4.0 Results

4.1 Plant protein extraction via ammonium sulfate precipitation

As shown in Figure 4.1, extracts from different types of leaves demonstrated three major peaks at different percentage of ammonium sulfate. Large, medium and small type leaf showed the most prominent peak in 50%, 20% and 40% fraction respectively. The fruits showed an ascending absorbance in different percentile of ammonium sulfate precipitation. The ammonium sulfate precipitation were then collectively pooled and later denoted as semi purified fraction 30 (consisting of 0-30%), 60 (31-60%) and 90 (61-90%).

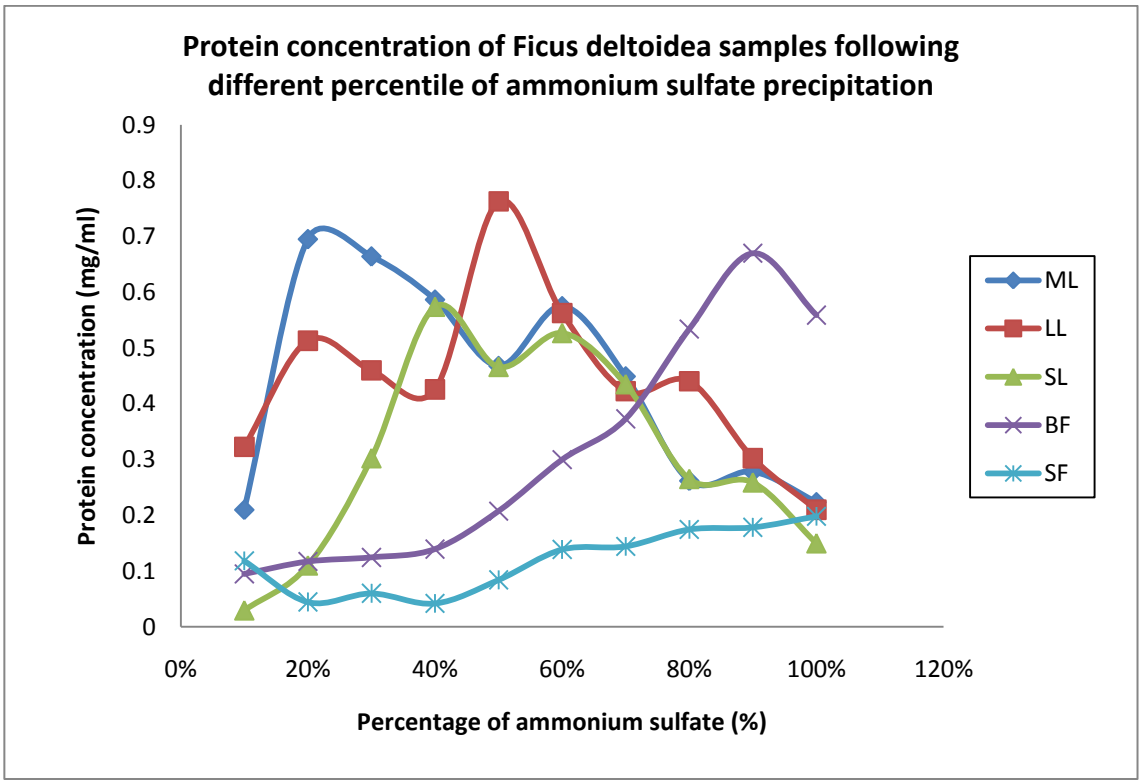


Figure 4.1: Protein concentration of *Ficus deltoidea* samples following different percentage of ammonium sulfate precipitation. The precipitated proteins were estimated by using BCA kit. The leaves showed similar pattern with three peaks while the fruits has higher absorbance towards higher percentage of ammonium sulfate precipitation.

4.2 Preliminary antioxidative studies

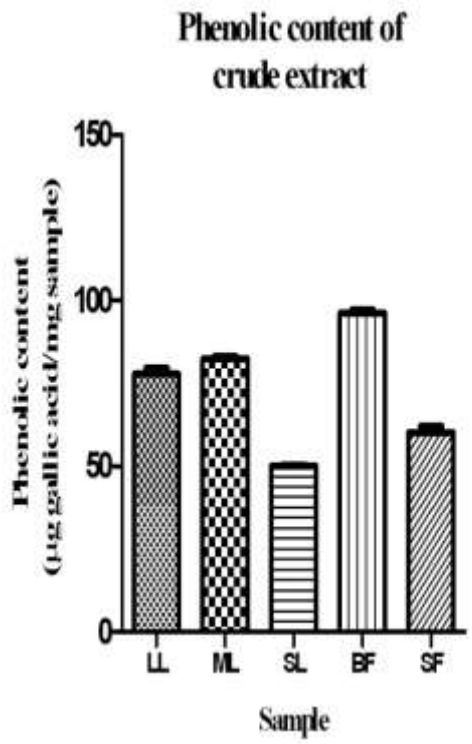
In preliminary studies, three antioxidant assays were conducted in order to determine the antioxidant properties of the crude (LL, ML, SL, BF, SF were crude extracts) and semi-purified fractions (LL30, ML30, SL30, BF30, SF30 were fraction 30; LL60, ML60, SL60, BF60, SF60 were fraction 60; LL90, ML90, SL90, BF90, SF90 were fraction 90). The assays consisted of total phenolic content assay, DPPH assay and lipid peroxidation assay.

4.2.1 Total phenolic content assay

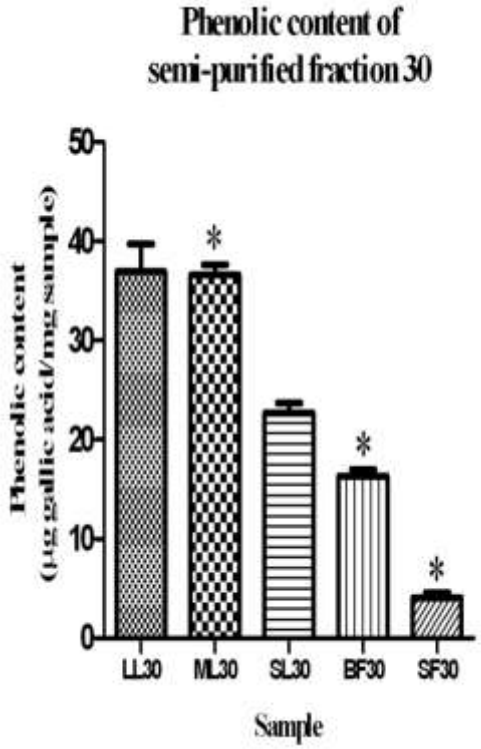
The total phenolic content of samples was claculated based on the gallic acid standard graph (Appendix B). Based on the total phenolic content results (Figure 4.2), crude extracts of both leaves and fruits have higher total phenolic content compared to the semi-purified fractions. Among the five crude samples (Figure 4.2 (a)), BF has the highest phenolic content followed by ML > LL > SF > SL. BF has the highest phenolic content with a value of 96.18 ± 0.98 μg gallic acid/mg sample while SL has the lowest with a value of 50.03 ± 0.35 μg gallic acid/mg sample. For the leaves extract, ML and LL has higher total phenolic content compared to SL in both crude and semi-purified fractions.

For semi-purified leaves fractions (Figure 4.2 (b)-(d)), different types of leaves shown to have the highest total phenolic content in fraction 60 compared to the other two fractions. In fruit extracts, phenolic content of BF is higher compared to SF. SF has the lowest total phenolic content among all the semi-purified fractions.

