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**Index**

# SYNTHESIS AND BIOLOGICAL SCREENING OF NOVEL ARYLOXYACETIC ACID ANALOGS

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**ABSTRACT:**
A series of 5-Bromo- 2-formyl phenoxyacetyl amino acids and peptides have been synthesized by coupling of the 5-Bromo- 2-formyl phenoxyacetic acid with amino acid/methyl esters/dipeptides/ tripeptides using DCC as coupling agent and NMM as base. The structures were elucidated FTIR and $^{1}$HNMR .The newly synthesized compounds were evaluated for their antibacterial, antifungal and anthelminitic activities. The compounds (**2, 6, 11 and 13**) were found to exihibit potent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* (gram positive bacteria) and *Escherichia coli* (gram negative) bacterias.The compounds (**5, 6 and 13**) were found to exihibit potent antifungal activity against *Candida albicans* and *Aspergillus niger*. The moderate to good anthelminitic activity was shown by the synthesized compounds (**7** and **13**) against *Eudrilus* spieces.

**Keywords:** Phenoxyacetic acid, amino acids, antibacterial, antifungal and anthelminitic.

Phenoxyacetic acid is among the most vital moieties which are associate with potent antidiabetic (Rival et al 2004), antimycobacterial (Yar et al 2007),diuretic (Lebedev et al 1985, Woltersdorf et al 1976, Bicking et al 1976), anti-inflammatory (Kunsch et al 2005, Shokol et al 2005), antibiotic (Grardin et al 1995), anti-obesity (Kiso et al 1999), diagnostic (Ohmomo et al 1989), inhibition of platelet aggregation (Meanwell et al 1993, Seiler et al 1994) activities. The review of literature has suggested that incorporation of amino acids and peptides into aromatic and heterocyclic congeners have resulted in compounds with potent bioactivities.

Introducing an amino acid or peptide into aromatic compounds can increase the potency, decrease the toxicity and prolong its action. Among aromatics, phenolic compounds have wide range of activities. Further phenoxylation the resulting compound phenoxyacetic acid is obtained, which is well known for their biological potential. Thus keeping in view the biological potency of phenoxyacetic acids as well as taking advantage of biodegradability and biocompatibility of a novel series of substituted phenoxyacetic acid derivatives of amino acids and peptides have been synthesized with an anticipation to get potent agents with

good therapeutic efficacy with negligible side effects.

The study of IR and $^{1}$HNMR spectrum gives us most of the required information of certain vibrational bands or characteristic groups present in the molecule. The IR spectrum of newly synthesized compounds showed characteristic bands in the region 3329-3318, 1654-1642, 1537-1532 $cm^{-1}$ which can be assigned as N-H stretching, C=O stretching and N-H bend respectively. In their $^{1}$HNMR spectra, a singlet appeared in the range 8.33-8.36 ppm corresponding to the CO-NH proton.

The compounds were found to exhibit potent antimicrobial and moderate anthelmintic activity in comparison to standard drugs against the same concentration.

**Results and discussion**

**Antibacterial Activity:**

The synthesized peptide derivatives were screened for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus and Bacillus subtilis* using modified Kirby-Bauer disc diffusion method (DMF as a solvent).The test samples were tested at the concentrations 25, 50, 100 µg/ml. The petri plates inoculated with bacterial cultures were incubated at 37ºC for 18 hrs. The diameters obtained for the test sample were compared with that produced by the standard drug ciprofloxacin. The results are shown in Table 2.

**Antifungal Activity:**

The synthesized peptide derivatives were screened for antifungal activity against *Candida albicans* and *Asperigillus niger*. DMSO is used as negative control. The test samples were tested at the concentrations 25, 50, 100 µg/ml. The petri plates inoculated with fungal cultures were incubated at 25ºC for 48 hrs. Diameters of the zone of inhibition were calculated in triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug griseofulvin. The results are shown in Table 2.

**Anthelmintic Activity:**

The anthelminitic activity was carried out against earthworms *Eudrilus* species by Garg and Atal method at 2 mg/ ml concentration. Suspension of samples was prepared by triturating synthesized cyclic peptide (200 mg) with Tween 80 (0.5 %) and distilled water. Suspension of the standard drug albendazole was prepared with the same concentration in a similar way. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50ºC) which stimulated the movement. The results were shown in Table 3.

The results of biological activities revealed that newly synthesized peptide derivative 73 at 50µg/ml concentration exhibited highest zone of inhibition against *Staphylococcus aureus* and 75 at 50µg/ml concentration exhibited highest zone of inhibition against *Candida albicans*. Morover, other compounds

showed moderate antimicrobial activities against tested organisms. Comparison of anthelmintic activity data revealed that peptide derivative 75 was found to exhibit potent anthelmintic activity and other peptide derivatives showed good to moderate activity.

**Experimental**

Melting points were determined and uncorrected. The amino acids, di-tert-butyl pyrocarbonate ($Boc_2O$), 5-Bromosalicyaldehyde, DCC and NMM were obtained from Spectrochem Limited, Himedia laboratories Limited mumbai and Sd-fine-chem Limited, Mumbai, India. The IR spectra were recorded on a Perkin Elmer Fourier transform infrared spectrophotometer using KBr pellets. The $^1$HNMR spectra were recorded on the Bruker Avance II-400 NMR spectrometer using $CDCl_3$ as the solvent. The purity of all the compounds was controlled by TLC on silica gel G plates. Chloroform:Methanol (9:1 v/v) was used as developing solvent system and dark brown spots were detected on exposure to iodine vapours in a tightly closed chamber. The physical data of synthesized compounds is listed in Table 1. The scheme of synthesis is given in Scheme 1.

**Synthesis of Boc amino acids (1-3):**

L-Leucine (1.31gm, 10mmol) was dissolved in 10 ml of sodium hydroxide (1 mol $L^{-1}$) and 10 ml of i-propanol. di-tert.butylpyrocarbonate (3 ml, 13 mmol) in 5 ml of i-propanol was added followed by 10 ml of sodium hydroxide (1 mol $L^{-1}$) to the resulting solution. The solution was stirred at room temperature for 2 hr, washed with 10 ml of light petroleum ether (b.p. 40-60°C), acidified to p H 3.0 with 1 mol $L^{-1}$ sulphuric acid and finally extracted with chloroform (3 x 20 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure to give crude product. The crude product was purified by recrystallization from methanol and ether at 0°C to get pure Boc-Leucine (**1**). Similarly, Boc-Serine (**2**) and Boc-Alanine (**3**) were prepared by stirring di-tert.butylpyrocarbonate (3 ml, 13 mmol) with Boc-Serine (1.05gm, 10 mmol) and Boc-Alanine (0.89gm, 10 mmol) respectively.

**Synthesis of L-amino acid methyl ester hydrochlorides (4-6) :**

Thionyl chloride (0.73mL, 10 mmol) was slowly added to methanol (50 mL) at 0°C and 1.15 gm of L- Proline (10 mmol) was added to the above solution. The resulting mixture was refluxed for 9 hrs at 110 ºC. Methanol was evaporated and the residue was triturated with ether at 0°C until excess dimethyl sulphite was removed. The crude product was purified by recrystallization from methanol and ether at 0°C to get L-proline methyl ester hydrochloride (**4**). Similarly, L-leucine methyl ester hydrochloride (**5**) and L-tryptophan methyl ester hydrochloride (**6**) was prepared by refluxing 1.31 gm of L-leucine (10 mmol) and 2.04 gm of L-tryptophan with 50 ml methanol in the

presence of 0.73 ml of thionyl chloride (10 mmol).

**Synthesis of Boc-dipeptide methyl esters (7-9) :**

To a mixture of 1.65 gm compound **4** (10 mmol) in 20 ml of chloroform, 2.3 ml of N- methylmorpholine (21mmol) was added at 0°C. The reaction mixture was stirred for 15 min. 2.31gm compound **1** (10mmol) in 20 ml chloroform and 2.1gm of DCC (10mmol) were added under stirring to the above mixture. After 36 hrs, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filterate. The filterate was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution (25 ml each). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to get Boc-Leu-Pro-OMe (**7**). Similarly Boc-Ser-Leu-OMe (**8**) and Boc-Ala-Pro-OMe (**9**) were prepared by stirring compounds **2** and **3** with amino acid methyl ester hydrochlorides **5** and **4,** respectively in the presence of DCC and NMM.

**Deprotection of dipeptides at carboxyl end (8a, 9a) :**

To a solution of 3.32 gm of compound **8** (10 mmol) in 36 ml of THF/$H_2O$ (1:1), 0.36 gm lithium hydroxide (15mmol) was added at 0°C. The mixture was stirred at room temperature for 1 hr, and acidified to [1] pH 3.5 with 0.5 mol $L^-$ $H_2SO_4$. The aqueous layer was extracted with diethyl ether (3 x 25 ml). Combined organic extracts were dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was recrystallized from methanol and ether to get Boc-Ser-Leu-OH (**8a**). Similarly compound **9** was hydrolyzed under alkaline conditions to obtain Boc-Ala-Pro-OH (**9a)**.

**Deprotection of dipeptide at amino end (7a):**

Compound **7** (3.42 gm, 10mmol) was dissolved in 15 ml of chloroform and treated with 2.28 gm of trifluoroacetic acid (20 mmol). The resulting solution was stirred at room temperature for 1 hr and washed with 25 ml of saturated sodium hydrogen carbonate solution. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by recrystallization from mixture of chloroform and light petroleum ether (b.p. 40-60°C) to get pure Leu-Pro-OMe (**7a**).

**Synthesis of Boc-tripeptide methyl esters (10, 11) :**

To synthesize Boc-Ser-Leu-Trp-OMe (10), 3.18 gm of dipeptide unit 8a (10 mmol) was coupled with 2.54 gm of amino acid methyl ester hydrochloride 6 (10 mmol) in the presence of DCC and NMM following the same procedure as adopted for the synthesis of Boc-dipeptide methyl esters 7-9. Similarly Boc-Ala-Pro-Pro-OMe (11) was

prepared by coupling 2.86 gm of deprotected dipeptide unit 9a and 1.65 gm of amino acid methyl ester hydrochloride 4 using DCC as the coupling agent and NMM as the base.

**Deprotection of tripeptides at amino end (10a, 11a):**

Compound **10** (5.18 gm, 10mmol) was dissolved in 15 ml of chloroform and treated with 2.28 gm of trifluoroacetic acid (20 mmol). The resulting solution was stirred at room temperature for 1 hr and washed with 25 ml of saturated sodium hydrogen carbonate solution. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by recrystallization from mixture of chloroform and light petroleum ether (b.p. 40-60°C) to get pure Ser-Leu-Trp-OMe (**10a**). Similarly Ala-Pro-Pro-OMe (**11a**) was prepared by stirring compound **11** with 2.28 gm of trifluoroacetic acid (20 mmol).

**Synthesis of free acid**

Sodium hydroxide (0.89gm, 22.4mmol) in 25 ml water was slowly added with stirring to 2.01gm of 5-bromo-2-hydroxyaldehyde (10mmol) and 0.94gm of chloroacetic acid (10mmol). The mixture was heated on heating mantle to remove all the liquid and the residue was treated with 30 ml water. The mixture was cooled and filtered and clear solution was acidified with dilute hydrochloric acid. The aqueous layer was extracted with diethyl ether (2 x 25 ml). Combined organic extracts were dried over anhydrous sodium sulphate. The crude product was recrystallized from ethanol and purified by recrystallization from ethanol-water (1:1) to get 5-Bromo-2-formyl-phenoxyacetic acid (**12**).

**Synthesis of 5-Bromo-2-formyl-phenoxyacetyl amino acid and peptide methyl esters**

L-Proline methyl ester hydrochloride (1.65 gm, 10mmol) was dissolved in Tetrahydrofuran (75 mL). To this, 2.3 ml of N-methylmorpholine (21mmol) was added at 0°C and the reaction mixture was stirred for 15 min. 2.01gm of compound **12** (10mmol) in tetrahydrofuran and 2.1gm dicyclohexylcarbodiimide (10mmol) were added under stirring to the above mixture. After 36 hrs, the reaction mixture was filtered and the residue was washed with 30 ml of tetrahydrofuran and added to the filterate. The filterate was washed with 5% $NaHCO_3$ and saturated NaCl solution (25 ml each). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and n-hexane followed by cooling at 0°C to get 5-Bromo-2-formyl-phenoxyacetyl-proline methyl ester (**13**). Similarly, 5-Bromo-2-formyl-phenoxyacetyl-leucyl-proline methyl ester (**14**), 5-Bromo-2-formyl-phenoxyacetyl-seryl-leucyl-tryptophan methyl ester (**15**) and 5-Bromo-2-formyl-phenoxyacetyl-alanyl-prolyl-proline methyl ester (**16**) were prepared by stirring compound **7a**, **10a** and **11a**

with compound **12** respectively in the presence of DCC and NMM.

