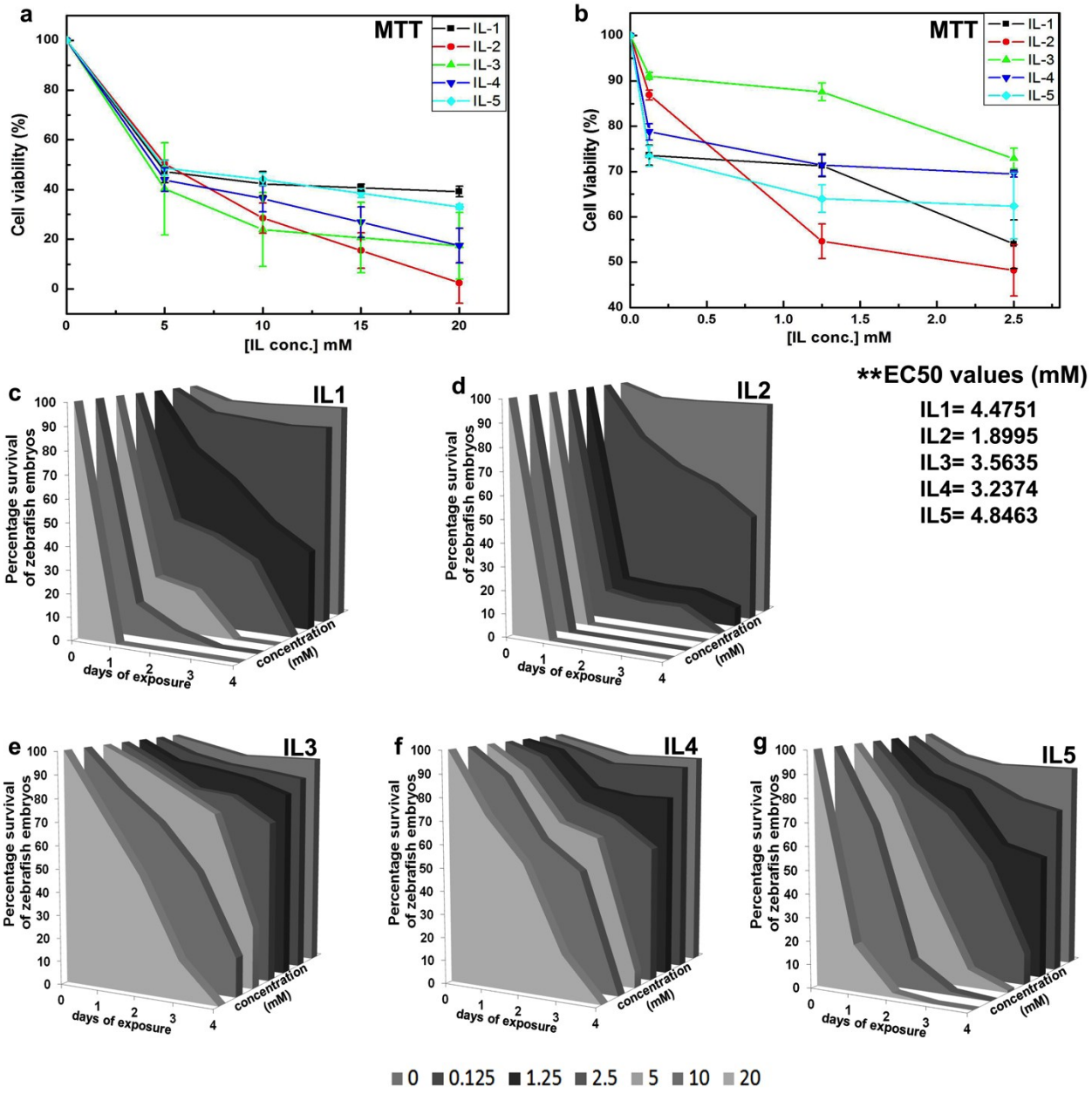
Electronic Supplementary Material (ESI) for RSC Advances.