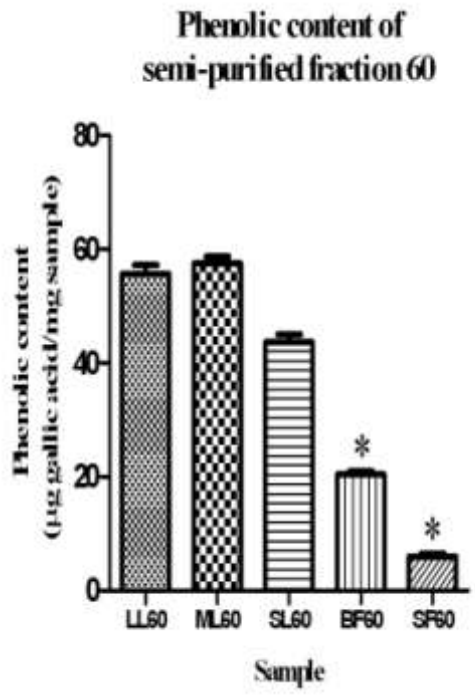
(a)


Phenolic content of crude extract
Phenolic content (µg gallic acid/mg sample)
150
100
50
0
LL
ML
SL
BF
SF
Sample

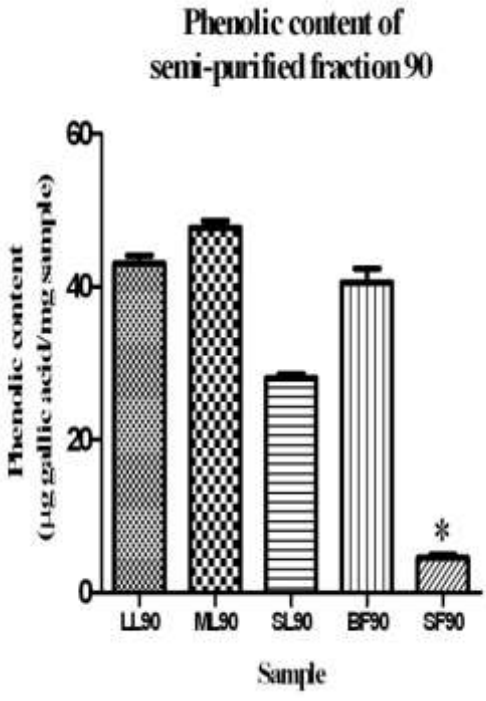
(b)


Phenolic content of semi-purified fraction 30
Phenolic content (µg gallic acid/mg sample)
50
40
30
20
10
0
*
*
*
LL30
ML30
SL30
BF30
SF30
Sample

(c)


Phenolic content of semi-purified fraction 60
Phenolic content (µg gallic acid/mg sample)
80
60
40
20
0
*
*
LL60
ML60
SL60
BF60
SF60
Sample

(d)


Phenolic content of semi-purified fraction 90
Phenolic content (µg gallic acid/mg sample)
60
40
20
0
*
LL90
ML90
SL90
BF90
SF90
Sample

4.2.2 DPPH assay

The IC_{50} values of each sample was estimated and calculated based on the dose-response inhibition curve (Appendix C). The DPPH assay indicates the scavenging capabilities of antioxidants towards DPPH radicals. Lower IC_{50} values indicate higher scavenging ability. In general, all crude samples were shown to have higher scavenging ability compared to semi-purified fractions. Interestingly, ML and LL showed almost similar scavenging activity. The same pattern was also reflected in their semi-purified fractions (Figure 4.3). The scavenging ability of crude samples were in descending order from BF (0.82±0.03 mg/ml), SF (2.54±0.03 mg/ml), LL (2.71±0.09 mg/ml), ML (2.74±0.11 mg/ml) and finally to SL (8.21±0.26 mg/ml). Even though fruits showed a higher scavenging activity in crude sample, the activities for the semi-purified fractions were less active compared to the leaves. The result was obviously shown by the SF. SF semi-purified fractions do not have the ability to scavenge 50% of the DPPH radical present in the mixture even though the crude sample was better compared to the leaves. On the other hand, ascorbic acid, a strong DPPH scavenger gave an IC_{50} value of 0.11 mg/ml.

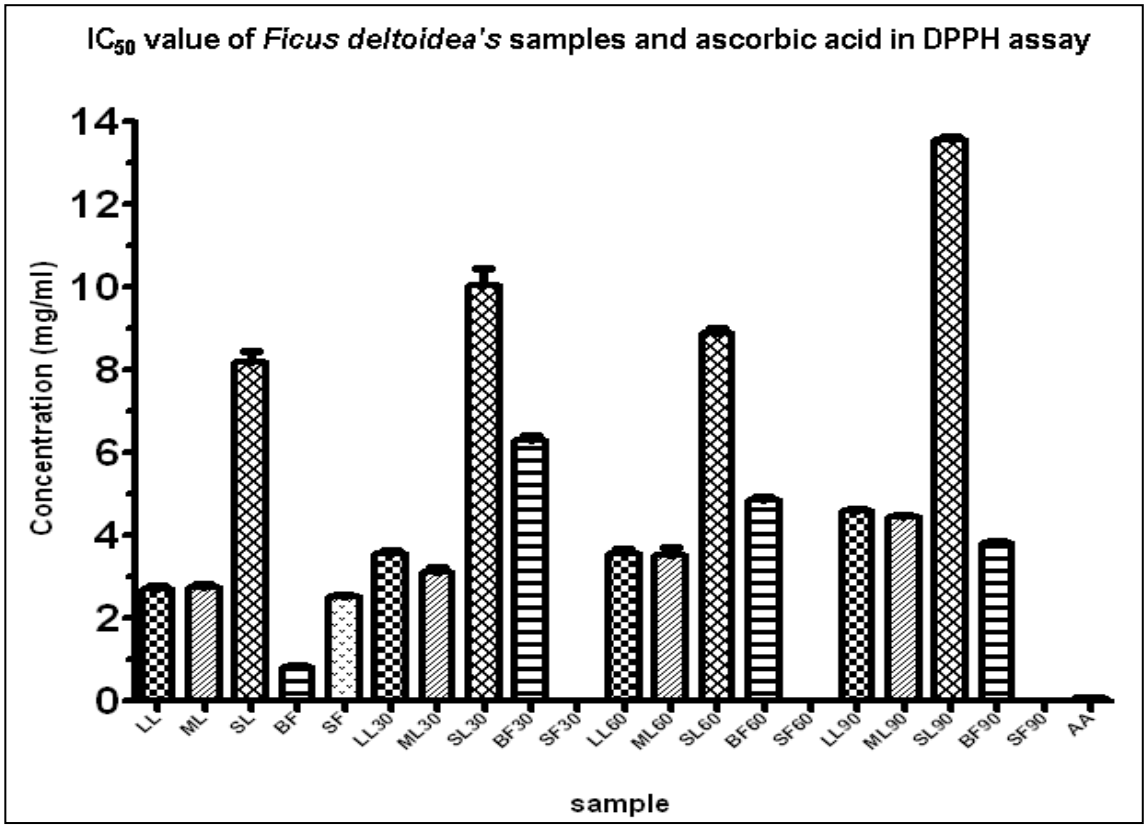


Figure 4.3: IC_{50} value of *Ficus deltoidea's* extracts in DPPH assay. The sample with lower IC_{50} value has higher radical scavenging ability. All SF fractions were not able to scavenge 50% of the DPPH present, no IC_{50} were obtained. Results are presented as mean ±SD (n=6). Statistical analysis was performed using one way ANOVA at $p<0.05$. LL: large type leaf, ML: medium type leaf, SL: small type leaf, BF: big type fruit, SF: small type fruit, AA: Ascorbic acid

4.2.3 Lipid peroxidation assay

The IC_{50} values of each sample was estimated and calculated based on the dose-response inhibition curve (Appendix D). Among the twenty samples, only two crude samples and three semi-purified fractions caused inhibition of lipid peroxidation (Figure 4.4). In crude samples, only the fruit samples had an effect towards the inhibition of lipid peroxidation. BF and SF gave a result of 15.05 ±0.12 mg/ml and 8.28 ±0.13 mg/ml respectively. Among the semi-purified fractions, two ML and one LL semi purified fractions showed an effect towards the inhibition of lipid peroxidation. Between the two ML semi-purified fractions, M30 (12.57 ±0.10 mg/ml) showed a better inhibition compared to M60 (15.44 ±0.20 mg/ml). Meanwhile, ascorbic acid which functions as positive control gave an IC_{50} value of 1.6 ±0.01 mg/ml.

