic extract of *Sesbania grandiflora* showed significant analgesic effect comparative to the standard drugs. These results provide support for the potential antidepressant and analgesic activity of *Sesbania grandiflora*.

Key words: Antidepressant, analgesic, Sesbania grandiflora, phytochemical screening.

Introduction

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic drugs. *Sesbania grandiflora* (family Leguminosae) is a short lived, quick growing, soft-wooded tree found widely in Tropical Asia and North Australia. In India it is found at Andhra Pradesh, Karnataka, West Bengal, and Assam. It is cultivated as an ornamental plant, grows wild in hedges and shady forests. The chemical constituents of the plant have been identified as amino acids, alkaloids, carbohydrates, Flavonoids, saponins, glycosides, tannins [1]. Different parts of *Sesbania grandiflora* have been used traditionally for the treatment of variety of diseases. These chemical constituents are well known for their potential health benefits and have been reported to possess valuable biological activities such as antibacterial and antifungal [2], antioxidant [3-4], antiurolithiatic [5], anticonvulsant and anxiolytic [6], hypolipidemic [7] hepatoprotective properties [8].However, there is no systematic scientific report

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published indicating utility of this plant material in the treatment of pain and depression. Thus the presence of therapeutically active flavonoids as major constituents was the basis of selection and evaluation of ethanol extract of *Sesbania grandiflora* flowers for their analgesic and antidepressant activity.

Materials and Methods

Plant material

Flowers of Sesbania grandiflora were collected from Maheshwaram, Ranga reddy (Dist) India in the month of October 2012. The plant material was taxonomically identified by Dr. Pasala Ratna Kumar, ICRISAT, A.P, India. A voucher specimen (No.6-09/12) has been preserved in our laboratory for future reference. The flowers were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the flowers was defatted with petroleum ether (60-80) and subsequently extracted with ethanol in a Soxhlet apparatus. The solvent was completely removed under reduced pressure and ethanol extract of flowers was obtained (yield 14.4%). Solution of Sesbania grandiflora was prepared freshly in distilled water and used for the studies.

Phytochemical screening

The Sesbania grandiflora flower extract was screened for the presence of various phytochemical constituents i.e. steroids, alkaloids, tannins, flavonoids, glycosides, etc by employing standard screening tests [9].

Chemicals and reagents

Ethanol and acetic acid were obtained from Merck Chemicals, Indomethacin obtained from Sigma Aldrich. Distilled water, which had been used in this experiment was laboratory prepared. Imipramine hydrochloride obtained from Sigma-Aldrich was used as reference standards for antidepressant activity.

Pharmacological Evaluation

Animals

Wistar albino rats of either sex weighing 180–200 g and Swiss albino mice of either sex weighing 18–25 g were used for animal studies. The animals were grouped in polyacrylic cages and maintained under standard laboratory conditions (temperature $25\pm2^\circ$C) and relative humidity ($50\pm5\%$) with dark and light cycle (14/10h).They were allowed free access to standard dry pellet diet and water *ad libitum*. The rats and mice were acclimatized to laboratory condition for 10 days before commencement of experiment. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Balaji College of Pharmacy, Anantapur, Andhrapradesh, India with CPCSEA Registration No: 1563 / PO / a / 11 / CPCSEA.

Acute toxicity test

The animals were divided into six groups containing six animals each. *Sesbania grandiflora* was dissolved in distilled water and administered orally as a single dose to mice at different dose levels viz. 500, 750, 1000, 1250, 1500, 2000, 2500 mg/kg of body weight (b.w.). The mice were observed periodically for symptoms of toxicity and death within 24 h and then daily for next 14 days [10]. The ethanolic extract of *Sesbania grandiflora* flowers were devoid of mortality of animals at dose of 2500 mg/kg.

Statistical analysis

The experimental data was expressed as mean ± SEM, the significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA followed by Dunnett's t-test using Graphat Instat Software, $p<0.05$ were considered as significant.

Analgesic activity by Acetic acid induced writing test [11]

An acetic acid induced abdominal constriction in mice (writhing effect) was determined by the following method. Fifteen mice of both sexes weighing 18-25 gm were divided in to three groups and pre-treated as follows: Group1 served as control, orally received distilled water in appropriate volumes, Group-2 administered with Indomethacin 20mg/kg p.o, Group-3 administered with *S.grandiflora* flower extract 250mg/kg p.o. After 30minutes, the animals were injected intraperitoneally with 0.7% of an aqueous solution of acetic acid (10ml/Kg of animal weight).The number of abdominal constrictions of injected mice was recorded during 30min of i.p injection. After an interval of fifteen minutes, this was given for absorption and no writhing was counted for 5 minutes. Then every mouse of all groups was observed carefully to count the number of writhing which made within 15 minutes (Table-1).

