

Contents

| Subjects | Page |
|--|------|
| List of abbreviations..... | II |
| List of Figures | VII |
| List of Tables | IX |
| • Aim of the work | 1 |
| • Review of Literature | |
| ♦ General introduction | 2 |
| ♦ Snake Venom Phospholipase A ₂ | 9 |
| ♦ Physiological Importance of PLA ₂ enzymes | 11 |
| ♦ Pharmacological Effects of Venom Phospholipase A ₂ Enzymes | 13 |
| ♦ Target Model and Pharmacological Specificity | 21 |
| ♦ Role of Enzymatic Activity in Pharmacological Effects | 23 |
| ♦ Classification of PLA ₂ Enzymes | 29 |
| ♦ Structure of PLA ₂ Enzymes | 35 |
| ♦ PLA ₂ and Its Complexes | 37 |
| ♦ Purification of PLA ₂ Enzymes | 44 |
| ♦ Mechanism of Catalysis | 45 |
| • Material and Methods | 50 |
| ♦ Crude venom | 50 |
| ♦ Protein estimation: Ultraviolet absorption method | 53 |
| ♦ Screening of the Phospholipase A ₂ activity for protein fractions obtained from various purification steps | 53 |
| ♦ Fractionation of crude Naja nigricollis venom and identification of the Phospholipase A ₂ activity: | |

| | |
|---|-----|
| i. Gel filtration chromatography on sephadex G-75..... | 57 |
| ii. Ion exchange chromatography..... | 62 |
| ◆ Determination of the purity of fraction Ib and its molecular weight by Disc SDS-PAGE..... | 65 |
| ◆ Assessment of carbohydrate content in fraction Ib..... | 70 |
| ◆ Determination of optimum pH of Phospholipase A ₂ fraction Ib..... | 72 |
| ◆ Effect of some cations on Phospholipase A ₂ activity of fraction Ib..... | 74 |
| ● Effect of some inhibitors on Phospholipase A ₂ activity of fraction Ib..... | 76 |
| ● Effect of adding CaCl ₂ on Phospholipase A ₂ activity of fraction Ib pre-incubated with EDTA..... | 78 |
| ● Results | 81 |
| ● Discussion | 104 |
| ● Recommendations | 119 |
| ● Summary | 120 |
| ● References | 123 |
| ● Arabic Summary | |

List of Abbreviations

| | |
|----------------------------------|---|
| AA | : Arachidonic Acid |
| AR | : Acrosome reaction |
| ART | : Assisted reproductive technology |
| Ba² | : Barium cation |
| C. adamanteus | : Calloselasma adamanteus snake |
| C. durissus collilineatus | : Crotalus durissus collilineatus snake |
| C. scutulatus scutulatus | : Crotalus scutulatus scutulatus snake |
| C. viridis concolor | : Crotalus viridis concolor snake |
| CAPT | : Crotoxin acceptor protein from <i>Torpedo</i> |
| cPLA₂ | : Cytosolic phospholipases A ₂ |
| CRISPs | : Cysteine rich secretory proteins |
| DEAE Sephadex | : Diethyl amino ethyl - sephadex |
| DEAE-Sephadex | : Diethyl amino ethyl-sephadex |
| E.C. NO | : Enzyme classification number |
| iPLA₂ | : Ca ²⁺ independent PLA ₂ |
| IVF | : In vitro fertilization |
| lp-PLA₂ | : Lipoprotein-associated PLA ₂ s |
| m.A | : Milliamber |
| mGX | : Mouse sperm, group X |

| | |
|-----------------------------|---|
| MiPLA1 | : Micropechis ikaheka PLA1 |
| MMP-2 | : Matrix metalloproteinase |
| nm | : Nanometer |
| OHVA-PLA₂ | : <i>Ophiophagus hannah</i> venom acidic PLA ₂ |
| PAF-AH | : Platelet activating factor acetylhydrolase |
| PAGE | : Polyacrylamide gel electrophoresis |
| p-BPB | : P-bromophenyl bromide |
| pI | : Isoelectric point |
| PLA₂ | : Phospholipase A ₂ |
| PLA₂s | : Phospholipases A ₂ |
| SDS | : Sodium dodecyl sulphate |
| SDS-PAGE | : Sodium dodecyl sulfate |
| sPLA₂ | : Secreted phospholipase A ₂ |
| Sr²⁺ | : Strontium |
| Sr²⁺ | : Strontium cation |
| TCBP-49 | : Taipoxin-associated Ca ²⁺ binding protein |
| β-Btx | : β-bungarotoxin |