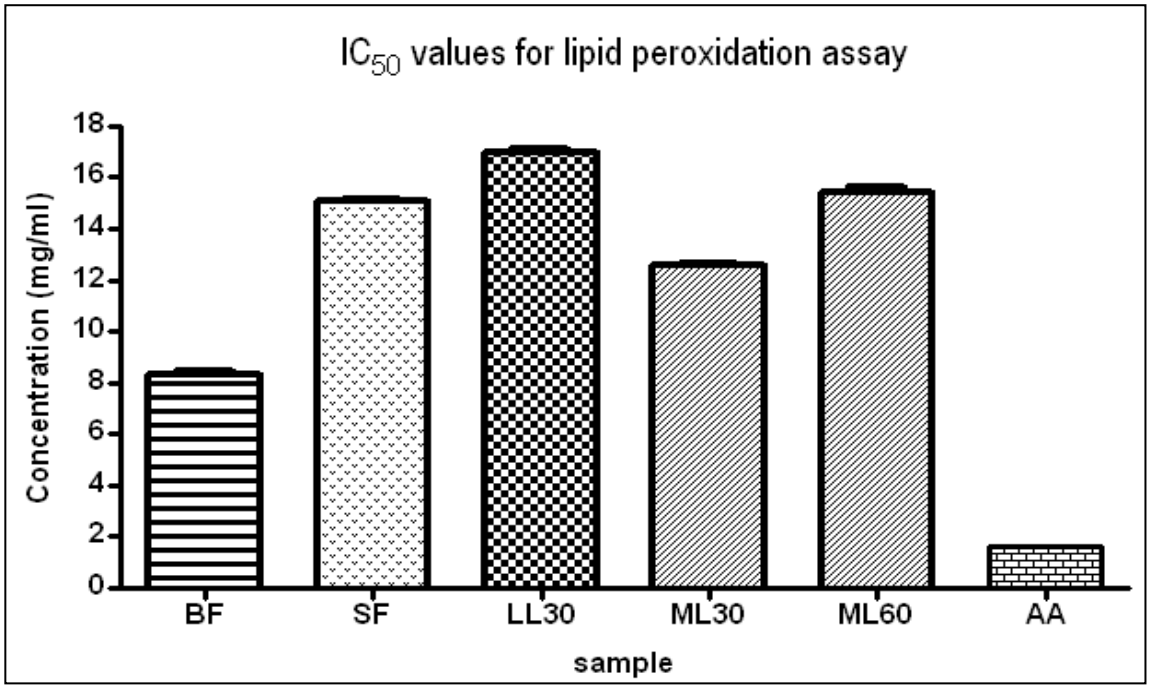


Figure 4.4: IC_{50} values for *Ficus deltoidea's* samples and ascorbic acid in lipid peroxidation assay. The sample with the lower IC_{50} value has better inhibition towards lipid peroxidation. Only five among twenty samples were able to show 50% inhibition. There were no IC_{50} obtained for the other fifteen samples. Results are presented as mean ±SD (n=6). Statistical analysis was performed using one way ANOVA at $p<0.05$. LL: large type leaf, ML: medium type leaf, SL: small type leaf, BF: big type fruit, SF: small type fruit, AA: Ascorbic acid

4.3 MTT assay

After 72 hours of treatement, the IC_{50} values were obtained based on the estimation of 50% viable cells (Appendix E). In MTT assays, lower IC_{50} values indicate higher toxicity towards cells. Even at high concentration (0.5 mg/ml), all crude extracts do not show any toxicity towards tested cell lines. Among the semi-purified fractions, only SF showed cytotoxic effect towards the cell lines (Figure 4.5). The SF semi-purified fractions works best in Hep G2 cells treatment followed by Chang Liver cells then finally Ca Ski cells. SF30 does not exert cytotoxic effect towards normal Chang Liver cells and needed a higher concentration to kill the cancer cells. SF60 was shown to have higher cytotoxic effect towards Hep G2 cells (225.33 ± 4.51 µg/ml) and Chang Liver cells (252.00 ± 3.61 µg/ml) compared to Ca Ski cells (476.00 ± 3.61 µg/ml). For SF90, the cytotoxic activity is considered as moderate compared to the other two semi-purified fractions.

For positive control, water soluble doxorubicin was used. Doxorubicin, a commonly used anticancer drug showed a potent effect towards the cell lines (Figure 4.6). It was able to kill Ca Ski cells in a low concentration of 0.67 ± 0.02 µg/ml. The disadvantage of doxorubicin was that it killed normal cell (1.45 ± 0.04 µg/ml) in a lower concentration as compared to Hep G2 (2.01 ± 0.06 µg/ml).

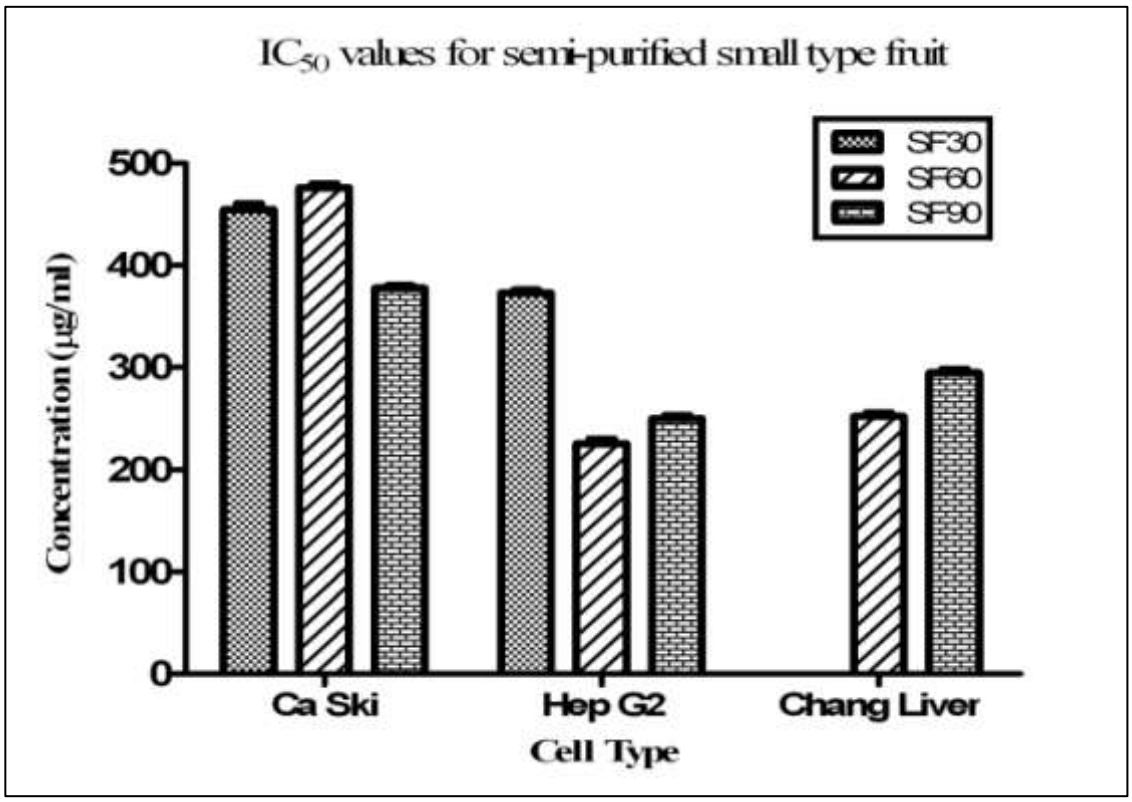


Figure 4.5: IC_{50} values for SF semi-purified fractions towards three different cell lines. Different type of samples acts differently towards different cell lines. There was no IC_{50} value obtained for SF30 towards Chang Liver cells indicated that the sample was non-toxic towards the cells. Results are presented as mean ±SD (n=9). Statistical analysis was performed using one way ANOVA at $p<0.05$.

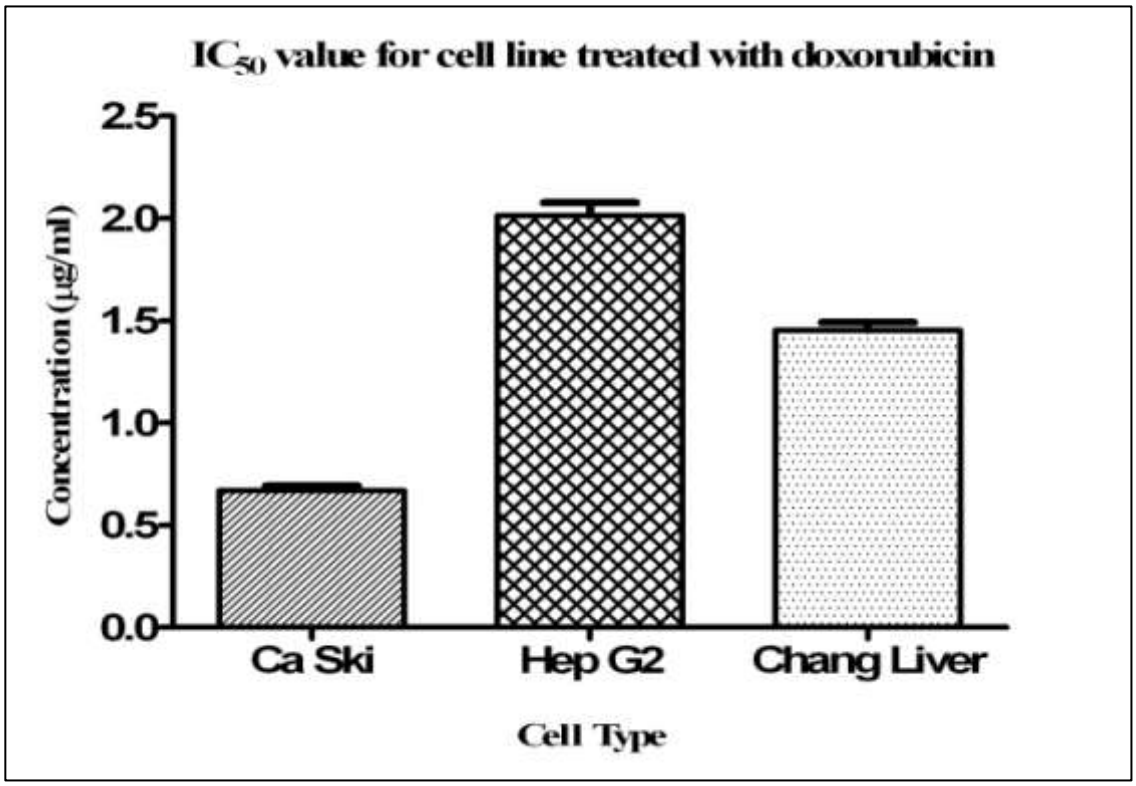


Figure 4.6: IC_{50} values of three different cell lines following treatment with doxorubicin. Results are presented as mean ±SD (n=9). Statistical analysis was performed using one way ANOVA at $p<0.05$.