**Deprotection of 5-Bromo-2-formyl-phenoxyacetyl-alanyl-prolyl-proline methyl ester at carboxyl end (16a) :**

To a solution of 4.39 gm of compound **16** (10 mmol) in 36 ml of THF/$H_2O$ (1:1), 0.36 gm lithium hydroxide (15mmol) was added at 0°C. The mixture was stirred at room temperature for 1 hr, and acidified to [1] pH 3.5 with 0.5 mol L$^-$$H_2SO_4$. The aqueous layer was extracted with diethyl ether (3 x 25 ml). Combined organic extracts were dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was recrystallized from methanol and ether to get 5-Bromo-2-formyl-phenoxyacetyl-alanyl-prolyl-proline (**16a**).

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**Table 1 : Physical data of the synthesized compounds**

| Compound No. | Physical state | Yield (%) | M.P (°C) | $R_F$ | Molecular formula |
|---|---|---|---|---|---|
| 12. | Pale yellow solid | 74.0 | 86-87 | 0.81 | $C_9H_7O_4Br$ |
| 13. | Brown solid | 68.2 | 72-75 | 0.94 | $C_{15}H_{16}NO_5Br$ |
| 14. | Semisolid mass | 70.7 | - | 0.61 | $C_{21}H_{27}N_2O_6Br$ |
| 15. | Semisolid mass | 63.2 | - | 0.62 | $C_{30}H_{35}N_4O_8Br$ |
| 16. | Semisolid mass | 74.3 | - | 0.78 | $C_{23}H_{28}N_3O_7Br$ |
| 16a. | Yellow brown solid | 67.3 | 73-75 | 0.80 | $C_{22}H_{26}N_3O_7Br$ |

**Table 2: Antimicrobial activity data of synthesized compounds**

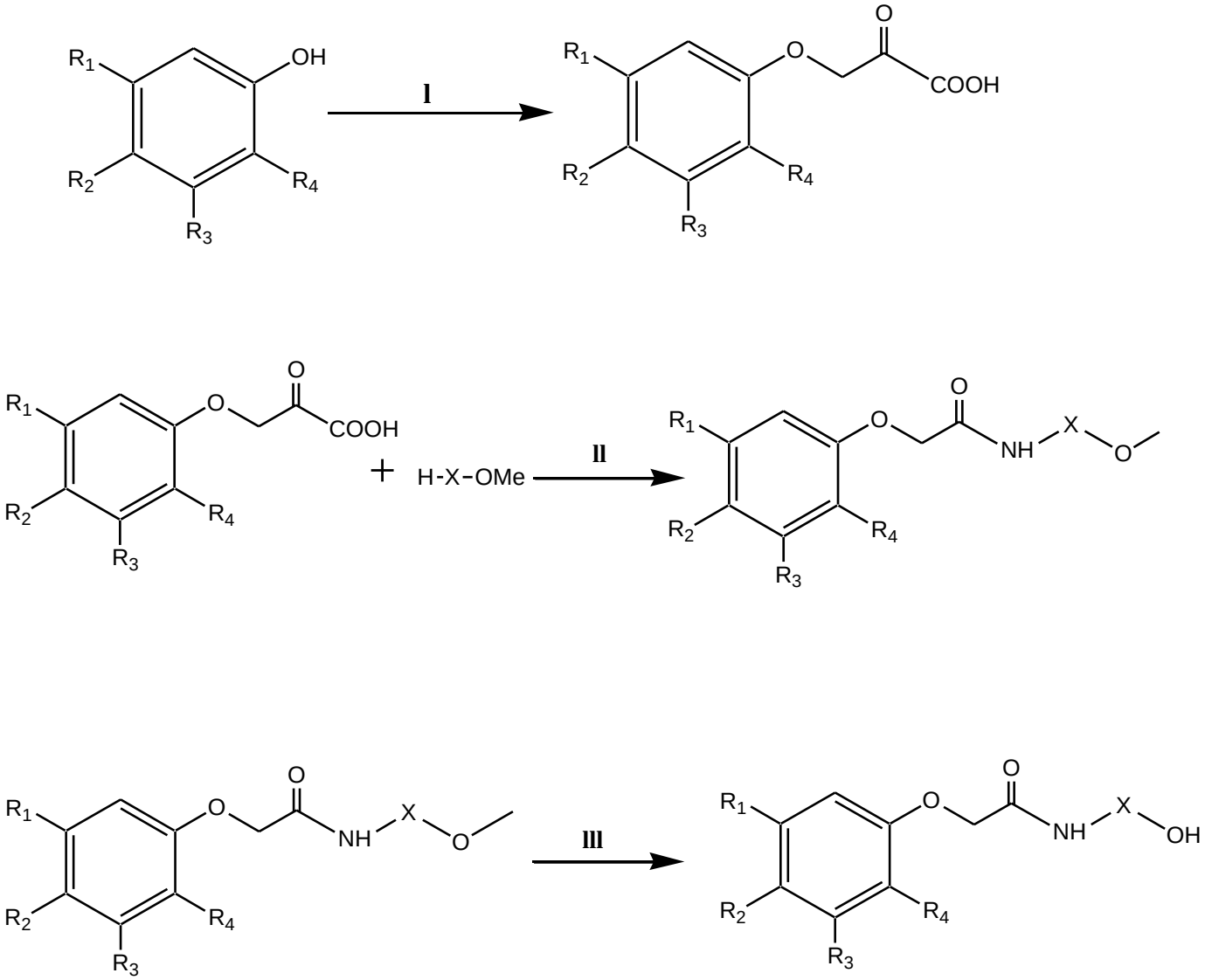
| Zone of inhibition in mm | | | | | |
|---|---|---|---|---|---|
| **Compound No.** | **Bacterial strains** | | | **Fungal strains** | |
| | ***B. subtillus*** | ***S. aureus*** | ***E.coli*** | ***C. albicans*** | ***A. niger*** |
| **12** | 13.00 | 12.67 | 10.33 | 14.33 | 13.33 |
| **13** | 9.33 | 5.33 | 11.67 | 11.67 | 5.67 |
| **14** | 10.30 | 10.33 | 10.67 | 6.33 | 6.67 |
| **15** | 12.67 | 20.67 | 11.33 | 8.00 | 13.33 |
| **16** | 17.30 | 12.67 | 10.67 | 16.67 | 11.33 |
| **16a** | 20.00 | 13.67 | 11.33 | 20.33 | 14.67 |
| **Control** | - | - | - | - | - |
| **Ciprofloxacin** | 17.67 | 17.33 | 18.33 | - | - |
| **Griseofulvin** | - | - | - | 16.67 | 15.33 |

**Table 3: Anthelmintic activity data of synthesized compounds**

| *Eudrilus species* | | |
|---|---|---|
| **Compound No.** | **Mean paralyzing time** | **Mean death time** |
| **12** | 14.15±1.62 | 28.18±1.52 |
| **13** | 15.54±0.28 | 30.87±0.24 |
| **14** | 11.73±0.90 | 23.82±1.34 |
| **15** | 10.32±0.51 | 21.64±2.63 |
| **16** | 9.66±0.34 | 18.13±1.00 |
| **16a** | 4.67±0.43 | 9.72±0.83 |
| **Control** | - | - |
| **Albendazole** | 10.89±0.48 | 22.78±0.56 |

**Table 4: Spectral data of synthesized compounds**

| **Compound No.** | **IR ($cm^{-1}$)** | **$^{1}$HNMR δ (ppm)** |
|---|---|---|
| 12. | 3310 (-OH, str), 1719 (-COOH, str), 1305 (-C-O, str), 1247 (C-O-C, asym), 1070 (C-O-C, sym) | 10.92 (1H, s, -OH), 4.50 (2H, s, $-OCH_2$) |
| 13. | 3329 (-NH, str), 1654 (-C=O, str), 1537 (-NH, bend) | - |
| 14. | 3322 (-NH, str), 1648 (-C=O, str), 1533 (-NH, bend) | 8.33 (2H, s, -CO-NH) |
| 15. | 3325 (-NH, str), 1653 (-C=O, str), 1534 (-NH, bend) | 8.36 (3H, s, -CO-NH) |
| 16. | 3318 (-NH, str), 1642 (-C=O, str), 1532 (-NH, bend) | 8.31 (3H, s, -CO-NH) |
| 16a | 3328 (-OH, str), 1718 (-COOH, str), 1326 (-C-O, str) | 10.75 (1H, s, -OH) |




**Scheme**

$R_1$ = Br; $R_2$ = H; $R_3$ = H; $R_4$ =CHO (**12-16a**)
**I** = ClCH2COOH, NaOH, RT 1hr, **II =** DCC, NMM, RT 36hrs, **III** = LiOH, THF: $H_2O$ (1:1) RT1hr
X = Pro (**13**), Leu -Pro (**14**), Ser-Leu-Trp (**15**), Ala-Pro-Pro (**16, 16a**)

# SYNTHESIS AND BIOLOGICAL SCREENING OF CYCLIC HEPTAPEPTIDE

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**ABSTRACT:**

A new bioactive cyclic heptapeptide cyclo(Gly-Tyr-Val-Pro-Leu-Trp-Pro) was synthesized using the solution phase technique by cyclization of the linear peptide Boc- Gly-Tyr-Val-Pro-Leu-Trp-Pro after proper deprotection at carboxyl and amino terminals. All the coupling reactions were performed at room temperature utilizing dicyclohexylcarbodiimide (DCC) as the coupling agent and N-methylmorpholine (NMM) as the base. Structures of all new compounds were characterized by IR and $^{1}$HNMR. The synthesized cyclopeptide was screened for antimicrobial and anthelminitic activities and found to exhibit good antibacterial activity against *Bacillus subtilis* and moderate antifungal activity against *Candida albicans* and *Asperigillus niger*. In addition the cyclic peptide was found to exhibit good anthelminitic activity against earthworms *Eudrilus* species.

**Keywords**: cyclic heptapeptide, antimicrobial activity, anthelminitic activity.

Cyclic congeners possess unusual or modified amino acid residues and exhibit there bioactivities through binding to corresponding enzyme. This characteristic feature can allow bioactive cyclopeptides to act as therapeutic agents in this resistant world. Cyclopepetides having multiple peptide bonds are concerned with a wide spectrum of biological activities such as antimicrobial, anti-inflammatory, antimalarial, cyctotoxic, and antifungal activities. Cyclic peptides are more important compounds for medicinal purposes and represent an important class of natural products. Since only minute quantities are obtained from natural resources many of these compounds were attempted to synthesize in the laboratory. Keeping in view the biological potential of cyclic peptide as well as to obtain a bioactive compound in a good yield, the present investigation aimed at synthesis of cyclic heptapeptide cyclo(Gly-Tyr-Val-Pro-Leu-Trp-Pro) in a convenient and economical manner. Synthesized cyclic heptapeptide was evaluated for pharmacological activities. The antibacterial and antifungal activities were carried out against variety of pathogenic microorganism like *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*, *Asperigillus niger*. The anthelminitic activity was carried out against *Eudrilus* species of earthworms.

## MATERIALS AND METHODS

Melting points were determined and uncorrected. The amino acids, di-tert-butyl pyrocarbonate ($Boc_2O$), p-nitrophenol (pnp), DCC and NMM were obtained from Spectrochem Limited and Sd-fine-chem Limited, Mumbai, India. The IR spectra were recorded on a Perkin Elmer Fourier transform infrared spectrophotometer using KBr pellets. The $^1$HNMR spectra were recorded on the Bruker Avance II-400 NMR spectrometer using $CDCl_3$ as the solvent. The purity of all the compounds was controlled by TLC on silica gel G plates. Chloroform:Methanol (9:1 v/v) was used as developing solvent system and dark brown spots were detected on exposure to iodine vapours in a tightly closed chamber. The physical data of synthesized compounds is listed in Table 1. The scheme of synthesis is given in Scheme 1.