# Supplementary Information




**Supplementary Figure S1. Effects due to acute and prolonged exposure to ILs ,on human breast adenocarcinoma cell line MCF-7 and zebrafish embryos** (a) Cell viability was measured using an MTT Assay (IL concentrations, ≤20mM). (b) Cell viability was measured using an MTT Assay (Lower IL concentrations, ≤2.5mM). For this, human breast adenocarcinoma cell lines MCF-7 were treated for 24 hours with the desired IL concentration gradient. Cell viability was calculated as the percentage of viable cells which were treated with ILs versus untreated cells. Origin was used for data anylsis (a-b). (c-g) Embryos were exposed to different concentrations of ILs from day 0 (6 hour old embryos) to day 4. The concentrations of each ionic liquid in water were 0.125mM, 1.25mM, 2.5mM, 5mM, 10mM and 20mM and were compared with 0mM IL (water). The embryos alive were counted each day and calculated as a percentage of total embryos at the beginning of the experiment. In each experiment, each condition or concentration the number (N) of embryos used was >50. Each experiment was repeated on three or more independent occasions. Average from >=3 experiments were calculated for analysis.3D wall graphs were plotted using MS Excel.

**Average percentage alive and standard error values of Fig. S1(c-g) (Effects due to acute and prolonged exposure to ILs ,on zebrafish embryos) have been listed in Table (S1-S5)**

**Table S1. 1-butyl-3-methylimidazolium tetrafluoroborate(IL1)**

| 1-butyl-3-methylimidazolium tetrafluoroborate(IL1) | | T=150 | Average percentage alive(%) | Standard Error |
|---|---|---|---|---|
| Days of exposure | IL1 Concentration (mM) | N | | |
| Day 1 | 0 | 141 | 94 | 3.05 |
| | 0.125 | 135 | 90 | 2.31 |
| | 1.25 | 113 | 75.33 | 4.05 |
| | 2.5 | 68 | 45.33 | 13.13 |
| | 5 | 34 | 22.67 | 7.86 |
| | 10 | 21 | 14 | 5.77 |
| | 20 | 0 | 0 | 0 |
| Day 2 | 0 | 140 | 93.33 | 2.67 |
| | 0.125 | 132 | 88 | 4.16 |
| | 1.25 | 94 | 62.67 | 7.33 |
| | 2.5 | 60 | 40 | 10.58 |
| | 5 | 28 | 18.67 | 6.36 |
| | 10 | 7 | 4.67 | 4.67 |
| | 20 | 0 | 0 | 0 |
| Day 3 | 0 | 140 | 93.33 | 2.67 |
| | 0.125 | 129 | 86 | 6.11 |
| | 1.25 | 68 | 45.33 | 22.7 |
| | 2.5 | 46 | 30.67 | 15.38 |
| | 5 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 |
| Day 4 | 0 | 140 | 93.33 | 2.67 |
| | 0.125 | 129 | 86 | 6.11 |
| | 1.25 | 51 | 34 | 17.09 |
| | 2.5 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 |

**Note:**
**T-Total number of zf embryos used (for all experiments)**
**N- Number of zf enbryos alive (from all experiments)**