4.4 Endogenous antioxidants

Based on MTT result, only SF showed cytotoxic effect towards the cell lines. Therefore, only the changes of endogenous antioxidants following SF (crude extract and semi purified fractions) and doxorubicin treatments at IC_{50} values were tested. The endogenous antioxidant measured were catalase, glutathione peroxidase and superoxide dismutase.

4.4.1 Catalase

Catalase concentrations in different treatment were estimated and calculated based on the catalase standard curve (Appendix F). Based on results obtained (Figure 4.7), Chang Liver has a lower catalase concentration (less than 0.25 μg/ml) compared to the other two cell lines. Following treatments at IC_{50} values, catalase concentration in Ca Ski cells were increased even though the changes were not significant compared to untreated cells. Unlike Ca Ski cells, the catalase concentrations were decreased in Hep G2 and Chang Liver following doxorubicin treatment. The catalase concentrations in cell lysate following all SF treatments were increased in Ca Ski and Chang Liver cells. On the other hand, catalase concentrations were decreased in HepG2 cells following SF and SF30 treatments while in SF60 and SF90 treatments, they remain unchanged.

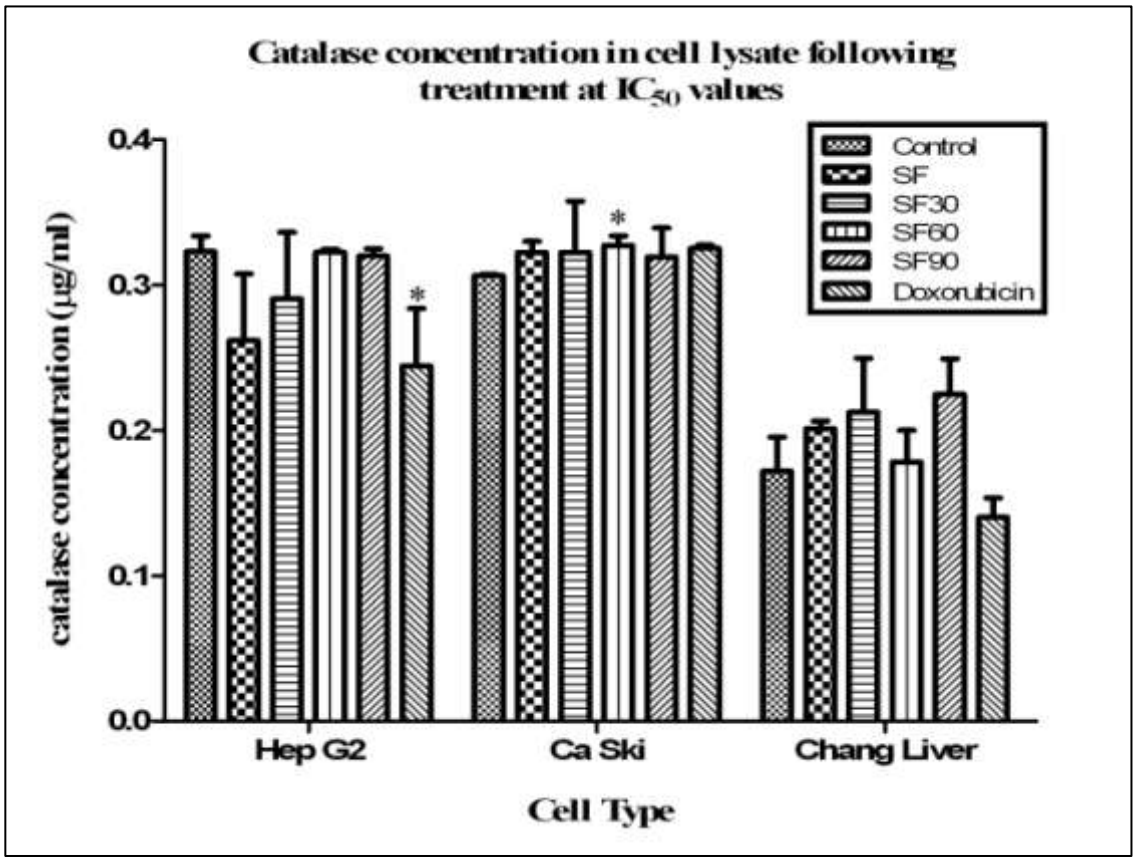


Figure 4.7: Catalase concentrations in untreated (control) and treated cells. Results are presented as mean±SD (n=9). Statistical analysis was performed using one way ANOVA at $p<0.05$.

4.4.2 Glutathione peroxidase

From the result (Figure 4.8), it can be seen that the GPx activities were consistent in all the cell lines for untreated cells which was around 33 Units/ml. GPx activities for all the cell lines were decreased following treatments with SF crude and semi-purified samples at IC_{50} values compared to untreated cells. The situations were similar in Hep G2 and Chang Liver cells following doxorubicin treatment. On the other hand, doxorubicin treated Ca Ski cells was the only sample which increased in GPx activity following treatment.

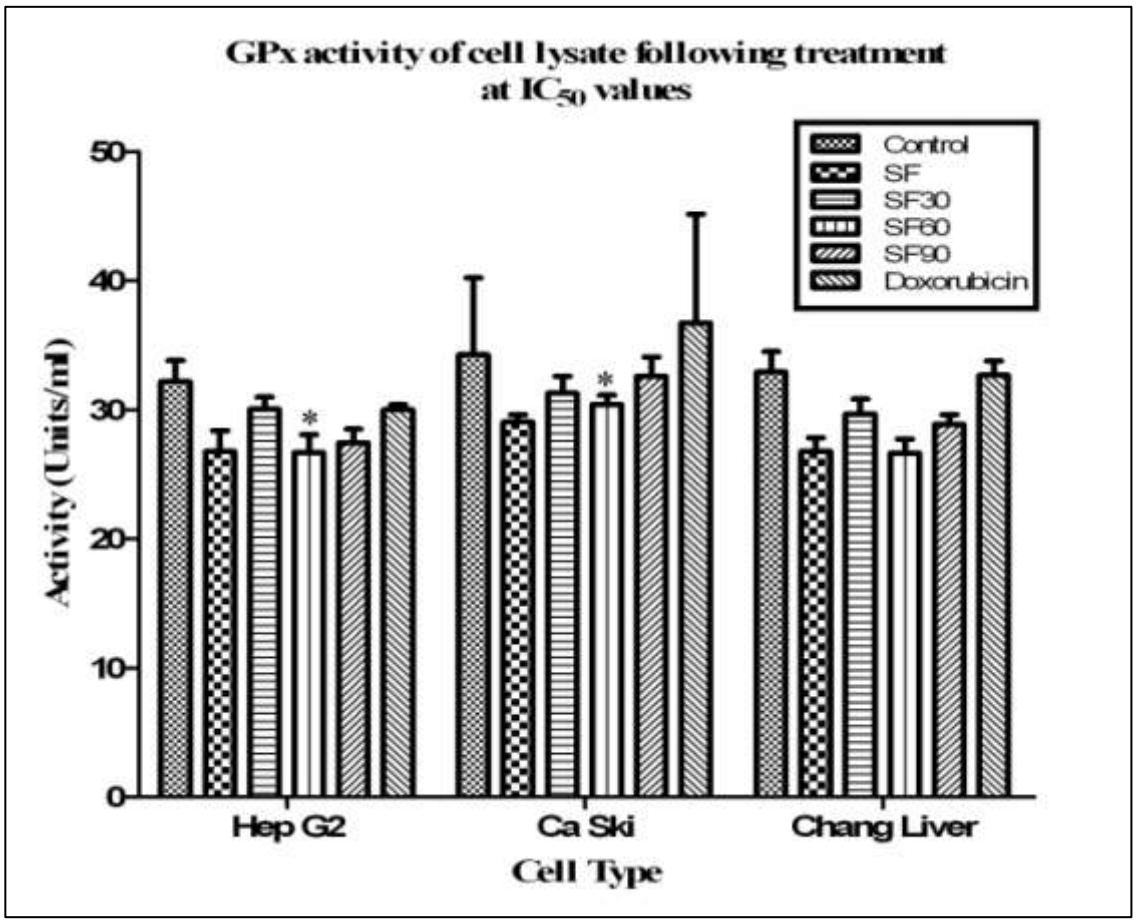


Figure 4.8: GPx activities in untreated (control) and treated cells. Results are presented as mean±SD (n=9). Statistical analysis was performed using one way ANOVA at $p<0.05$.