List of Figures

| <u>No.</u> | <u>Figure</u> | <u>Page</u> |
|------------|---|-------------|
| <u>1</u> | <i>Naja nigricollis</i> snake. | 4 |
| <u>2</u> | Venom producing apparatus of snake | 6 |
| <u>3</u> | Enzymatic contribution of a PLA ₂ enzyme to observed pharmacological effects. | 24 |
| <u>4</u> | Non enzymatic mechanism of a PLA ₂ enzyme that produces pharmacological effects. | 25 |
| <u>5</u> | Three-dimensional structures of snake venom PLA ₂ enzymes generated and modified using ViewerLite software. | 32 |
| <u>6</u> | Schematic representation of PLA ₂ complexes. | 38 |
| <u>7</u> | Disc gel electrophoresis apparatus. | 69 |
| <u>8</u> | Standard curve of lecithin. | 81 |
| <u>9</u> | Gel filtration chromatography of crude <i>Naja nigricollis</i> venom (100 mg), carried out on sephadex G-75 column (1.5 X 87 cm), equilibrated with ammonium acetate buffer (0.02 M, pH 4.8) and eluted by downward flow at a rate of 3 drops/min at 4°C. | 82 |
| <u>10</u> | Fraction Va, 2ml (3.5 mg/ml), obtained from gel filtration chromatography, was applied on DEAE sephadex A-50 (1.5 x 20 cm). | 85 |
| <u>11</u> | Disc SDS- PAGE of fraction Ib obtained from ion exchange chromatography. | 89 |
| <u>12</u> | Standard curve for sucrose. | 91 |
| <u>13</u> | Optimum pH of PLA ₂ activity of fraction Ib. | 94 |

| <u>No.</u> | <u>Figure</u> | <u>Page</u> |
|-------------------|---|--------------------|
| <u>14</u> | Effect of temperature on PLA ₂ activity of fraction Ib. | 96 |
| <u>15</u> | PLA ₂ Michaelis–Menten kinetics curve. | 102 |
| <u>16</u> | PLA ₂ Lineweaver-Burk plot curve. | 103 |
| <u>17</u> | Reaction catalyzed by phospholipase A ₂ (PLA ₂). | 104 |

List of Tables

| <u>No.</u> | <u>Table</u> | <u>Page</u> |
|------------|--|-------------|
| <u>1</u> | Screening fractions obtained from gel filtration chromatography for PLA ₂ activity | 84 |
| <u>2</u> | Screening fractions obtained from Ion exchange chromatography for PLA ₂ activity. | 87 |
| <u>3</u> | Summary of purification steps of PLA ₂ from Naja Nigricollis venom. | 88 |
| <u>4</u> | Determination of carbohydrate content in fraction Ib. | 92 |
| <u>5</u> | Determination of optimum pH of PLA ₂ activity of fraction Ib. | 93 |
| <u>6</u> | Effect of temperature on PLA ₂ activity of fraction Ib. | 95 |
| <u>7</u> | Effect of some cations on PLA ₂ activity of fraction Ib. | 97 |
| <u>8</u> | Effect of some inhibitors on PLA ₂ activity of fraction Ib. | 98 |
| <u>9</u> | Effect of adding metal ions on PLA ₂ activity of fraction Ib pre-incubated with EDTA. | 100 |



Aim of the Work





Review of Literature





Materials and Methods





Results





Discussion





Recommendations





Summary





References