### Synthesis of Boc amino acids (1-3) :

L-tyrosine (1.81gm, 10mmol) was dissolved in 10 ml of sodium hydroxide (1 mol $L^{-1}$) and 10 ml of i-propanol. di-tert.butylpyrocarbonate (3 ml, 13 mmol) in 5 ml of i-propanol was added followed by 10 ml of sodium hydroxide (1 mol $L^{-1}$) to the resulting solution. The solution was stirred at room temperature for 2 hr, washed with 10 ml of light petroleum ether (b.p. 40-60°C), acidified to pH 3.0 with 1 mol $L^{-1}$ sulphuric acid and finally extracted with chloroform (3 x 20 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure to give crude product. The crude product was purified by recrystallization from methanol and ether at 0°C to get pure Boc-Tyrosine (**1**). Similarly, Boc-leucine (**2**) and Boc-proline (**3**) were prepared by stirring di-tert.butylpyrocarbonate (3 ml, 13 mmol) with Boc-proline (1.15 gm, 10 mmol) and Boc-leucine (1.31gm, 10 mmol) respectively.

### Synthesis of L-amino acid methyl ester hydrochlorides (4-7) :

Thionyl chloride (0.73mL, 10 mmol) was slowly added to methanol (50 mL) at 0°C and 1.15gm of L- proline (10 mmol) was added to the above solution. The resulting mixture was refluxed for 9 hrs at 110 ºC. Methanol was evaporated and the residue was triturated with ether at 0°C until excess dimethyl sulphite was removed. The crude product was purified by recrystallization from methanol and ether at 0°C to get L-proline methyl ester hydrochloride (**4**). Similarly, L-valine methyl ester hydrochloride (**5**), L-tryptophan methyl ester hydrochloride (**6**) and glycine methyl ester hydrochloride (**7**) were prepared by refluxing 1.17 gm of L-valine (10 mmol), 2.04 gm of L-tryptophan (10 mmol) and 0.75 gm of glycine (10 mmol) with 50 ml methanol in the presence of 0.73 ml of thionyl chloride (10 mmol).

### Synthesis of Boc-dipeptide methyl esters (8-10):

To a mixture of 1.67gm compound **5** (10 mmol) in 20 ml of chloroform, 2.3 ml of N- methylmorpholine (21mmol) was added at 0°C. The reaction mixture was stirred for 15 min. 2.81gm compound **1** (10mmol) in 20

ml chloroform and 2.1gm of DCC (10mmol) were added under stirring to the above mixture. After 36 hrs, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filterate. The filterate was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution (25 ml each). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to get Boc-Tyr-Val-OMe (**8**). Similarly Boc-Leu-Trp-OMe (**9**) and Boc-Pro-Gly-OMe (**10**) were prepared by stirring compounds **2** and **3** with amino acid methyl ester hydrochlorides **6** and **7,** respectively in the presence of DCC and NMM.

**Deprotection of dipeptides at carboxyl end (8a, 9a) :**

To a solution of 3.94 gm of compound **8** (10 mmol) in 36 ml of THF/$H_2O$ (1:1), 0.36 gm lithium hydroxide (15mmol) was added at 0°C. The mixture was stirred at room temperature for 1 hr, and acidified to [1] pH 3.5 with 0.5 mol $L^-H_2SO_4$. The aqueous layer was extracted with diethyl ether (3 x 25 ml). Combined organic extracts were dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was recrystallized from methanol and ether to get Boc-Tyr-Val-OH (**8a**). Similarly compound **9** was hydrolyzed under alkaline conditions to obtain Boc-Leu-Trp-OH (**9a)**.

**Deprotection of dipeptide at amino end (10a):**

Compound **10** (2.86 gm, 10mmol) was dissolved in 15 ml of chloroform and treated with 2.28 gm of trifluoroacetic acid (20 mmol). The resulting solution was stirred at room temperature for 1 hr and washed with 25 ml of saturated sodium hydrogen carbonate solution. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by recrystallization from mixture of chloroform and light petroleum ether (b.p. 40-60°C) to get pure Pro-Gly-OMe (**10a**)

**Synthesis of Boc-tri/tetrapeptide methyl esters (11, 12) :**

To synthesize Boc-Tyr-Val-Pro-OMe (11), 3.80 gm of dipeptide unit 8a (10 mmol) was coupled with 1.66 gm of amino acid methyl ester hydrochloride 4 (10 mmol) in the presence of DCC and NMM following the same procedure as adopted for the synthesis of Boc-dipeptide methyl esters 8-10. Similarly Boc-Leu-Trp-Pro-Gly-OMe (12) was prepared by coupling 3.36 gm of deprotected dipeptide unit 9a and 1.86 gm of 10a using DCC as the coupling agent and NMM as the base.

**Synthesis of Boc-heptapeptide methyl ester (13):**

To synthesize Boc-Tyr-Val-Pro-Leu-Trp-Pro-Gly-OMe (**13)**, 4.77 gm of tripeptide unit **11** mmol was deprotected at carboxyl end to get Boc-Tyr-Val-Pro-OH (**11a**) following the same procedure as adopted for the synthesis of compounds **8a** and **9a** from compounds **9** and **10**, respectively. Tetrapeptide unit (**12**) (5.85 gm, 10 mmol) was deprotected at amino end to get Leu-Trp-Pro-Gly-OMe (**12a**)

following the same procedure as adopted for the synthesis of compounds **10a** from compound **10**. The deprotected tripeptide unit **11a** (4.77 gm, 10 mmol) and 4.85 gm of tetrapeptide unit (10 mmol) were coupled in the presence of DCC and NMM to get linear heptapeptide unit **13** under the same experimental conditions as adopted for the synthesis of Boc-dipeptide methyl esters (**8-10**).

**Synthesis of cyclic heptapeptide, cyclo(Gly-Tyr-Val-Pro-Leu-Trp-Pro) (14):**

To synthesize cyclo(Gly-Tyr-Val-Pro-Leu-Trp-Pro) (14), 9.44 gm of linear heptapeptide unit(10 mmol) (**13**) was deprotected at carboxyl end using 0.36 gm of LiOH (15 mmol) to get Boc-Tyr-Val-Pro-Leu-Trp-Pro-Gly-OH (**13a**) following the same procedure as adopted for the synthesis of compounds **8a** and **9a** from compounds **8** and **9** respectively.

The deprotected heptapeptide unit **13a** (4.65 gm, 5 mmol) was dissolved in 50 ml of $CHCl_3$ at 0ºC. To the above solution, 0.94 gm of pnp (6.7 mmol) was added and stirred at room temperature for 12 hrs. The reaction mixture was filtered and the filtrate was washed with 10% $NaHCO_3$ solution (3 x 15 ml) until excess of pnp was removed and finally washed with 5% HCl (2 x 10 ml) to get the corresponding p-nitrophenyl ester Boc-Tyr-Val-Pro-Leu-Trp-Pro-Gly-O-pnp (**13b**). To compound **13b** (4.20 gm, 4 mmol) dissolved in 35 ml of $CHCl_3$, 0.91 gm of TFA (8 mmol) was added, stirred at room temperature for 1 hr and washed with 10% $NaHCO_3$ solution (2 x 25 ml). The organic layer was dried over anhydrous sodium sulphate to get Tyr-Val-Pro-Leu-Trp-Pro-Gly-O-pnp (**13c**), which was dissolved in 25 ml of $CHCl_3$ and 2.3 ml of NMM (21 mmol) was added. Then all the contents were kept at 0ºC for 7 days. The reaction mixture was washed with 10% $NaHCO_3$ until the byproduct p-nitrophenol was removed completely and finally washed with 5% HCl (3 x 15 ml). The organic layer was dried over anhydrous sodium sulphate. Finally, chloroform was distilled off and the crude cyclized product was crystallized from $CHCl_3$ and n-hexane to get the pure compound **14**.

**Antibacterial Activity:**

The synthesized cyclic peptide was screened for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus and Bacillus subtilis* using modified Kirby-Bauer disc diffusion method. A spore suspension in sterile distilled water was prepared from five days old culture of the test bacteria growing on nutrient broth media. About 20 ml of the growth was transferred into sterilized petri plates and inoculated with 1.5 ml of the spore suspension. Each petri plate was divided into five equal portions along the diameter to place one disc. Three discs of test sample were placed on three portions together with one disc reference drug ciprofloxacin (50µg/ml) and a disc impregnated with the solvent DMF as negative control. The test samples were tested at the concentrations 25, 50, 100 µg/ml. The petri plates inoculated with bacterial cultures were incubated at 37ºC for 18 hrs. Diameters of the zone of inhibition were calculated in triplicate sets. The

diameters obtained for the test sample were compared with that produced by the standard drug ciprofloxacin. The results are shown in Table 2.

**Antifungal Activity:**

The synthesized cyclic peptide was screened for antifungal activity against *Candida albicans* and *Asperigillus niger*. A spore suspension in normal saline was prepared from culture of the test fungi on sabouraud's broth media. After transferring growth media, petri plates were inoculated with spore suspension. After drying, wells were made using agar punch and the test samples, reference drug (griseofulvin) (50µg/ml) and negative control (DMSO) were placed in labeled wells in each petri plate. The test samples were tested at the concentrations 25, 50, 100 µg/ml. The petri plates inoculated with fungal cultures were incubated at 25ºC for 48 hrs. Diameters of the zone of inhibition were calculated in triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug griseofulvin. The results are shown in Table 2.

**Anthelmintic Activity:**

The anthelminitic activity was carried out against earthworms *Eudrilus* species by Garg and Atal method at 2 mg/ ml concentration. Suspension of samples was prepared by triturating synthesized cyclic peptide (200 mg) with Tween 80 (0.5 %) and distilled water. The resulting mixture was stirred using mechanical stirrer for 30 min. The suspensions were diluted to contain 0.4 % w/v of the test samples. Suspension of the standard drug albendazole was prepared with the same concentration in a similar way. Three sets of five earthworms of almost similar sizes were placed in petri plates containing 50 ml suspension of Tween 80 (0.5 %) and distilled water. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50ºC) which stimulated the movement. The results were shown in Table 3.

## RESULTS AND DISCUSSION

The results revealed that newly synthesized cyclic peptide **14** at 50 µg $ml^{-1}$ concentration exhibited highest zone of inhibition against *B. subtilis*. The cyclic peptide **14** at 50 µg $ml^{-1}$ concentration exhibited moderate antifungal activity against *A. niger* and *C. albicans*. Anthelmintic activity data revealed that cyclic peptide **14** possessed moderate anthelmintic activity against *Eudrilus sp*. comparable to that of reference drug. Cyclization of linear heptapeptide fragment **13** was indicated by disappearance of absorption bands at 1736 $cm^{-1}$ (C=O stretching of ester) and 1392, 1367 $cm^{-1}$ (C-H band of $^{t}$butyl group) and presence of additional Amide I and Amide II bands at 1659 and 1539 $cm^{-1}$ in IR spectrum of synthesized cyclic peptide **14**. Formation of cyclic peptide was further confirmed by disappearance of singlets at 3.62 and 1.60 ppm corresponding to three protons of methyl ester group and nine protons of $^{t}$butyl group of di-tert.butylpyrocarbonate in $^{1}$HNMR spectrum of the cyclic heptapeptide **14** showed characteristic peaks confirming the presence of all seven amino acid moieties.