4.4.3 Superoxide dismutase

In Hep G2 cells, all the treatments were shown to decrease the inhibition of NBT. This indicates that the amount of SOD decreased following treatments. For Ca Ski and Chang Liver cells, SF30 and SF60 treatment lowered NBT inhibition while the other two SF samples showed an opposite effect. Meanwhile, doxorubicin treatment decreased the SOD levels in Hep G2 and Chang Liver cells but not in Ca Ski cells. (Figure 4.9)

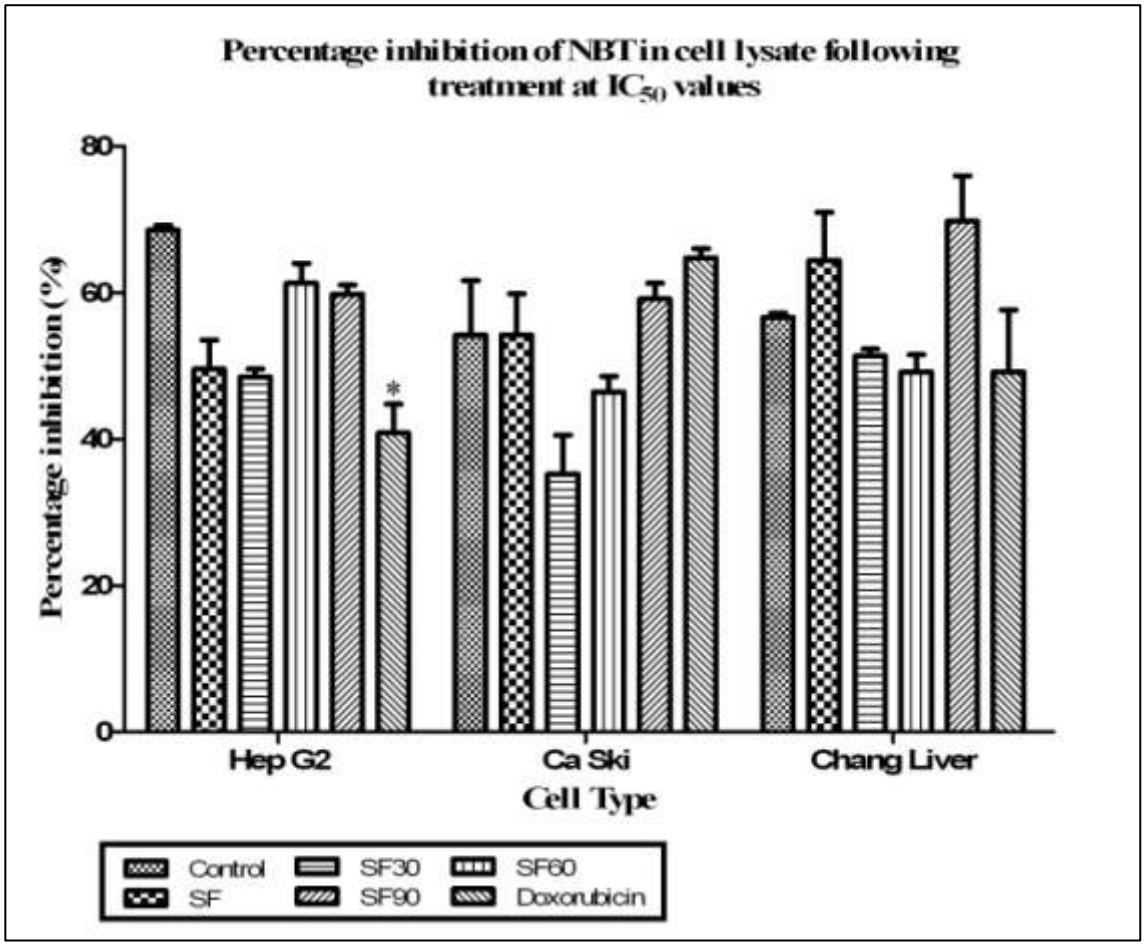


Figure 4.9: Percentage inhibition of NBT in untreated (control) and treated cells. High inhibition of NBT indicate high SOD present in the cell lysate as SOD will compete with NBT in the conversion of superoxide radicals to oxygen. Results are presented as mean±SD (n=9). Statistical analysis was performed using one way ANOVA at $p<0.05$.

4.5 DNA fragmentation

Treatment with doxorubicin and SF extracts at different concentrations showed that DNA fragmentation pattern was different in the various cell lines and treatment concentrations (Appendix G). Following treatment with doxorubicin, DNA laddering was shown to be apparent only in Hep G2 cells (Figure 4.10). As for SF, the crude and semi-purified fractions have an effect on DNA fragmentation for cancer cells. Crude samples showed the effect at the higher concentration compared to semi-purified fractions. Among the cell lines, Chang Liver cells showed the least or no DNA fragmentation following treatment (Figure 4.11).

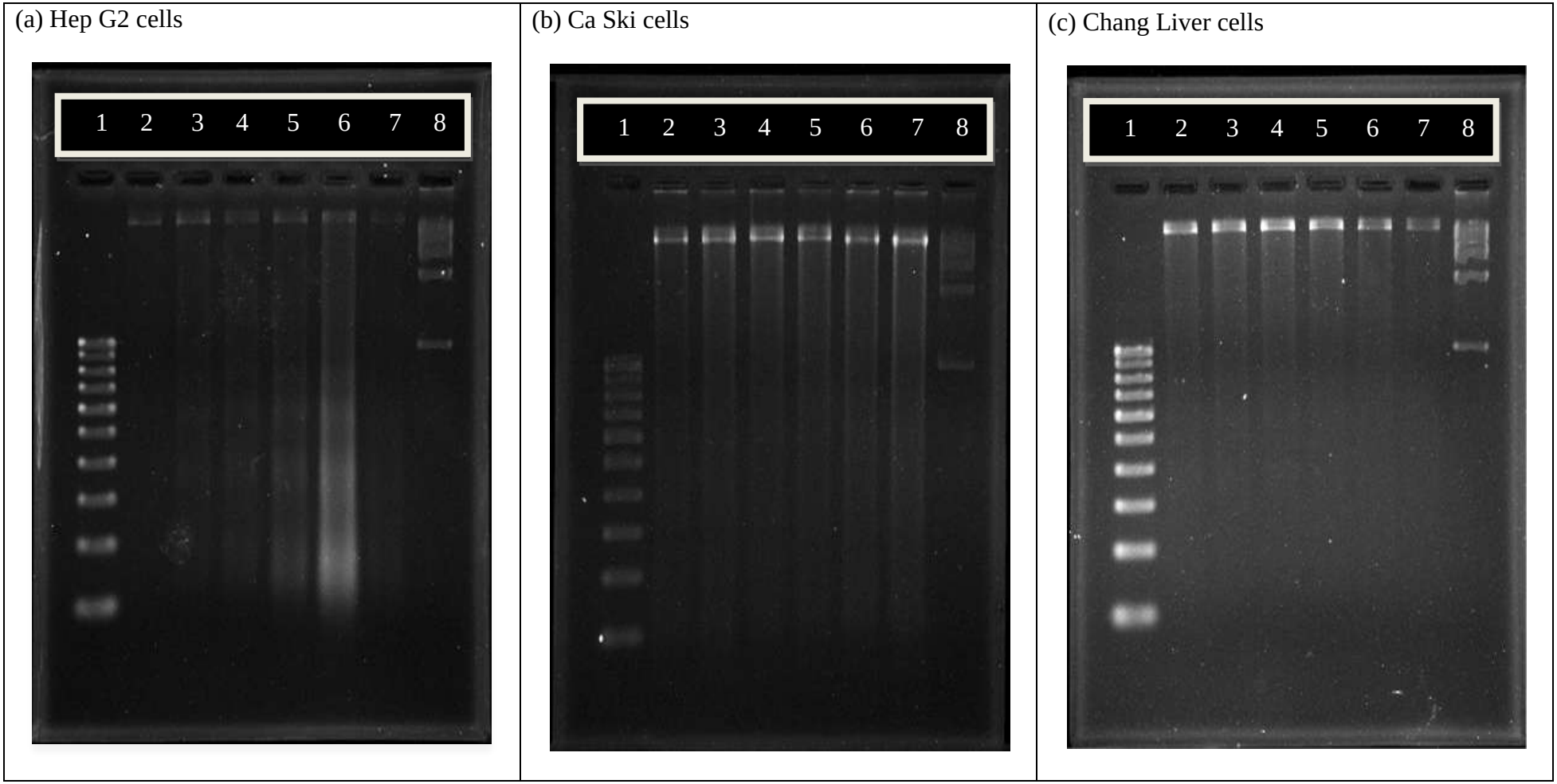


Figure 4.10: DNA fragmentations following treatment with doxorubicin. First lane is 100 bp marker while lane 8 is 1 kb marker. Treatment concentration lane 2: 0.125 µg/ml; lane 3: 0.25 µg/ml; lane 4: 0.5 µg/ml; lane 5: 1 µg/ml; lane 6: 2 µg/ml. Lane 7 as control.