Forced swimming test (FST) [12]

Rats of either sex were individually forced to swim in an open cylindrical container (diameter 10cm,height 25cm), containing 19cm of water at $25\pm1^\circ$C. All the rats of either sex were divided in six different groups. The first group assigned as control receiving only vehicle (NaCl 5ml/kg). The other four groups received acute dose of ethanolic extract of *Sesbania grandiflora* (100, 250mg/kg). The sixth group received standard drug Imipramine (30mg/kg). The total duration of immobility was recorded during the last 6min of the 10min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect.

Table-1

Group	Avg. Body Wt of mice (gm)	Writhing Counting	Mean	% Writhing	SD	SE	SEM	Mean ±SEM	% of inhibition
Group1		26 29 25 27 29	27.2	100	1.6	0.8	0.8	27.2± 0.80	0
Group2	18-25	10 11 09 08 06	8.8	32.35	1.72	0.86	0.86	8.8± 0.86	67.65
Group3		13 18 16 15 19	16.2	59.55	2.13	1.06	1.06	16.2± 1.06	40.45

Table-2

Group	Dose (i.p;mg/kg)	Time of immobility in seconds
Control	5ml/kg	149±2.469
Imipramine	30mg/kg	117±2.875**
SGF100	100mg/kg	134±3.276*
SGF 250	250mg/kg	125±3.055**

SGF-Ethanolic extract of Sesbania grandiflora. One way ANOVA followed by Dunnet's test. Values are mean ± S.E.M (n = 6), in each group *p < 0.05, **p < 0.01 when compared to control.

Results and Discussion

In this study analgesic activity of ethanol extract of *Sesbania grandiflora* flowers was evaluated by in *vivo* screening method. Preliminary phytochemical screening of the ethanol extract of *Sesbania grandiflora flowers* revealed the presence of amino acids, alkaloids, carbohydrates, flavonoids, saponins, glycosides, tannins. Further separation of specific phytochemicals is in progress. In the acute toxicity assay no deaths were observed during the 72h period at the doses tested. At these doses, the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, ataxia, diarrhoea or increased diuresis thus the median lethal dose (LD50) was determined to be higher than the dose tested i.e. 2.0g/ kg b.w. In this study, use of scientific methods to elucidate the analgesic properties of *S. grandiflora* flower extract has been attempted. The data obtained clearly indicated that the plant extract has analgesic activity by the highly significant responses. The mean number of abdominal constriction after I.P injection of acetic acid was 27.2 in vehicle treated control animals. Indomethacin treatment produced 67.65% inhibition of writhing response. At the dose of 250mg/kg inhibition of writhing response was observed 40.45%. The experiment proved that the flower extract of *S. grandiflora* has analgesic activity.

It was observed that *Sesbania grandiflora* at doses of 100mg and 250mg/kg exhibited significant reduction in immobility time when compared to control in dose dependent manner. Similarly the animals treated with Imipramine (30mg/kg) as expected showed significant decrease in immobility time.

The prevention and management of stress disorders remains a major clinical problem. Hence it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. In this work, it was demonstrated that the administration of different doses of the ethanolic extract of *Sesbania grandiflora* in mice was able to induce antidepressant effects. It was found that *Sesbania grandiflora* can produce antidepressant like activity at a dose of 100mg and 250mg/kg body weight in a dose dependent manner. The decrease in the immobility time is accompanied with the increase in swimming time. Imipramine was widely used as antidepressant drug and agreed with studies in animal models, such as forced swimming test. Antidepressant drug reduce the exploratory behaviour depending upon the concentration. At present, the study revealed that the ethanolic extract of SGF significantly reduces the number of head dippings which is indication of exploratory behaviour. The findings from the present investigation indicate that SGF possesses significant antidepressant activity as shown by its mitigating effects on different experimentally induced stress models in mice.

Conclusion

In this study, use of scientific methods to elucidate the analgesic properties of *S. grandiflora* flower extract has been attempted. The data obtained clearly indicated that the plant extract has analgesic activity by the highly significant responses and forced swimming test is more sensitive and better reflects the state of depression. Our present study confirmed that the *Sesbania grandiflora* ethanolic extract has the antidepressant activity as it significantly reduces the immobility time and increases the exploratory behaviour during depression in animal models.

Acknowledgment

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