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**TABLE 1 : PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS**

| Compound No. | Physical state | Yield (%) | M.P (°C) | $R_F$ | Molecular formula |
|---|---|---|---|---|---|
| 1. | Light brown solid | 72.6 | 136-138 | 0.29 | $C_{14}H_{19}NO_5$ |
| 2. | Semisolid mass | 82.6 | - | 0.31 | $C_{11}H_{21}NO_4$ |
| 3. | White crystals | 83.4 | 122-124 | 0.26 | $C_{10}H_{19}NO_4$ |
| 4. | Semisolid mass | 82.4 | - | 0.41 | $C_6H_{12}NO_2Cl$ |
| 5. | White solid | 83.2 | 137-139 | 0.66 | $C_6H_{14}NO_2Cl$ |
| 6. | Light brown solid | 76.7 | 181-183 | 0.79 | $C_{12}H_{15}N_2O_2Cl$ |
| 7. | White crystals | 82.4 | 118-120 | 0.27 | $C_3H_8NO_2Cl$ |
| 8. | Semisolid mass | 83.9 | - | 0.89 | $C_{20}H_{30}N_2O_6$ |
| 9. | Brown Solid | 84.5 | 176-178 | 0.69 | $C_{23}H_{33}O_5N_3$ |
| 10. | Semisolid mass | 66.0 | - | 0.71 | $C_{13}H_{22}O_5N_2$ |
| 11. | Semisolid mass | 82.6 | - | 0.88 | $C_{25}H_{37}N_3O_7$ |
| 12. | Semisolid mass | 79.4 | - | 0.75 | $C_{30}H_{43}N_5O_7$ |
| 13. | Semisolid mass | 79.3 | - | 0.73 | $C_{49}H_{68}N_8O_{11}$ |
| 14. | Brown Solid | 75.2 | 90-92 | 0.48 | $C_{43}H_{56}N_8O_8$ |

**Table 2 : ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF COMPOUND 14:**

| **Zone of inhibition (mm)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **Bacterial strains** | | | | | | | | |
| | *Escherichia coli* | | | *Staphylococcus aureus* | | | *Bacillus subtilis* | | |
| | 25 µg/ml | 50 µg/ml | 100 µg/ml | 25 µg/ml | 50 µg/ml | 100 µg/ml | 25 µg/ml | 50 µg/ml | 100 µg/ml |
| Control | - | - | - | - | - | - | - | - | - |
| Ciprofloxacin | - | 17.67 | - | - | 17.00 | - | - | 17.37 | - |
| 14 | 4.33 | 9.30 | 13.67 | 3.33 | 10.67 | 16.30 | 8.67 | 15.33 | 18.00 |
| **Compound** | **Fungal strains** | | | | | | | | |
| | *Candida albicans* | | | *Asperigillus niger* | | | | | |
| | 25 µg/ml | 50 µg/ml | 100 µg/ml | 25 µg/ml | 50 µg/ml | 100 µg/ml | | | |
| Control | - | - | - | - | - | - | | | |
| Griseofulvin | - | 16.33 | - | - | 15.67 | - | | | |
| 14 | 4.67 | 8.33 | 12.00 | 3.67 | 7.33 | 13.33 | | | |

**TABLE 3 : ANTHELMINTIC ACTIVITY OF COMPOUND14 :**

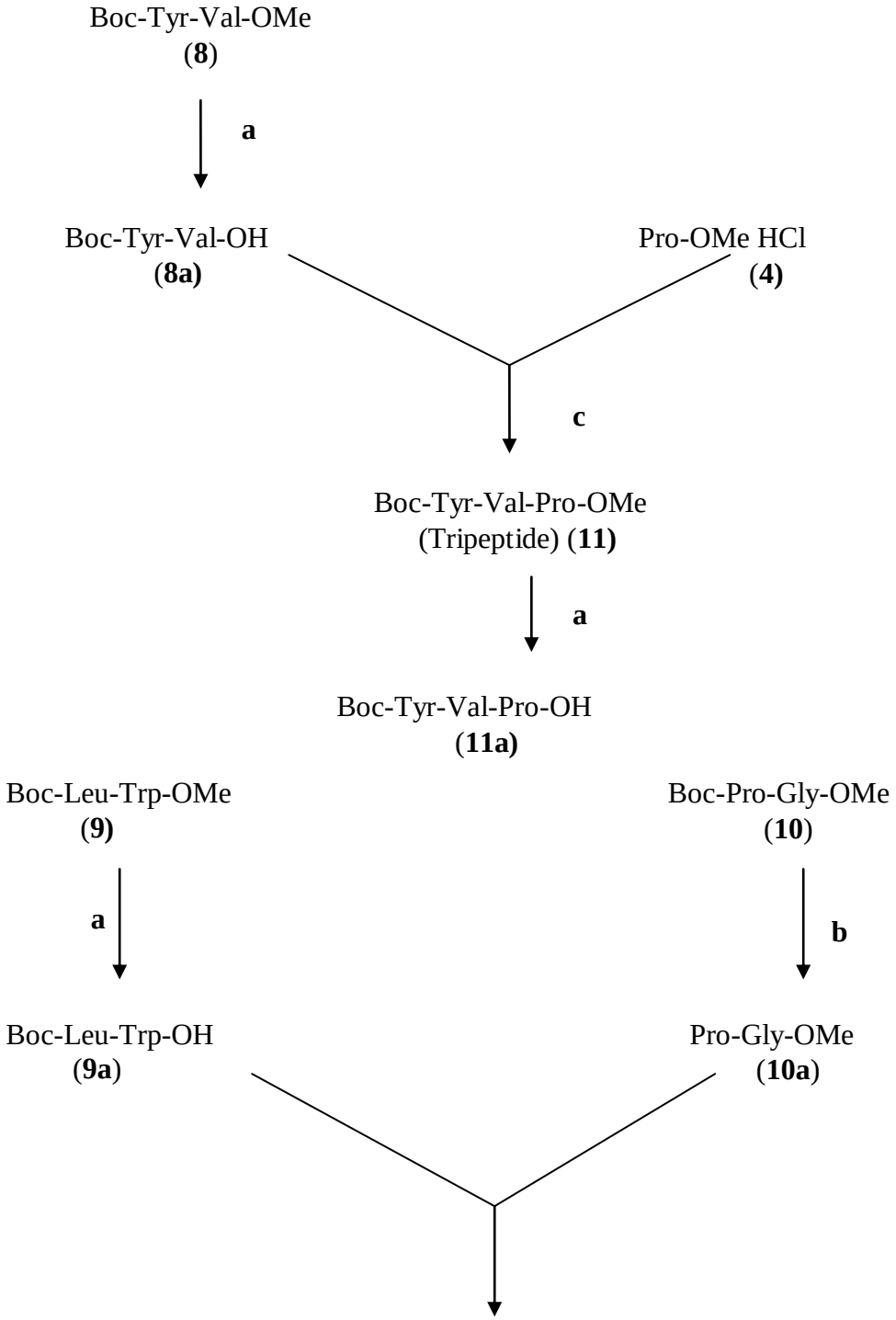
| *Eudrilus species* | | |
|---|---|---|
| **Compound** | **Mean paralyzing time ± S.D (min)** | **Mean death time ± S.D (min)** |
| Control | - | - |
| Albendazole | 10.89± 0.48 | 22.89 ± 1.16 |
| 14 | 13.23 ± 0.45 | 27.65 ± 0.10 |

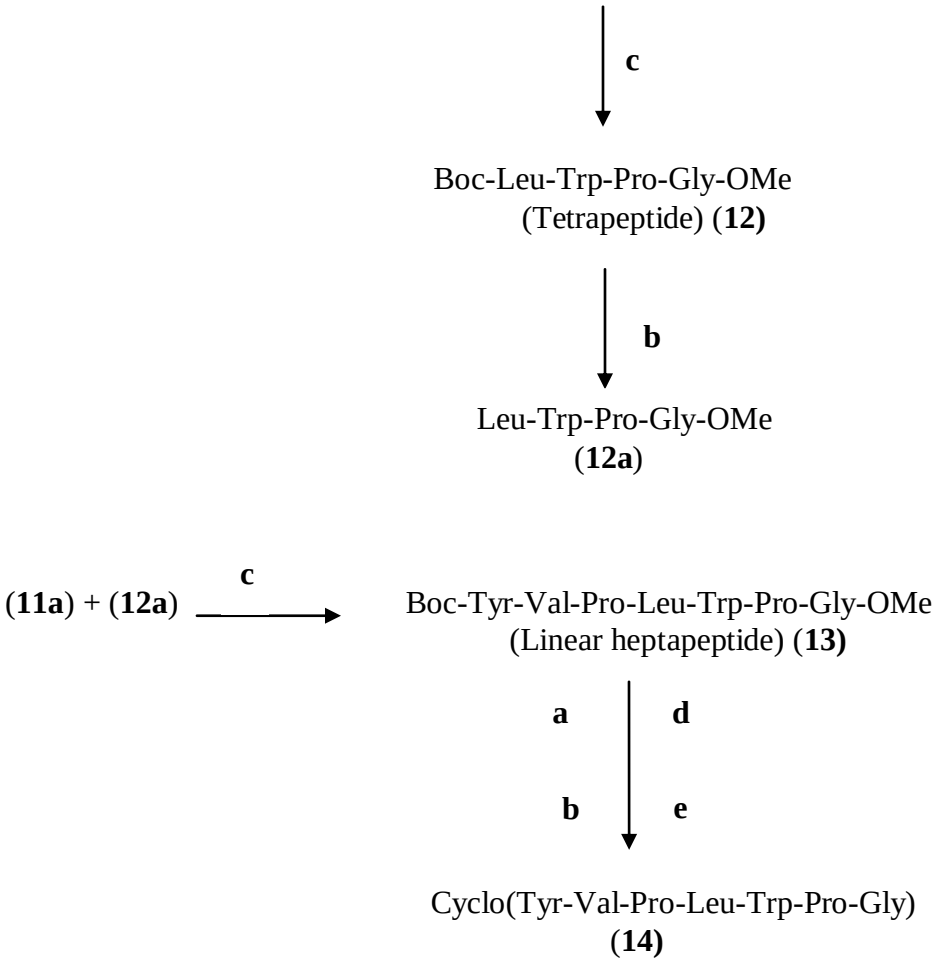
**TABLE 4: SPECTRAL DATA OF SYNTHESIZED COMPOUNDS**

| **Compound No.** | **IR ($cm^{-1}$)** | **$^{1}$HNMR δ (ppm)** |
|---|---|---|
| 8 | 3322 (-NH, str), 1660 (-C=O, str), 1526 (-NH, bend) | 8.11 (2H, s, -CO-NH) |
| 9 | 3318 (-NH, str), 1661 (-C=O, str), 1521 (-NH, bend) | 8.12 (2H, s, -CO-NH) |
| 10 | 3314 (-NH, str), 1685 (-C=O, str), 1532 (-NH, bend) | 8.22 (1H, s, -CO-NH) |
| 11 | 3307 (-NH, str), 1655 (-C=O, str), 1515 (-NH, bend) | 8.49 (2H, s, -CO-NH) |
| 12 | 3317 (-NH, str), 1655 (-C=O, str), 1520 (-NH, bend) | 8.87 (3H, s, -CO-NH) |
| 13 | 3314 (-NH, str), 1645 (-C=O, str), 1528 (-NH, bend) | 8.49 (5H, s, -CO-NH) |
| 14 | 3307 (-NH, str) | 8.25 (6H, s, -CO-NH) |

**Scheme (1)**

Synthesis of Cyclo(glycyl-tyrosinyl-valyl-prolyl-leucyl-tryptophanyl-prolyl)






**a** = LiOH, THF: $H_2O$ (1:1), RT, 1h
**b** = TFA, $CHCl_3$, RT, 1h
**c** = DCC, TEA, $CHCl_3$, RT, 24h
**d** = pnp, $CHCl_3$, RT, 12h
**e** = NMM, $CHCl_3$, 7days, 0ºC

# ASSESSMENT OF THE TREATMENT PATTERN, CLINICAL OUTCOME, AND QUALITY OF LIFE IN PATIENTS WITH BLADDER OUTLET OBSTRUCTION IN A TERTIARY CARE TEACHING HOSPITAL

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**ABSTRACT**

**Aim:**

This study provides an insight into the assessment of the treatment pattern, clinical outcome and quality of life in patients with Bladder outlet obstruction and to assess the safety and efficacy of (0.2 mg) and (0.4 mg) tamsulosin in female patients with Bladder outlet obstruction in a tertiary care teaching hospital.

**Method:** Institutional ethics committee approval was obtained from kasturba hospital, Manipal for conducting this study. This was a randomised controlled study conducted for eight months at Dept. of Urology, Kasturba Hospital, Manipal. Inclusion criteria were all male patients in age group greater than 45 years with bladder outlet obstruction symptoms due to BPH and all the female patients aged greater than 35 years with bladder outlet obstruction were included for the study. Exclusion criteria were all Male patients with Stricture urethra, acute cystitis, Carcinoma of prostate, Neurogenic voiding dysfunction and all female patients with Pelvic masses, previous pelvic surgery, and acute cystitis were excluded from the study. The American Urological Association Symptom Score (AUASS) was adopted to assess the severity of urinary symptoms and to check the effectiveness of the treatment. The percentage of improvement of treatment was also assessed in terms of both objective and subjective parameters like Maximum Flow Rate (MFR), Post Voidal Residual Volume (PVR) and bladder thickness. Female patients were randomised into two groups' first group of patients receiving (0.2 mg of tamsulosin) while second group of patients receiving (0.4 mg of tamsulosin) daily for a period of two months with periodic follow up at $2^{nd}$ , $4^{th}$ and $8^{th}$ week, assessed with IPSS (International Prostate Symptom Score) and uroflowmetry and

ultrasonography at 8 th week. The quality of life in patients with bladder outlet obstruction was evaluated by using the quality of life questionnaire.