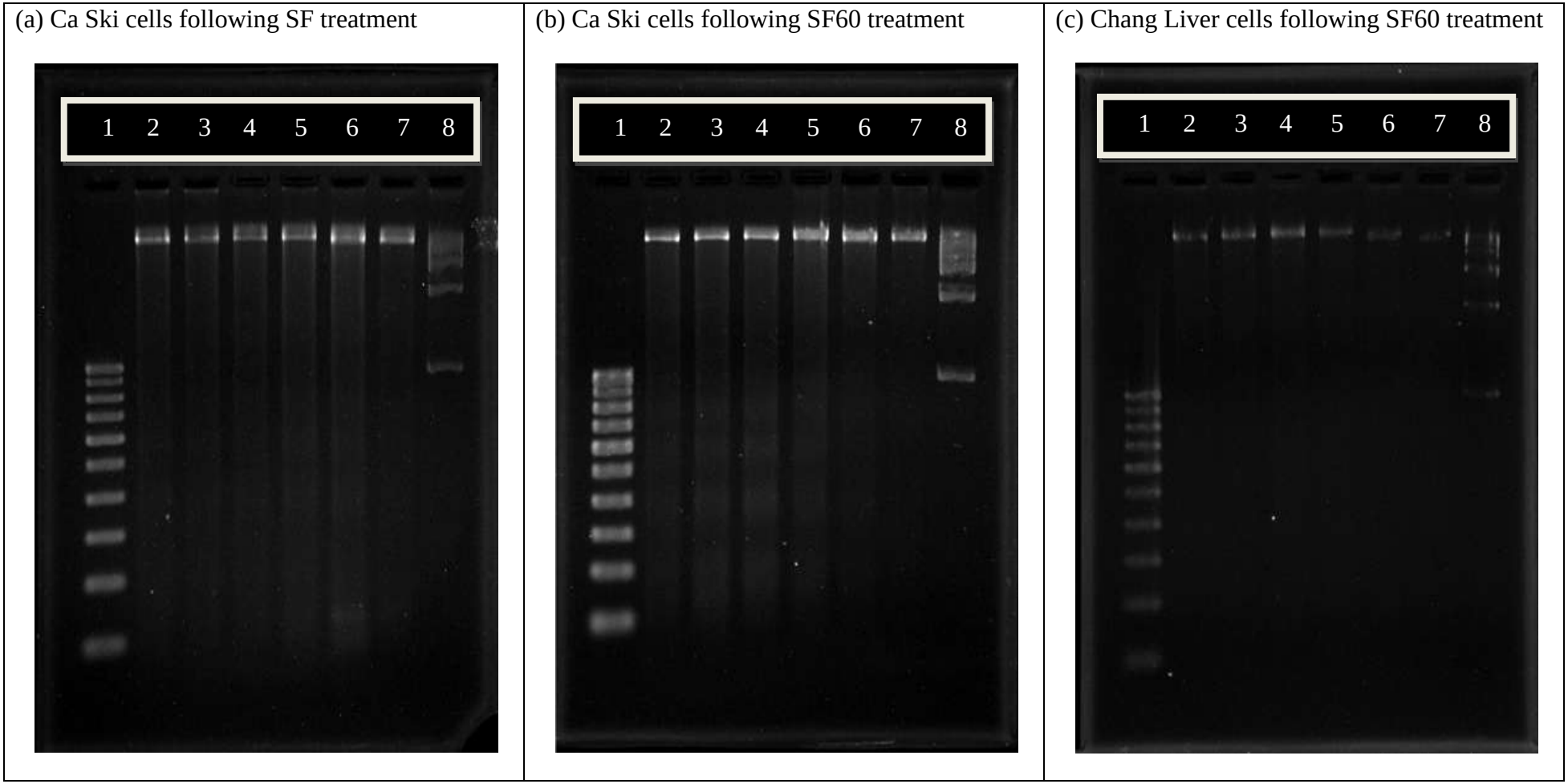


Figure 4.11: DNA fragmentations following treatment with semi-purified and crude sample in different cell lines. Semi-purified sample forms DNA laddering at lower concentration compared to crude sample. Semi-purified treatment showed no DNA fragmentation in Chang Liver cells. First lane is 100 bp marker while lane 8 is 1 kb marker. Treatment concentration lane 2: 0.125 mg/ml; lane 3: 0.25 mg/ml; lane 4: 0.5 mg/ml; lane 5: 0.75 mg/ml; lane 6: 1 mg/ml. Lane 7 as control.

4.6 Gel image analysis

The two-dimensional (2D) gels obtained after horizontal isoelectric focusing (IEF) and vertical gel electrophoresis were analyzed using ImageMaster Platinum version 7.0. For each cell line, the gels were compared among treated and untreated samples. For each sample, seven replicates were subjected to the analysis. As for the result, the spot was considered significant whenever it was deregulated for more than two folds and with p value which was less than 0.001 ($p<0.001$). The number of spots that were deregulated following treatments was shown as the table 4.1.

Based on Table 4.1, among the three semi-purified fractions, SF60 caused the most significant change in all the three cell lines. Most of the deregulated proteins following semi-purified fractions treatment were up-regulated while doxorubicin treatment showed the opposite effect. These gave us an indication that the samples might undergo different pathways in halting the growth of the cells as compared to doxorubicin. Profile of 2-DE of different untreated cell lines are presented as in Figure 4.12 (Hep G2 cells), Figure 4.13 (Ca Ski cells) and Figure 4.14 (Chang Liver cells). The treated cell lines were shown in Appendix H. In order to illustrate the fold changes, the respective deregulated protein were shown in Table 4.2 (Hep G2 cells), Table 4.3 (Ca Ski cells) and Table 4.4 (Chang Liver cells).

Table 4.1: Number of deregulated proteins following treatment at IC_{50} values

Cell Type	Treatment	Up-regulated	Down-regulated	Total
Hep G2	SF30	20	6	26
	SF60	28	2	30
	SF90	16	4	20
	Doxorubicin	19	53	72
Ca Ski	SF30	1	5	6
	SF60	7	7	14
	SF90	6	5	11
	Doxorubicin	20	48	68
Chang Liver	SF30	3	2	5
	SF60	28	9	37
	SF90	-	8	8
	Doxorubicin	19	35	54