**Table S2. 1-butyl-3-methylimidazolium hexafluorophosphate(IL2)**

| 1-butyl-3-methylimidazolium hexafluorophosphate (IL2) | | T=150 | Average percentage alive(%) | Standard Error |
|---|---|---|---|---|
| Days of exposure | IL2 Concentration (mM) | N | | |
| Day 1 | 0 | 141 | 94 | 3.05 |
| | 0.125 | 116 | 77.33 | 7.05 |
| | 1.25 | 22 | 14.67 | 7.69 |
| | 2.5 | 18 | 12 | 6.43 |
| | 5 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 |
| Day 2 | 0 | 140 | 93.33 | 2.67 |
| | 0.125 | 98 | 65.33 | 10.91 |
| | 1.25 | 20 | 13.33 | 6.76 |
| | 2.5 | 14 | 9.33 | 4.81 |
| | 5 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 |
| Day 3 | 0 | 140 | 93.33 | 2.67 |
| | 0.125 | 87 | 58 | 8.08 |
| | 1.25 | 20 | 13.33 | 6.76 |
| | 2.5 | 14 | 9.33 | 4.81 |
| | 5 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 |
| Day 4 | 0 | 140 | 93.33 | 2.67 |
| | 0.125 | 68 | 45.33 | 6.96 |
| | 1.25 | 12 | 8 | 4.62 |
| | 2.5 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 |

**Note:**
**T-Total number of zf embryos used (for all experiments)**
**N- Number of zf enbryos alive (from all experiments)**

**Table S3. 1-Butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide (IL3)**

| 1-Butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide (IL3) | | T=150 | Average percentage alive(%) | Standard Error |
|---|---|---|---|---|
| Days of exposure | IL3 Concentration (mM) | N | | |
| Day 1 | 0 | 144 | 96 | 1.15 |
| | 0.125 | 140 | 93.33 | 2.4 |
| | 1.25 | 137 | 91.33 | 3.53 |
| | 2.5 | 137 | 91.33 | 3.53 |
| | 5 | 138 | 92 | 2 |
| | 10 | 125 | 83.33 | 1.76 |
| | 20 | 112 | 74.67 | 7.42 |
| Day 2 | 0 | 138 | 92 | 4 |
| | 0.125 | 134 | 89.33 | 4.05 |
| | 1.25 | 133 | 88.67 | 3.71 |
| | 2.5 | 122 | 81.33 | 10.09 |
| | 5 | 124 | 82.67 | 6.36 |
| | 10 | 105 | 70 | 7.57 |
| | 20 | 75 | 50 | 6.93 |
| Day 3 | 0 | 138 | 92 | 4 |
| | 0.125 | 131 | 87.33 | 5.92 |
| | 1.25 | 128 | 85.33 | 6.36 |
| | 2.5 | 119 | 79.33 | 12.02 |
| | 5 | 110 | 73.33 | 9.33 |
| | 10 | 76 | 50.67 | 12.67 |
| | 20 | 25 | 16.67 | 11.21 |
| Day 4 | 0 | 138 | 92 | 4 |
| | 0.125 | 127 | 84.67 | 4.37 |
| | 1.25 | 119 | 79.33 | 4.37 |
| | 2.5 | 103 | 68.67 | 6.96 |
| | 5 | 40 | 26.67 | 3.33 |
| | 10 | 25 | 16.67 | 8.35 |
| | 20 | 0 | 0 | 0 |

**Note:**
**T-Total number of zf embryos used (for all experiments)**
**N- Number of zf enbryos alive (from all experiments)**