**Results:**

A total number of 177 patients diagnosed with bladder outlet obstruction were aged between 55 to 65 years and males (n=152) predominated over females (n=25). Weak stream urinary symptoms were found to be the highest complained symptom. The co morbidities associated with bladder outlet obstruction was Hypertension. Surgery was the main line of treatment in patients with bladder outlet obstruction. The drug tamsulosin was the most implicated drug in the medical group. The most common surgical procedure performed was transurethral resection of the prostate. The safety and efficacy of tamsulosin in female patients showed that patients were having AUASS score between 8-19 (moderate) before treatment and most of them showed an improvement in the score from moderate to mild (1-7) after treatment. The subjective parameters showed that the female patients had an MFR value of < 15ml/s before treatment and have improved after treatment from < 15ml/s to >15ml/s. The PVR values were >50ml in all patients before treatment and < 50ml after treatment. The bladder thickness showed a reduction from > 5mm to < 2.5mm. Our results show tamsulosin to be very effective in the management of bladder outlet obstruction in female patient if detected early.

**Conclusions:** In female patients' use of tamsulosin was found to be a very safe, well tolerated showing significant improvement in urinary outflow symptoms, reducing post void urine volume and decreasing IPSS with minimal tolerable adverse events. The correct and timely diagnosis of bladder outlet obstruction in women was difficult, since clinical features are similar to those of other voiding disorders and diagnostic modalities are often inconclusive or even misleading.

**Key words:** bladder outlet obstruction, Medical management, tamsulosin, urodynamics.

## Introduction

Bladder outlet obstruction (BOO) is a blockage at the base of the bladder that reduces or prevents the flow of urine into the urethra, the tube that carries urine out of the body[1]. Numerous gender-specific etiologies are responsible for bladder outlet obstruction. BOO may be induced by specific functional and anatomic causes. The resulting obstruction frequently produces lower urinary tract symptoms (LUTS), although the degree of bother by LUTS is highly variable and not predictable on the basis of the specific inciting etiology[2]. Induced LUTS symptoms may be predominantly obstructive, irritative, or often a combination of both. Typically, obstructive symptoms include hesitancy, sensation of incomplete bladder emptying, diminished urinary stream, and post voiding urinary dribbling.

Irritative complaints include urinary urgency, frequency of urination, occasional dysuria, and nocturia. Rarely are symptoms related to BOO isolated; often the individual experiencing LUTS presents with a variety of mixed symptoms of obstruction and irritation. BOO may also occur in the complete absence of symptoms and be first identified in the scenario of urinary retention or decompensation of the upper urinary tracts.

BOO often occur in both male and females; it is more common in aging men. urodynamic evaluation and pressure flow evaluation is the gold standard diagnostic tool, other modalities may also be used, including post void residual analysis, urinary flow rates, cystoscopy, and selected radiologic ones. Patient self-appraisal of symptoms using various inventories such as the American Urologic Association Symptom Index[3] or the International Prostate Symptom Score is relevant to the initial assessment and subsequent follow up. Analysis of secondary symptoms of obstruction in women is often performed using a subjective symptom appraisal and is determined urodynamically, assessing the pressure-flow relation during voiding. The complete assessment of LUTS arising from BOO often includes several of these modalities to fully define the obstructive impact on the individual's urinary function and quality of life[4].

There are a number of treatment options in both male and female patients with bladder outlet obstruction. The treatment options in male patients includes watchful waiting[5] (A strategy of management in which the patient is monitored but receives no active treatment), medical treatment which includes alpha blocker therapy which inhibit contraction of prostatic smooth muscle and finasteride therapy, an enzyme inhibitor that lowers prostatic androgen levels and decreases prostate size, surgical treatment which includes balloon dilation (A catheter with a balloon at the end is inserted through the urethra and into the prostatic urethra. The balloon is then inflated to stretch the urethra narrowed by the prostate), Transurethral Incision of the Prostate (TUIP) an endoscopic surgical procedure in which patients with smaller prostates (<30 g) is inserted through the urethra to make one or two cuts in the prostate and reduce the constriction on the urethra, Transurethral Resection of the Prostate (TURP) is an surgical removal of the prostate's inner portion by endoscopic approach through the urethra. This is the most common and the gold standard of treatment and open prostatectomy is a surgical removal of the prostate via an incision in the lower abdomen. The main treatment options in females are urethral dilatation under local or regional anaesthesia.

Alpha blockers is currently considered the preferred treatment alternative for those individuals who lack absolute indications for surgery. Although alpha blockers have proved to be very efficacious in males[6, 7] but very few

studies are done to prove the efficacy of alpha blocker in females.[8] This observational, cross sectional descriptive study provides an insight into the assessment of the treatment pattern, clinical outcome and quality of life in patients with Bladder outlet obstruction in a tertiary care teaching hospital.

**Material and Methods**

The study was approved by the Institutional ethics committee at kasturba hospital, manipal. A suitably designed Informed Consent Form (ICF) in different languages namely English, Kannada and Malayalam was prepared and used for the purpose of the study. In order to record the data for the study, a separate Case Record Form (CRF) was designed and used, which contains the details of the patients demographics, medical history, medication history, final diagnosis, laboratory investigations, study specific investigations, patient outcome analysis chart, adverse event reporting card, discharge medication and details of follow up visits. The American Urological Association Symptom Score[3] (AUASS) was used to assess the severity of urinary symptoms and to check the effectiveness of the treatment. The AUA symptom index questionnaire consists of 7 questions. For the purpose of this study questions 2, 4 and 7 were assigned to irritative symptoms, while questions 1, 3, 5 and 6 were assigned to obstructive symptom sub scores. Total scores were classified as mild 0 to 7, moderate 8 to 19 and severe 20 to 35 symptoms, as recommended by the AUA measurement committee[3]. This questionnaire has been adopted worldwide and it is also known as the International Prostate Symptom Score (IPSS). The quality of life questionnaires[4] were classified as delighted with score (0), pleased with score (1), mostly satisfied with score (2), mixed with score (3), mostly dissatisfied with score (4), unhappy with score (5) and terrible with score (6) was used to assess the quality of life of the patient with bladder outlet obstruction.This was a randomised controlled study conducted in the Dept. of Urology of Kasturba Hospital, Manipal from September 2008 to April 2009. Our first step was to identify the female patients with bladder outlet obstruction and male patients with BPH during ward rounds or with the help of the physician. Once the patient was identified, they were enrolled according to the study criteria and subject information sheet was explained in detail to the patient or patient's legally acceptable representatives and written inform consent was obtained from qualified patients prior to their enrolment. A detailed history, clinical examination, study specific investigations which includes Maximum Flow Rate (MFR), Post Voidal Residual Volume (PVR) and bladder thickness was done and the follow up details for all the patients was recorded in the CRF followed by assessment of American Urological Association symptom score (AUASS). The treatment patterns were analyzed with respect to medical treatment or surgical procedures adapted to the patient. Medical treatment includes the various drugs like alpha

blockers, 5-alpha reductase inhibitors, and combination of both or sometimes anticholinergic and the surgical procedures include Transurethral Resection of the Prostate (TURP), cystoscopy and dilatation, bladder neck resection etc.

The incidence of bladder outlet obstruction was obtained in terms of gender and age group wise distribution. The occurrences of urinary symptoms were analyzed based on the number of patients presenting with each symptoms. The co morbidities most commonly associated with bladder outlet obstruction were assessed based on number of patients presented with co morbidities. The patterns of treatment the patient received such as medical or surgical treatment and drug class implicated, compliance, benefit and adverse effects of each therapy were analyzed. Outcome analysis of different treatment patterns were based on percentage of improvement in the AUAS score, MFR (>25ml/s), bladder wall thickness (<2.5mm) and PVR values (nil) after treatment.

Female patients were randomised into two groups' first group of patients receiving (0.2 mg of tamsulosin) while second group of patients receiving (0.4 mg of tamsulosin) in order to assess the safety and efficacy of 0.2 mg and 0.4 mg of tamsulosin in female patients with bladder outlet obstruction. The clinical outcome of this therapy in terms of both subjective and objective improvement was evaluated by estimating the improvement in the AUASS, MFR, and ultrasound reports like bladder wall thickness and PVR values, before and after treatment in both groups and the quality of life in the female patients with bladder outlet obstruction was evaluated by using the quality of life questionnaire.

**Results**

A total of 177 patients (n=152) males with Benign Prostatic Hyperplasia (BPH) as the risk factor for causing bladder outlet obstruction and (n=25) females with bladder outlet obstruction (BOO) were included in the study. Male predominance was observed over females. Only females' patients were studied to assess the safety and efficacy of 0.2 mg and 0.4 mg of tamsulosin with bladder outlet obstruction.

There was difference in the mean age in years $\pm$ standard deviation of patients with males (62.16 $\pm$ 11.233) with that of females (53.76 $\pm$ 9.418). Majority of bladder outlet obstruction patients were aged between 55 to 65 years. Results are summarized in Table 1. In the present study, the most severe obstructive urinary symptoms associated with bladder outlet obstruction were weak stream (72%) followed by irritative urinary symptoms nocturia (49%) and frequency (45%) as presented in Table 2. our study has detected a higher prevalence of hypertension (28%) as co morbidities associated with bladder outlet obstruction followed by diabetes (13%), respiratory diseases (6%), cardiac diseases other than hypertension (5%) and the least presented comorbidities associated with bladder outlet

obstruction was parkinsonism as summarized in Table 3.

Table 4 shows the various treatment pattern associated with bladder outlet obstruction in our study and results show that maximum number of patients were on surgical treatment (68%) followed by medical treatment (13%), medical treatment followed by surgical procedures (11%) and other treatment (8%) such as watchful waiting and life style modifications. The drug class most commonly prescribed in medical treatment was alpha blockers (55%) followed by anticholinergics (14%), combination treatment with alpha blocker and 5-alpha reductase inhibitors (14%), 5-alpha reductase inhibitor alone (5%) as summarized in Table 5. In our study tamsulosin followed by combination of Tamsulosin + dutasteride was the most implicated drug prescribed in the medical treatment; Table 6. A total of 139 patients underwent various surgical procedures, of which 110 patients had underwent transurethral resection of prostate, of which 28 patients had acute retention and had undergone catheterization for retention. 19 patients had undergone cystoscopy and dilatation and 10 patients had undergone bladder neck resection as there was reoccurrence of symptoms within few months or years as summarized in Table 7.

In our study, the results of both objective and subjective parameters for all types of treatment in the bladder outlet obstruction showed that highest percentage of improvement in the surgical group i.e. 69% improvement in the (AUASS) American urological association symptom score, 70% improvement in the (MFR) Maximum flow rate values, 74% improvement in the (PVR) Post Void Residual Volume and 78% improvement in the (BT) bladder thickness as summarized in table 8.

We analyzed the safety and efficacy of 0.2 mg and 0.4 mg of tamsulosin with bladder outlet obstruction. The results showed that patients were having AUA score between 8-19 (moderate) before treatment and all of them showed an improvement in the score from moderate to mild (1-7) after treatment. Similarly the subjective parameters showed that the female patients had an MFR value of < 15ml/s before treatment and have improved after treatment from < 15ml/s to >15ml/s. The PVR values were >50ml in all patients before treatment and < 50ml after treatment. The bladder thickness showed a reduction from > 5mm to < 2.5mm. No suspected adverse drug reactions were reported during the study period. The results of the quality of life questionnaire showed that most of the patients were unhappy by their disease state before treatment and mostly satisfied after treatment. There was a significant improvement in the urinary symptoms after treatment in both groups as shown in the figure 1.