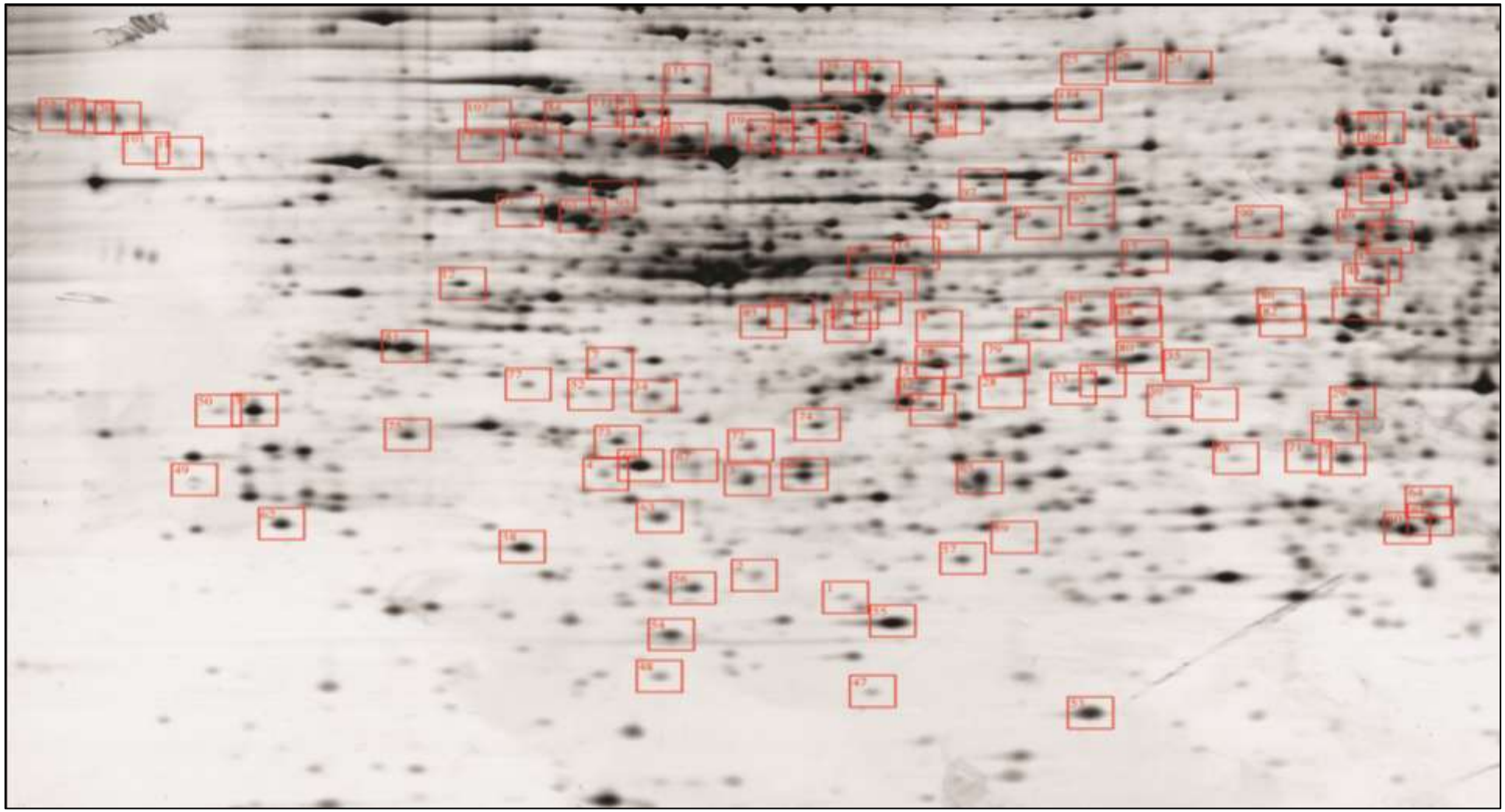


Figure 4.12: Deregulated proteins with at least two fold changes following different treatments at IC_{50} values for HepG2 cells. Statistical analysis was performed using ANOVA at $p<0.001$ (n=7). The deregulated proteins and fold change are shown in Table 4.2.

Table 4.2: Image of deregulated proteins following different treatments in Hep G2 cells

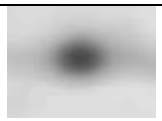
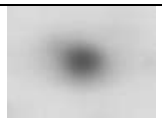
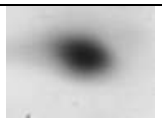
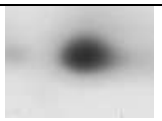
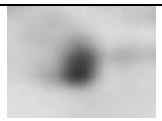
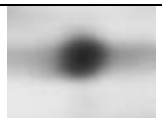
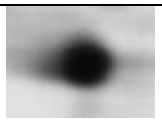
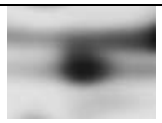
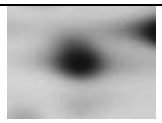
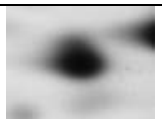
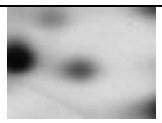
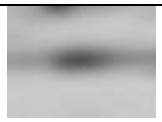
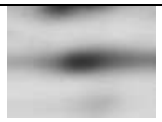
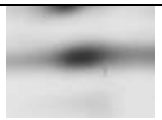
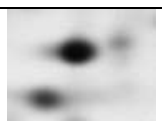
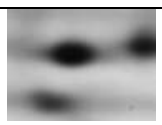
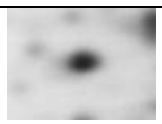
Spot #	Control	SF30	SF60	SF90	Doxorubicin
1		 2.17	---	---	---
2		 3.18	 2.31	 3.38	 5.76
3		 2.4	---	 2.55	---
4		 6.29	 6.09	 5.38	---
5		 -2.09	---	---	---
6		 3.22	 3.84	---	---
7		 -3.25	---	---	 -3.36
8		 2.82	 3.23	 2.95	---
9		 2.12	 2.72	---	---
10		 -2.50	---	---	---
11		 2.30	 5.09	---	---

Table 4.2, continued

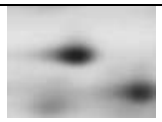
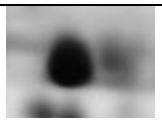
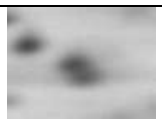
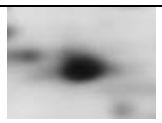
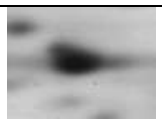
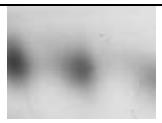
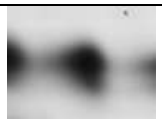
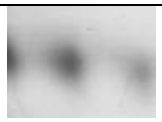
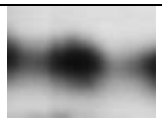
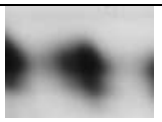
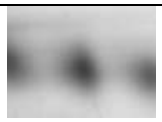
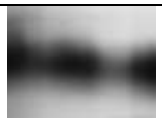
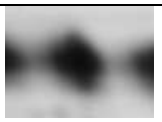
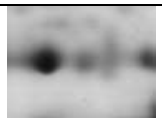
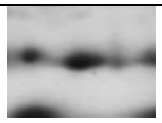
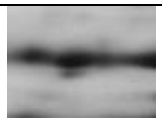
12		 -4.86	---	---	---
13		 2.66	 3.83	 2.90	---
14		 2.54	---	---	---
15		 2.18	---	---	---
16		 2.30	 2.49	---	---
17		 -3.39	---	---	---
18		 2.13	---	---	---
19		 -4.18	---	---	 -4.90
20		 2.89	---	---	---
21		 2.20	---	---	 4.77
22		 2.02	---	---	 4.54
23		 2.86	 3.29	---	---

Table 4.2, continued

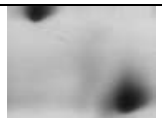
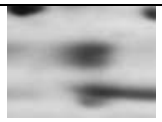
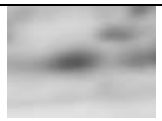
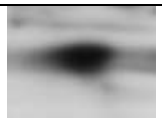
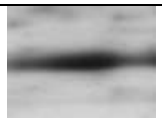
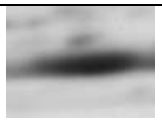
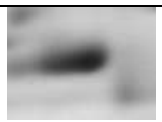
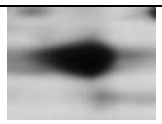
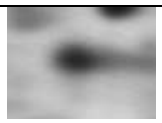
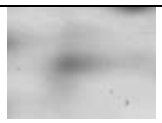
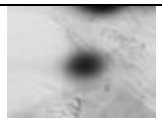
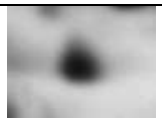
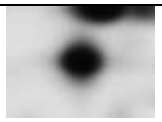
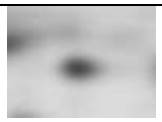
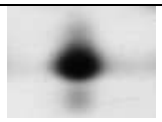
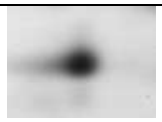
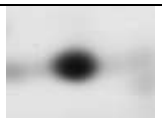
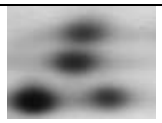
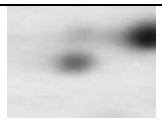
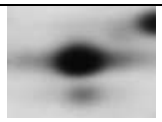
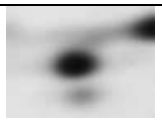
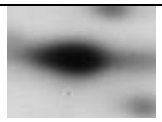
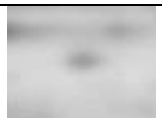
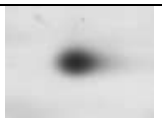
24		 3.94	---	---	---
25		 3.16	 2.42	 2.37	---
26		 3.46	---	---	---
27		---	 2.16	---	---
28		---	 3.60	---	---
29		---	 2.79	 2.07	---
30		---	 2.67	---	---
31		---	 -2.41	 -2.72	 -2.24
32		---	 3.61	---	---
33		---	 3.71	 2.45	 -3.03
34		---	 3.66	 2.73	---
35		---	 -2.01	---	 3.79