**Table S4. 1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (IL4)**

| 1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (IL4) | | T=150 | Average percentage alive(%) | Standard Error |
|---|---|---|---|---|
| Days of exposure | IL4 Concentration (mM) | N | | |
| Day 1 | 0 | 144 | 96 | 1.15 |
| | 0.125 | 143 | 95.33 | 1.33 |
| | 1.25 | 144 | 96 | 1.15 |
| | 2.5 | 141 | 94 | 1.15 |
| | 5 | 134 | 89.33 | 2.4 |
| | 10 | 132 | 88 | 3.05 |
| | 20 | 110 | 73.33 | 1.76 |
| Day 2 | 0 | 138 | 92 | 4 |
| | 0.125 | 134 | 89.33 | 3.71 |
| | 1.25 | 120 | 80 | 3.05 |
| | 2.5 | 113 | 75.33 | 2.67 |
| | 5 | 102 | 68 | 2.31 |
| | 10 | 94 | 62.67 | 2.9 |
| | 20 | 82 | 54.67 | 3.53 |
| Day 3 | 0 | 138 | 92 | 4 |
| | 0.125 | 134 | 89.33 | 3.71 |
| | 1.25 | 116 | 77.33 | 1.76 |
| | 2.5 | 104 | 69.33 | 2.4 |
| | 5 | 94 | 62.67 | 2.9 |
| | 10 | 76 | 50.67 | 8.51 |
| | 20 | 28 | 18.67 | 10.41 |
| Day 4 | 0 | 138 | 92 | 4 |
| | 0.125 | 134 | 89.33 | 3.71 |
| | 1.25 | 116 | 77.33 | 1.76 |
| | 2.5 | 86 | 57.33 | 7.69 |
| | 5 | 11 | 7.33 | 7.33 |
| | 10 | 0 | 0 | 0 |
| | 20 | 0 | 0 | 0 |

**Note:**
**T-Total number of zf embryos used (for all experiments)**
**N- Number of zf enbryos alive (from all experiments)**

**Table S5. 1-Butyl-4-methylpyridinium tetrafluoroborate (IL5)**

| 1-Butyl-4-methylpyridinium tetrafluoroborate (IL5) | | T=200 or(¥=250) | Average percentage alive(%) | Standard Error |
|---|---|---|---|---|
| Days of exposure | IL5 Concentration (mM) | N | | |
| Day 1 | 0 | 228¥ | 91.2 | 3.26 |
| | 0.125 | 178 | 89 | 5.07 |
| | 1.25 | 170 | 85 | 4.43 |
| | 2.5 | 164 | 82 | 4 |
| | 5 | 157 | 78.5 | 3.5 |
| | 10 | 136 | 68 | 1.82 |
| | 20 | 50¥ | 20 | 12.26 |
| Day 2 | 0 | 219¥ | 87.6 | 3.49 |
| | 0.125 | 166 | 83 | 3.41 |
| | 1.25 | 158 | 79 | 3.11 |
| | 2.5 | 141 | 70.5 | 4.11 |
| | 5 | 81 | 40.5 | 9.74 |
| | 10 | 25 | 12.5 | 7.32 |
| | 20 | 5¥ | 2 | 2 |
| Day 3 | 0 | 219¥ | 87.6 | 3.49 |
| | 0.125 | 149 | 74.5 | 5.79 |
| | 1.25 | 113 | 56.5 | 18.98 |
| | 2.5 | 100 | 50 | 16.85 |
| | 5 | 15 | 7.5 | 4.5 |
| | 10 | 3 | 1.5 | 1.5 |
| | 20 | 0¥ | 0 | 0 |
| Day 4 | 0 | 219¥ | 87.6 | 3.49 |
| | 0.125 | 141 | 70.5 | 7.13 |
| | 1.25 | 104 | 52 | 18.27 |
| | 2.5 | 27 | 13.5 | 7.89 |
| | 5 | 2 | 1 | 1 |
| | 10 | 0 | 0 | 0 |
| | 20 | 0¥ | 0 | 0 |

**Note:**
**T-Total number of zf embryos used (for all experiments)**
**N- Number of zf enbryos alive (**from **all experiments)**

**Table S6. Primers for quantitative real time RT PCR**

| Gene | Sequence | |
|---|---|---|
| | Forward Primer | Reverse Primer |
| *nppb* | 5′- CATGGGTGTTTTAAAGTTTCTCC-3′ | 5′- CTTCAATATTTGCCGCCTTTAC-3′ |
| *lfabp* | 5′- GAGCCATCTCTCTGCCAGAAGA-3′ | 5′- GGAGTTTTGGAGGTGATGGTGA-3′ |
| *hepcidin* | 5′- TCTGGCTGCTGTCGTCAT-3′ | 5′- TGGTTCTCCTGCAGTTCTTCAC-3′ |