**Discussion**

Bladder outlet obstruction (BOO) is becoming an increasingly recognized entity over the past several years. The goal of treatment of bladder outlet

obstruction is primarily to provide a rapid and sustained improvement in lower urinary tract symptoms (LUTS) and to reduce the long term complications. LUTS associated with BOO are a common disorder in urology. Surgical removal of prostate tissue or pharmacological prostate/bladder neck smooth muscle relaxation with alpha$_{1A}$-AR antagonists relieves outlet obstruction but does not improve irritative symptoms.[9] Tamsulosin and nonselective subtype antagonists have the ability to relieve obstructive and irritative symptoms in men, while in women there is also some evidence for this. Although, recent studies suggest that it is an under diagnosed cause of female lower urinary tract symptoms.[10] our study showed that the male patients were having Benign Prostatic Hyperplasia (BPH) as the main risk factor for BOO. Symptomatic BPH is thought to be due to bladder outflow obstruction and is often referred to as lower urinary tract symptoms suggestive of BOO[11]. This study systemically explored the treatment pattern and clinical outcome of treatment in patients with BOO in the Urology Department of Kasturba Hospital, Manipal. A total of 177 patients with BOO were evaluated during the eight month study period.

On studying the overall gender wise distribution of the 177 patients with BOO, it was observed that there was male (n=152) predominance over female (n=25). This was because of under diagnosis and failure of the female patients to visit the Urology Department. In males, the occurrence of BOO was more common because gonadal androgens play a major role. The hormone testosterone is converted to dihydrotestosterone (DHT) in males as the age progresses and this DHT has a pivotal role in the development of BPH. This observation was consistent with observation made by McConnell[12] which revealed about a relationship between DHT and BPH.

The male to female ratio was 63:1 with a mean age of 62.16 $\pm$ 11.23 years for males and 53.76 $\pm$ 9.41 years for females. A study by Nunzio et al[5] showed similar results with males having mean age 64.5 $\pm$ 7.6 years and a study by Pummangura and Kochakarn[13]showed that mean age of females affected by bladder outlet obstruction is 45.3 $\pm$ 12.9. In this study, majority (42%) of the patients with BOO were in the age group of 55-65 years. This was because the chance of occurrence of this disease increases with the progression of age. Verhamme et al[14] has observed that BPH is one of the most common condition associated with ageing men and approximately 40% of men are affected at the age fifties.

The study patients were evaluated for the distribution of obstructive and irritative urinary symptoms with BOO. However, in our study these symptoms, which were mainly of the obstructive type (weak urinary stream), were reported. This finding is in accordance with the

study carried out by Lee et al[15] which showed a similar observation with weak stream as the most commonly observed obstructive urinary symptom.

The result of our study further supports the co morbidity between Hypertension and bladder outlet obstruction. The prevalence of BPH and arterial Hypertension increases with age and hence both are frequent disease state in the elderly people with bladder outlet obstruction. This study result was consistent with a study carried out by Nicolas et al[16] which confirms that there is a statistically significant relationship between Hypertension and LUTS secondary to BPH and correlation between mean blood pressure and prostate size.

In our study majority of the patients had undergone surgery for bladder outlet obstruction because their urinary symptoms were moderate to severe in AUA index score and also patients presented with reoccurrence of urinary symptoms which suggestive of clear indication for surgery. This may be due to the fact that the treatment pattern followed was similar to the AUA guidelines in the management of Bladder outlet obstruction and also the European Urology guidelines on BPH. As per the guidelines, watchful waiting is recommended for patients with mild symptoms, medical treatment for patients with mild to moderate symptoms and surgery for patients who failed medication or conservative management and who have moderate to severe symptoms and/or complications of BPH. Although in our study men with LUTS due to BPH are generally managed with watchful waiting or medical therapy, some of the patients presented with progressive disease that can result in symptom deterioration, acute urinary retention, urinary tract infection, renal failure, and thereby indicated for surgery.

Our study results showed that majority of the patients were prescribed with Tamsulosin, in the treatment of BOO and several studies have shown its superiority for treatment of lower urinary tract symptoms associated with Bladder outlet obstruction. Recently tamsulosin was found to be effective in patients with neurogenic lower urinary tract dysfunction; the drug decreased the maximal urethral pressure significantly in the long term follow up and improves cystometric parameters related to bladder storage and emptying[17]. Interestingly, in our study it is preferred by the physicians because of the propensity to cause adverse drug reaction with alpha blocker is comparatively less with that of other classes of drugs.

Transurethral Resection of Prostate (TURP) is the gold standard of treatment for BPH. In our study most of the patients with BOO in whom BPH was the risk factor had bothersome BPH symptoms refractory to medical treatment and because of the symptom severity and reoccurrence of symptoms there was a clear indication for transurethral Resection of Prostate

surgery. This finding is consistent with the European Urology guidelines where it is clearly indicated that TURP is the most frequently performed surgical procedure for those patients who have bothersome symptoms.[18] Most of the female patients in our study were presented with urethral and meatal narrowing, which causes obstruction in them and in these patients cystoscopy and urethral dilatation has been preferred as an empirical treatment. Bladder neck resection surgery has been preferred because of reoccurrence of symptoms.

Critical analysis of our result data very clearly illustrates the safety and efficacy of tamsulosin in medical management of bladder outlet obstruction. Being an uroselective antagonist it controls both irritative and obstructive symptoms thereby giving a total therapeutic effect in relieving urinary symptoms. The result of our study in comparable to many international studies conducted. Abrams et al [19] in their randomized placebo control clinical trial on 296 patients found tamsulosin to be well tolerated drug showing significant improvement in UFR and symptoms score. Lee et al [20] from Korea found tamsulosin to be a safe drug showing statistically significant improvement in urine flow and symptom score as compared to placebo. The Japanese study conducted by Kawabe et al[21] and the Chinese study conducted by Yan Jun et al[22] showed results similar to our result.

Our study showed that AUA score decreased from 23.9 to 16.1 after treatment with tamsulosin, mean MFR was 11 ml/s before treatment and 22 ml/s after treatment with tamsulosin. Similarly, there was an improvement in PVR and bladder thickness as early as 2 nd week and improvement was maintained throughout the treatment period. The improvement was seen both in 0.2 mg and 0.4 mg tamsulosin after treatment. The improvement was seen both in IPSS and urodynamic parameters. Tamsulosin was found to be very effective, safe and well tolerated drug with no significant differences with 0.2 mg and 0.4 mg as far as the vital signs and adverse events were concerned. Quality of life improved among the patient prescribed with tamsulosin due to improved flow rate and decreased symptom score. Many of the patients in our hospital were prescribed tamsulosin for management of their features of prostatism. In our study about 70% of patients taking Tamsulosin showed significant overall improvement in both objective and subjective parameters.

**Conclusions**

The AUA symptom index score may be useful as a bothersomeness index in women with bladder outlet obstruction. A complete urodynamic evaluation is essential for making the diagnosis, although standard urodynamic definitions are still lacking. Further epidemiological and pathophysiological investigations are needed to evaluate the causes and risk factors for bladder outlet obstruction in women. Better understanding the pathophysiological

mechanisms associated with bladder outlet obstruction in women may provide the possibility of using appropriate diagnostic and treatment modalities, thus, avoiding any unnecessary intervention.

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**Table.1.Age group and gender wise distribution of Bladder outlet obstruction.**

| Age group (years) | Males | Percentage (%) | Mean age ± standard deviation | Females | Percentage (%) | Mean age ± standard deviation |
|---|---|---|---|---|---|---|
| 35-45 | 0 | 0 | 62.16 ±11.23 | 4 | 16 | 53.76 ± 9.418 |
| 45-55 | 26 | 17 | | 4 | 16 | |
| 55-65 | 59 | 39 | | 15 | 60 | |
| 65-75 | 46 | 30 | | 2 | 8 | |
| 75-85 | 18 | 12 | | 0 | 0 | |
| 85-95 | 3 | 2 | | 0 | 0 | |
| | **n=152** | | | **n=25** | | |

**Table.2.The distribution of obstructive and irritative urinary symptoms associated with bladder outlet obstruction.**

| Obstructive and irritative Urinary Symptoms | Occurrence of symptoms | Percentage (%) |
|---|---|---|
| Weak stream | 126 | 72 |
| Nocturia | 86 | 49 |
| Frequency | 79 | 45 |
| Dysuria | 67 | 38 |
| Straining to void | 42 | 24 |
| Intermittency | 40 | 23 |
| Hesitancy | 36 | 20 |
| Incomplete emptying | 34 | 19 |
| Dribbling | 12 | 6 |

**Table.3.Co morbidities associated with bladder outlet obstruction.**

| Co morbidities | Number of patients with co morbidities | Percentage (%) |
|---|---|---|
| Hypertension | 50 | 28 |
| Diabetes | 23 | 13 |
| Respiratory diseases | 10 | 6 |
| Cardiac diseases excluding | 9 | 5 |
| Parkinsonism | 4 | 2 |

**Table.4.Treatment pattern associated with bladder outlet obstruction.**

| Treatment pattern | Number of patients | Percentage (%) |
|---|---|---|
| Surgery | 120 | 68 |
| Medical treatment | 23 | 13 |
| Medical treatment followed by surgical procedures | 19 | 11 |
| Other treatment | 15 | 8 |
| **Total** | **n= 177** | |

**Table.5. Drug Class most commonly prescribed in patients with bladder outlet obstruction.**

| Drug class | No. of patients with drugs | Percentage (%) |
|---|---|---|
| Alpha blockers | 23 | 55 |
| Anticholinergics | 10 | 24 |
| Alpha blockers + 5-alpha reductase inhibitors | 6 | 14 |
| 5- alpha reductase inhibitors | 2 | 5 |
| Alpha blocker + 5-alpha reductase inhibitor + anticholinergics | 1 | 2 |
| | **Total n=42** | |

**Table.6.Drugs implicated in the medical treatment with bladder outlet obstruction.**

| Drugs implicated | No. of patients prescribed with drugs |
|---|---|
| Tamsulosin | 14 |
| Alfuzosin | 1 |
| Dutasteride | 1 |
| Tamsulosin + Dutasteride | 3 |
| Tolterodine | 1 |
| Bethanachol | 3 |
| | **Total 23** |

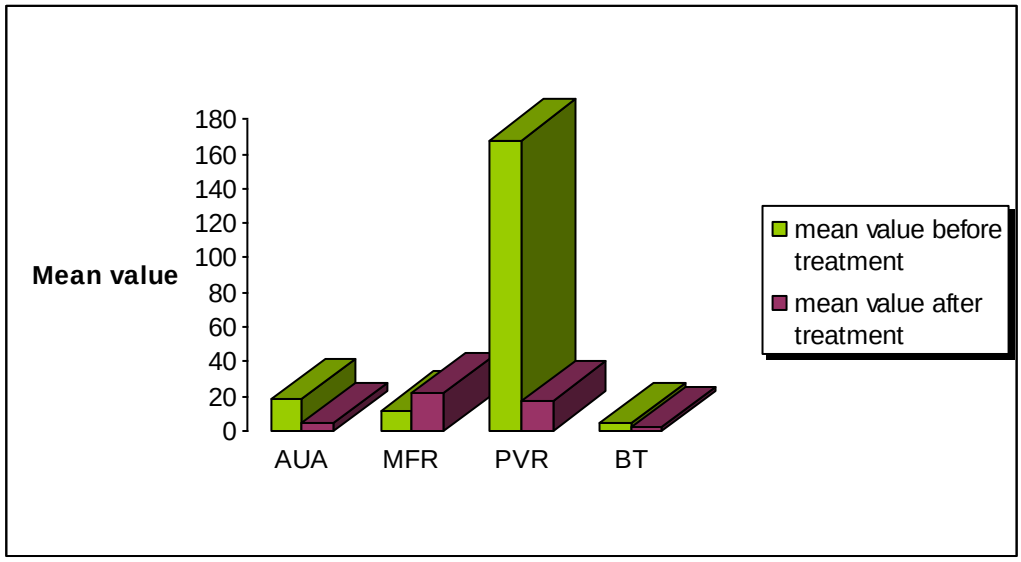
**Table.7.Surgical procedures performed in bladder outlet obstruction.**

| Surgical procedure | No. of patients underwent surgery | Percentage (%) |
|---|---|---|
| Transurethral resection of prostate | 110 | 79 |
| Cystoscopy + dilatation | 19 | 14 |
| Bladder neck resection | 10 | 7 |
| | **N=139** | |

**Table.8. Objective and subjective improvement for all types of treatment in bladder outlet obstruction.**

| Treatment type | AUASS (%) | MFR (%) | PVR (%) | BT (%) |
|---|---|---|---|---|
| Surgical | 69 | 70 | 74 | 78 |
| Medical | 15 | 16 | 15 | 11 |
| Medical followed by surgical | 13 | 9 | 10 | 9 |
| Other treatments | 3 | 5 | 1 | 2 |

**Figure 1 Safety and efficacy of tamsulosin in female patients with bladder outlet obstruction.**




- AUA - American Urological Association symptom score
- MFR – Maximum Flow Rate
- PVR – Post Void Residual Volume
- BT – Bladder Thickness

## SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF SOME NEW BENZOPYRONE DERIVATIVES

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**ABSTRACT**

A new series of benzopyrone derivatives were synthesized and the structure of these compounds was established on the basis of spectral data. The title compounds were evaluated for

antitubercular. Some of these compounds have shown excellent antitubercular activity.

**Keywords:**Benzopyrone, antitubercular, CHN analysis.

## INTRODUCTION

A number of natural and synthetic benzopyrone derivatives have been reported to exert anti microbial, anti tubercular and anti diabetic activity.[1] Benzopyrones having chromen (γ-benzopyrone) moiety are associated with interesting physiological activities such as anti microbial, anti tubercular, antidiaetic, antiviral, anticancer, anti-inflammatory [2] etc.In view of these observations and our interest in the synthesis of biologically active biheterocycles possessing benzopyrone nucleus we modified benzopyrone ring to explore activities associated with this nucleus & evaluated them for anti tubercular activity.

## Material and Methods

### ANTITUBERCULAR ACTIVITY: [3,5]

The antitubercular screening was carried out by Middle brook 7H9 agar medium against $H_{37}$Rv. Strain. Middle brook 7H9 agar medium containing different derivatives, standard drug as well as control, Middle brook 7H9 agar medium was inoculated with *Mycobacterium tuberculosis* of $H_{37}$Rv Strain. The inoculated bottles were incubated for 37°C for 4 weeks. At the end of 4 weeks they were checked for growth.

## EXPERIMENTAL

Melting points were determined in open capillary method and are uncorrected. Purity of the compound was checked on Silica gel TLC plates. IR spectra were recorded on Thermo Nicolate IR 200 spectrophotometer using KBr disc method. $^1$H NMR spectra were recorded on BRUKER amx-400, DMSO $d_6$ as internal standard. Combustion analyses were found to be within the limits of permissible errors.

### Synthesis of 6-Chloro-2-phenyl-4-benzopyron (II) [2]

To 36.6 g (0.13 mol) of 3-phenyl-4-chloro-2-acrylophenone in 325 ml methanol, 65 ml of 20 % aq. Sodium hydroxide solution was added & this mixture was cooled to 0 $^0$. To this mixture, 65 ml of 30% hydrogen peroxide was added dropwise maintaining temp. Below $10^0$. The contents were further stirred for 2 hr at temp below $10^0$ and then poured on crushed ice. The mixture was then neutralized with dil. hydrochloric acid. The solid product was filtered washed with ice-cold water, dried and recystallized from rectified spirit, m.p. 72-74$^0$, yield 73%.

### Synthesis of 6-Chloro-3-(4-chlorophenyl)-2-phenyl-4H-benzopyran-4-one (III) [4]

A solution of potassium hydroxide [5.6 g (100 mol) in 5 ml $H_2O$ ] was added to a mixture of 6-Chloro-2-phenyl-4-benzopyron (16.6 g, 100 ml) and the aromatic aldehydes (100 mmol) in ethanol (50 ml), and the reaction mixture was stirred for half an hr. The formed yellow precipitate was filtered off.

Washed thoroughly with water, dried and recrystallized from ethanol, m.p. 90-$92^0$, yield 64%.

**Synthesis of 8-Chloro-3-(chlorophenyl)-4-(Diphenyl , phenyl)-1, 2, 3, 4-tetrahydrobenzopyrano [4, 3] Pyrazole ($C_1$-$C_2$).**

A suspension of **III** (2 mmol) in glacial acetic acid 10 ml was treated with phenyl hydrazine/Hydrazine hydrate 0.2 ml (0.22 mmol). The reaction mixture was refluxed for 2 hr, poured into water and solid formed was separated and recystallized from ethanol.

**Synthesis of 8-Chloro-4-(4 chlorophenyl)-5-phenyl-3,4-dihydro-1H-benzopyron [4,3] pyrimidine-2 (5H)-thiones ($C_3$-$C_4$).**

To a mixture of **III** (10 mmol) and thiourea /urea 1 g (13 mmol) in ethanol (50 ml), there was added a solution of potassium hydroxide 1g (18 mmol) in H2O (1 ml). The mixture was refluxed for 4 hr, poured into water and the solid formed was filtered off, dried and recrystallized from dioxan.

Synthesis of 2-Amino-8-chloro-4-(4-chlorophenyl)-5-phenyl-5H-benzopyron (4, 3)-3-carbonitrile ($C_5$)

A few drops of triethylamine were added to a mixture of **III** 3g (10mmol), $CH_2(CN)_2$ (10mmol) and ammonium acetate 0.62 g (80 mmol) in absolute ethanol (50 ml). The reaction mixture was refluxed for 8 hrs, allowed to cool and poured gradually while stirring in cold water. The solid formed was collected, dried and recystallized from dioxan.

Synthesis of ($C_6$).

A mixture of **II** (10 mmol), $CH_2(CN)_2$, 0.66 g (10mmol) and β-alanine (50 mg ) in ethanol (25 ml) was refluxed for 3hrs. The reaction mixture was cooled and poured in cold water. The solid formed was washed with water, dried and recystallized from the ethanol.

**Result and Discussion**

Substituted acetophenones and substituted aldehydes were reacted to get the chalcones which were treated with $H_2O_2$ then with other intermediate to get the various derivatives. The structures of these compounds were confirmed by IR, NMR. and CHN analysis. These compounds were subjected to anti tubercular activity by using LJ media with $H_{37}R_v$ strain. Compounds $C_3$, $C_5$ and $C_6$ have found to be potential anti tubercular agent when compared with standard Streptomycin $C_1$, $C_4$, $C_2$ have shown moderate anti tubercular activity. With suitable molecular modification these compounds may prove as potent anti tubercular drug in future.

**Acknowlegement**

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**SCHEME**

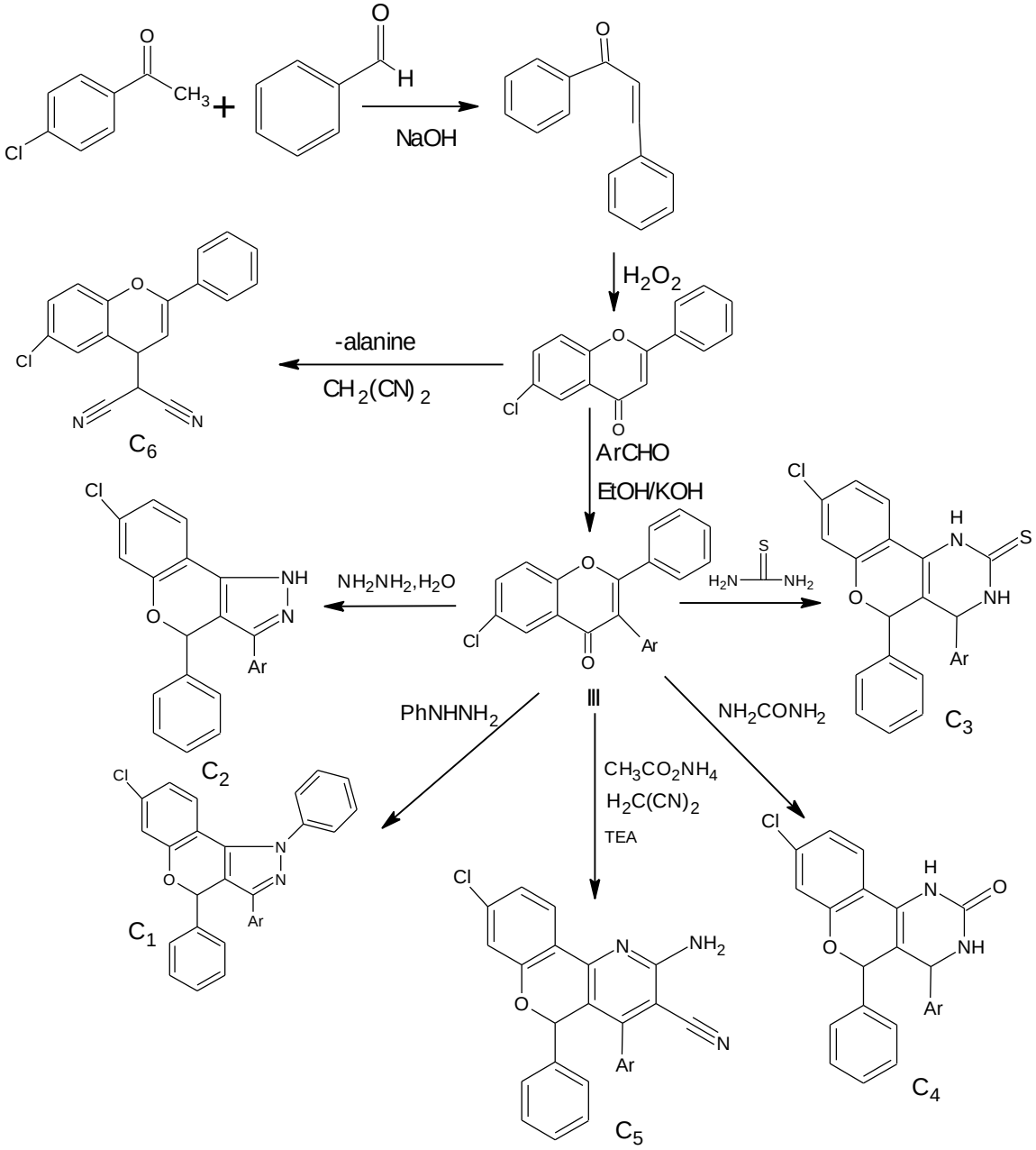
NaOH
$H_2O_2$
-alanine
$CH_2(CN)_2$
ArCHO
EtOH/KOH
$NH_2NH_2,H_2O$
$PhNHNH_2$
$NH_2CONH_2$
$CH_3CO_2NH_4$
$H_2C(CN)_2$
TEA
III
$C_1$
$C_2$
$C_3$
$C_4$
$C_5$
$C_6$

**Table no.1: Analytical data of the synthesized compounds ($C_1$-$C_5$)**

| Compd. | Mol. Formula | Mol. Wt. | Yield % | m.p. ° C | Elemental analyses Calcd. (Found) | | |
|---|---|---|---|---|---|---|---|
| | | | | | C | H | N |
| **$C_1$** | $C_{22}H_{14}N_2OCl_2$ | 469.36 | 88 | 132 | 71.65 | 3.87 | 5.97 |
| **$C_2$** | $C_{28}H_{18}N_2OCl_2$ | 393.26 | 86 | 104 | 67.19 | 3.59 | 7.12 |
| **$C_3$** | $C_{23}H_{17}N_2OSCl_3$ | 475.81 | 56 | 178 | 58.06 | 3.60 | 5.89 |
| **$C_4$** | $C_{23}H_{16}N_2O_2Cl_2$ | 423.29 | 64 | 111 | 65.26 | 3.81 | 6.62 |
| **$C_5$** | $C_{25}H_{15}N_3OCl_2$ | 444.31 | 48 | 108 | 67.58 | 3.40 | 9.46 |
| **$C_6$** | $C_{18}H_{11}N_2OCl$ | 306.74 | 32 | 114 | 70.48 | 3.61 | 9.13 |

The combustion analysis of compounds synthesized is within the limits of permissible errors.

**Table No.2: Anti-tubercular activity of the synthesized compounds:**

| SL. No. | *Compounds* | 25 *µcg* /mL | 50 *µcg* /mL |
|---|---|---|---|
| 1. | **$C_1$** | R | S |
| 2. | **$C_2$** | R | S |
| 3. | **$C_3$** | **S** | S |
| 4. | **$C_4$** | R | S |
| 5. | **$C_5$** | **S** | S |
| 6. | **$C_6$** | S | S |
| **STD.** | **Streptomycin** | **S** | **S** |

**R**- Resistance; **S**- Sensitive

**SPECTRAL DATA**

$C_1$:IR (KBr) $cm^{-1}$ : 3249 (N-H str.), 3055 (C-H str.Ar),1500 (C-C str.Ar),1331 (C-Ostr.), 1255 (C-N str.), 762 (Cl).$^1$ H NMR (d ppm): 6.91-7.3(17 H ,m,Ar-H),5.55(1H,s,CH),3.0(1H, s,CH), 2.5(1H,s,NH).

$C_2$: IR (KBr) $cm^{-1}$ : 3276 (N-H str.), 3062 (C-H str.Ar),1517 (C-C str.Ar),1358 (C-Ostr.), 1219 (C-N str.), 764 (Cl).

$C_3$:$^1$H NMR (d ppm): 6.92-7.38(12 H, m, Ar-H), 5.56 (1H,s, CH), 4.51 (1H, s,CH), 9.72-12.01(2H,s,NH).

$C_5$:IR (KBr) $cm^{-1}$ : 3232 (N-H str.), 3055 (C-H str.Ar),1680 (C=O str.),1515 (C-C str.Ar),1331 (C-Ostr.), 1254 (C-N str.), 761 (Cl).$^1$ H NMR (d ppm): 7.0-8.12 (12 H ,m,Ar-H),6.12 (1H,s,CH), 6.79 (2H, s,CH).

# GARLIC: A WONDER NUTRACEUTICAL

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**History**

Thousand years ago garlic (*Allium sativum)* has been used as a food product and known for its medicinal values by civilizations around the world. Old existing corpus can supports its use in Chinese, Egyptian, French & Ayurvedic medicine. Garlic is indigenous to central Asia, where people live the longest, and the occurrence of cancer is the lowest known is not a seer coincidence. Garlic was included in the diet of the slaves for strength and endurance who built the pyramids in Egypt.[13] Evidence shows that Egyptians worshiped garlic, they placed clay models of the bulb in Tutankhamen's tomb. It is said that Hippocrates himself used garlic vapors to treat certain cancers.[11]

It was included as one of the first medicines in the *Codex Ebers*, a 1550 B.C. papyrus that consider the oldest medical text, and was mentioned even earlier in clay, cuneiform tablets from the library of Nineveh in Mesopotamia. Pliny, in his *Natural History* listed garlic as a remedy for sixty-one ailments.[16] Egyptian medical papers dating back to 1550 B.C. mentioned about eight hundred formulas for the therapeutic uses of garlic.[19] Gravediggers in early eighteenth-century in France drank a concoction of crushed garlic in wine to protect themselves from getting the plague that killed many people in Europe. During World Wars I and II, soldiers were given garlic to prevent gangrene.[2]

**Regulatory Status**[22]

US: generally recognized as sage
UK: included in general sales list
Canada: over-the-counter drug status
France: traditional medicinal use
Germany: commission E approved as over-the counter drug

**Medicinal value**

It is found that garlic (pulp) contains more than 200 chemical compounds including volatile oil with sulphur-containing compounds like allicin, alliin, and ajoene. It also contains enzymes such as allinase, peroxidase and myrosinase. It is considered that Allicin is responsible for antibiotic properties and strong odor; it also shows fibrinolytic activity which reduces platelet aggregation by inhibiting prostaglandin E2. Ajoene also contributes to the anticoagulant action of garlic. Apart from this garlic also contains citral, geraniol, linalool, Aphellandrene and B phellandrene. The allyl contained in garlic is also found in several members of the onion family and is considered a very valuable therapeutic compound.[22] Alliin and diallyl dysulphur are highly unstable substances and melt easily into liquids and gases. When transported by the blood, they infuse all tissues and organs of the body, thus they act on the whole body.[13]

*Allium sativum* pulp contains vitamins especially B-1, vitamin C, vitamin A, flavonoids, ascorbic acid, phosphorous, potassium, sulphur, selenium, calcium, magnesium, germanium, sodium, iron, manganese and trace iodine. Seventeen

amino acids are found in it, including eight essential ones.[22]

**Garlic as nutraceutical**

It is evident that garlic has been used as a food product across the globe. Today it became inseparable part of our diet. Scholars around the world have proved garlic for its medicinal use in treatment & prevention of certain diseases. It possesses both curative and preventative properties; new focus is on its use in prevention of heart attack and cancer. By studying these properties of garlic it can be categorised as nutraceuticals.

**Clinical applications**

From centuries garlic is being used in the treatment of bronchitis, respiratory problems, gastrointestinal problems, flatulence, leprosy, menstrual cramps, high blood pressure, diabetes and externally for warts, corns, arthritis, muscle pain, neuralgia and sciatica. In Ayurvedic medicine garlic is considered heating, diuretic, diaphoretic (enduces sweating), expectorant, carminative, anti-coagulant, anthelmintic and immune-enhancing. Homeopathically, garlic is used to treat upper respiratory tract inflammation, rheumatism and digestive problems.[16] Researchers are studying the use of garlic in prevention and treatment of several diseases.

**In cardiovascular diseases**

Dioscorides was a well-known first century physician who wrote that garlic "clears the arteries and opens the mouths of veins."[11] Today, studies around the globe have suggested that garlic consumption can reduce the risk of heart disease caused by the hardening of the arteries.[14] Garlic is considered good for the health of heart because it lowers the cholesterol and blood fats called triglycerides in the bloodstream. According to Yu-Yan Yeh, Ph.D., professor of nutrition science at Pennsylvania State University in University Park, many of garlic protective effects take place in the liver, where cholesterol is produced. In laboratory studies, rats given garlic extract produced 87% less cholesterol and 64% fewer triglycerides. In a review of 16 studies involving 952 people, British researchers found that eating garlic, whether fresh or in powdered form lowered total cholesterol an average of 12 to 13%.[21]Thirty years of research has shown garlic to be affective in reducing cholesterol levels. It is known to reduce systolic blood pressure and lower the blood sugar.[20]

Sulfur compounds of garlic, including diallyldisulfide (DADS) which seem to help smooth blood flow by preventing platelets from sticking together and clotting. In a Brown University study, researchers gave 45 men with high cholesterol aged garlic extract (the equivalent of about 5-6 cloves of fresh garlic). When they examined the men's blood, they saw that the rate at which platelets clumped and stuck together had dropped anywhere from 10 - 58%.[21]

It decreases the level of LDL cholesterol in the blood, because it makes its absorption by the intestine more difficult. It has been proven that in the hours following a breakfast of toast with butter, the level of cholesterol increases 20%, however when the bread is rubbed with garlic, even if it has butter, this increase does not take place.[13] Garlic also has been shown to protect blood vessels from the deleterious effects of free radicals. This antioxidative

activity has also been linked to its blood cholesterol-lowering action and its ability to decrease deposits of cholesterol on the walls of blood vessels.[5]

A garlic-supplementation trial on 432 patients over a three-year period done by Arun Bordia, M.D. a cardiologist at India's Tagore Medical College, shows that 10-percent drop in blood cholesterol and in blood pressure, and less expressions of angina in the population who ate garlic daily. While the non-garlic eaters saw no cardiovascular changes.[5]

**As an anticancer**

Recent researches have supported the fact that garlic shows excellent potential in the prevention of cancer. Laboratory tests on animal studies suggest that garlic may have some anti-cancer activity. Population-based observation studies suggest that people who have more raw or cooked garlic in their diet are less likely prone to colon, stomach breast, prostate, and laryngeal cancers.[2] Researchers have found that certain enzymes contained in some cancers are totally inhibited by alliinase and other compounds contained in garlic. Several Japanese experiments suggest that injecting garlic into rats with certain types of sarcoma blocked tumor cell reproduction and caused mutations in the cancer cells themselves. Garlic's role in simulating the body's immune defenses may also be linked to cancer control and prevention, in laboratory experiment, the natural killer cells of garlic-eating subjects destroyed 159 percent more tumor cells than those who had not consumed garlic. [22]

A 1994 study done on 41,000 women who consumed a weekly serving of garlic demonstrated a 35% decrease in the risk of colon cancer.[20] In animal studies by Weisberger and Pensky of Western Reserve University, as reported in *Science,* mice injected with cancer cells died within 16 days. When cancer cells were treated with Garlic extract and injected into the animals, no deaths occurred for a period of 6 months. In other studies, feeding fresh Garlic to female mice completely inhibited the development of mammary tumors. It is considered that the high germanium content of garlic may also play a role in cancer treatment and prevention. [22] The ajoene in garlic is showing some promise in the treatment of skin cancer.[18]

According to John Milner, Ph.D., professor and head of the department of nutrition at the Pennsylvania State University, s-allycysteine of garlic appears to stop the metabolic action that causes a healthy cell to become cancerous. The substance called DADS chokes cancer cells until their numbers are reduced and they start dying. Another substance in garlic is diallyl trisulfide (DATS), which is 10 times more powerful than DADS at killing human lung cancer cells. Its effectiveness is comparable to that of 5 flluorouracil, a widely used chemotherapy agent. In his view, garlic contains compounds that help prevent nitrites, common substances found in some foods and pollutants from transforming themselves into nitrosamines, harmful compounds that can trigger cancerous changes in the body[21]

**As an antibiotic**

Louis Pasteur first verified garlic's antibacterial properties scientifically in 1858, and showed how it killed bacteria under laboratory conditions.[16] Several modern extensive studies confirm that

garlic has definite antibiotic properties and is effective against many bacteria, fungi and viruses. According to Wright State University, garlic is approximately one per cent as potent an antibiotic as penicillin. The significant advantage of using garlic as antibiotic is that, the body does not seem to build up a resistance to it as it does to many modern antibiotics.[1] Garlic is effective against diverse types of fungi, yeasts, and some viruses, including herpes. The active principles of garlic are supposed to interact with the nucleic acids of the virus, thus limiting its proliferation.[13]

Researchers around the globe are extensively studying the role of Garlic in enhancing the immune system, as antidiabetic, and anti oxidant ability to neutralize damaging free radicals before they can harm healthy cells.

**As an antidiabetic**

Few studies suggest that garlic may have some mild blood-sugar-lowering properties. It is believed that garlic lower blood sugar levels by decreasing the rate at which insulin is inactivated and degraded by the body, effectively increasing quantities of circulating insulin and decreasing blood glucose levels. Overall, these effects do not appear to be strong enough to warrant use of garlic as a blood-sugar-lowering agent.[45] In general, sulfur compounds in garlic are believed to exert hypoglycemic activity by competing with insulin.[44]

**Safety**

Garlic may increase bleeding, especially in patients already taking certain anti-clotting medications. Rarely, gastrointestinal upset symptoms are considered as possible side effects.[32] It is advised that people taking warfarin, regular doses of aspirin, or other blood thinners should not use garlic for anything other than seasoning.[35]

At the National Institutes of Health's (NIH) HIV clinic, one woman who was on ritonavir (Norvir) treatment and then started garlic supplements was developed severe nausea and vomiting, which resolved after stopping the garlic. It is considered that the garlic may have increased the levels of ritonavir. It was also supported in one more case study. Still it is unclear, if garlic was increasing the risks of ritonavir related side effects or if it was the actual cause of them. Subsequently, small single-dose studies of ritonavir and garlic do not suggest a serious herb-drug interaction.[36]

Garlic may also increase the risk of side effects associated with other anti-HIV drugs. It is assumed that garlic has an effect on p450 enzyme. Garlic supplements should not be used while taking saquinavir as the sole protease inhibitor due to the risk of decreased saquinavir plasma concentrations.[30] Moreover, people using the supplement with anti HIV drugs who experience serious stomach problems might consider stopping it to see if these symptoms lessen.[36]

Garlic may intensify the effects of drugs that decrease blood sugar levels (hypoglycemic drugs, such as insulin and glipizide) causing an excessive decrease in blood sugar levels (hypoglycemia).[25]

**Dosage and dosage form**

Garlic supplement preparations are available in oil, extract, powder, capsules and tablet forms. It is observed that chemical composition of these preparations may not mirror the

composition of fresh garlic clove. Hence it is always advisable to take daily dose equivalent to 4 g[41] of fresh garlic cloves, which is about the size of one average clove of garlic. Average daily dosage: fully-dried powder, 400-1200 mg; fresh (air-dried) bulb, 2-5 g; garlic oil, 2-5 mg. Fully-dried powder, 400-1200 mg, fresh (air-dried) bulb, 2-5 g; garlic oil, 2-5 mg.[28]

**Conclusion**

Garlic is being used from thousand of years for its medicinal properties. Numerous researches have proved its beneficial role in cardiovascular condition. Indeed, garlic does indeed have cardio-protective properties. Researches also proved its active role as anticancer, natural immunity booster, antioxidant, antibiotic & antidiabetic product. On other hand studies also report some side effects of garlic if it is used with blood-thinners, anti HIV, or hypoglycemic drugs. This observation suggests that more research is needed in safety and active use of garlic as a Nutraceutical.

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