Table 4.2, continued

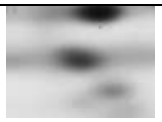
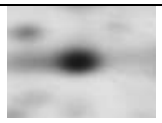
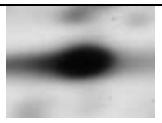
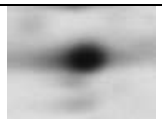
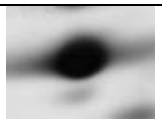
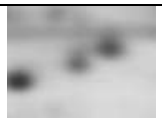
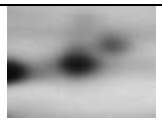
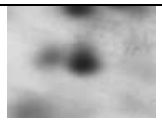
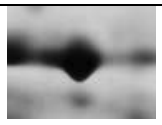
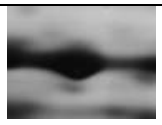
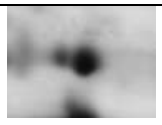
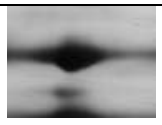
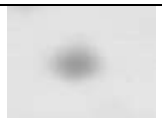
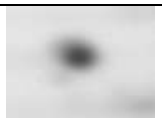
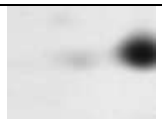
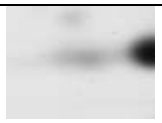
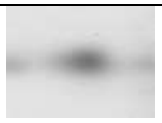
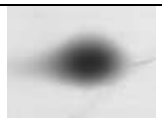
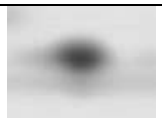
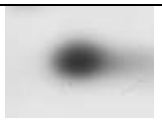
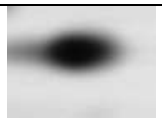
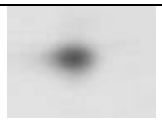
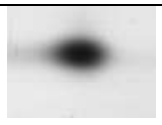
36		---	 2.70	 2.70	---
37		---	 3.25	 3.08	---
38		---	 2.30	 2.37	---
39		---	 3.11	 2.58	---
40		---	 5.76	---	---
41		---	 3.68	---	---
42		---	 2.22	---	---
43		---	 4.17	---	---
44		---	 2.13	---	---
45		---	 2.59	---	---
46		---	 2.42	---	---
47		---	---	 -2.38	 4.28

Table 4.2, continued

48		---	---	 -2.50	---
49		---	---	 2.42	---
50		---	---	 -2.82	---
51		---	---	 2.93	---
52		---	---	 2.11	---
53		---	---	---	 -2.48
54		---	---	---	 2.24
55		---	---	---	 -3.15
56		---	---	---	 -3.41
57		---	---	---	 -2.73
58		---	---	---	 -3.08
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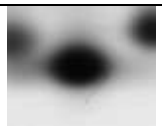
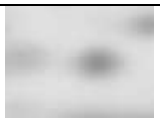
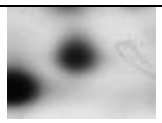
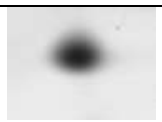
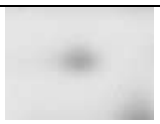
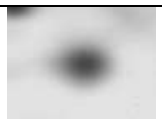
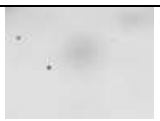
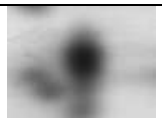
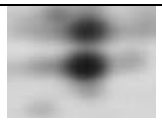
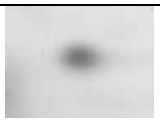
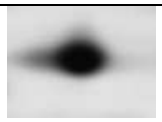
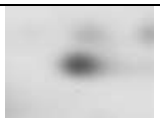
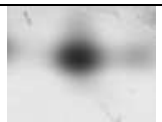
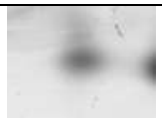
60		---	---	---	 -3.31
61		---	---	---	 -2.09
62		---	---	---	 -2.06
63		---	---	---	 -2.53
64		---	---	---	 -2.82
65		---	---	---	 -2.22
66		---	---	---	 -2.60
67		---	---	---	 -3.98
68		---	---	---	 -2.98
69		---	---	---	 -2.47
70		---	---	---	 -3.30
71		---	---	---	 -3.38

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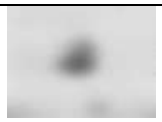
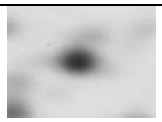
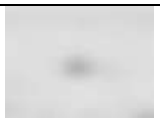
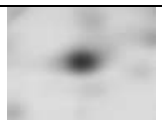
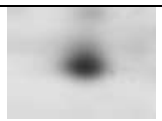
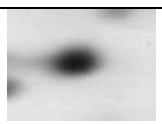
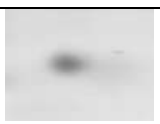
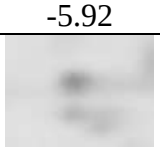
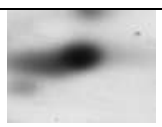
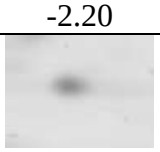
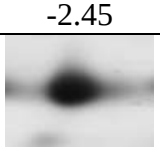
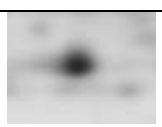
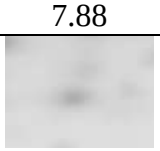
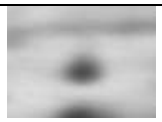
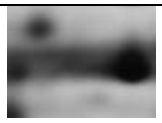
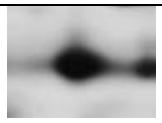
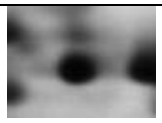
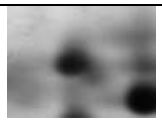
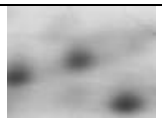
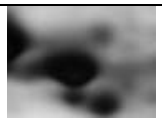
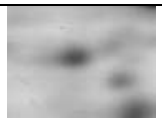
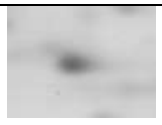
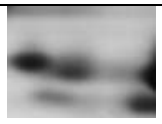
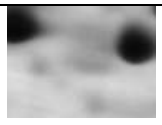
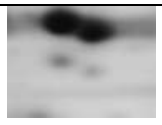
72		---	---	---	 -4.19
73		---	---	---	 -2.90
74		---	---	---	 -4.35
75		---	---	---	 -2.95
76		---	---	---	 -3.48
77		---	---	---	 -3.60
78		---	---	---	 -5.92
79		---	---	---	 -2.20
80		---	---	---	 -4.02
81		---	---	---	 -2.45
82		---	---	---	 7.88
83		---	---	---	 -2.39

Table 4.2, continued

84		---	---	---	 -3.97
85		---	---	---	 -2.46
86		---	---	---	 -3.16
87		---	---	---	 8.35
88		---	---	---	 -2.49
89		---	---	---	 -3.59
90		---	---	---	 -3.39
91		---	---	---	 2.50
92		---	---	---	 2.48
93		---	---	---	 4.13
94		---	---	---	 2.04
95		---	---	---	 -2.20

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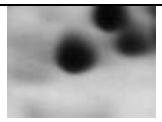
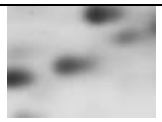
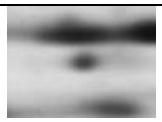
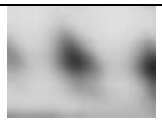
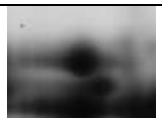
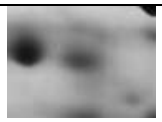
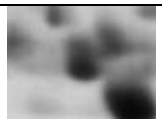
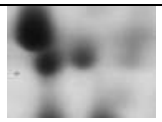
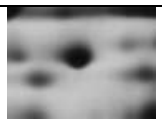
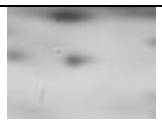
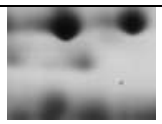
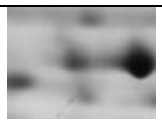
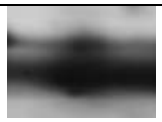
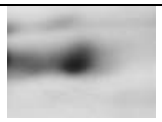
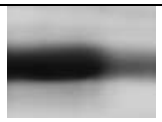
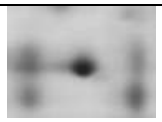
96		---	---	---	 -2.21
97		---	---	---	 -2.06
98		---	---	---	 -2.35
99		---	---	---	 -5.56
100		---	---	---	 -3.57
101		---	---	---	 4.60
102		---	---	---	 -3.62
103		---	---	---	 7.67
104		---	---	---	 -2.81
105		---	---	---	 -2.24
106		---	---	---	 -2.28
107		---	---	---	 5.88

Table 4.2, continued

108		---	---	---	 -2.63
109		---	---	---	 -3.11
110		---	---	---	 2.64
111		---	---	---	 -2.55
112		---	---	---	 -3.75
113		---	---	---	 4.27
114		---	---	---	 3.54
115		---	---	---	 -3.87

Positive numbers indicate up-regulation while negative numbers indicate down-regulation.

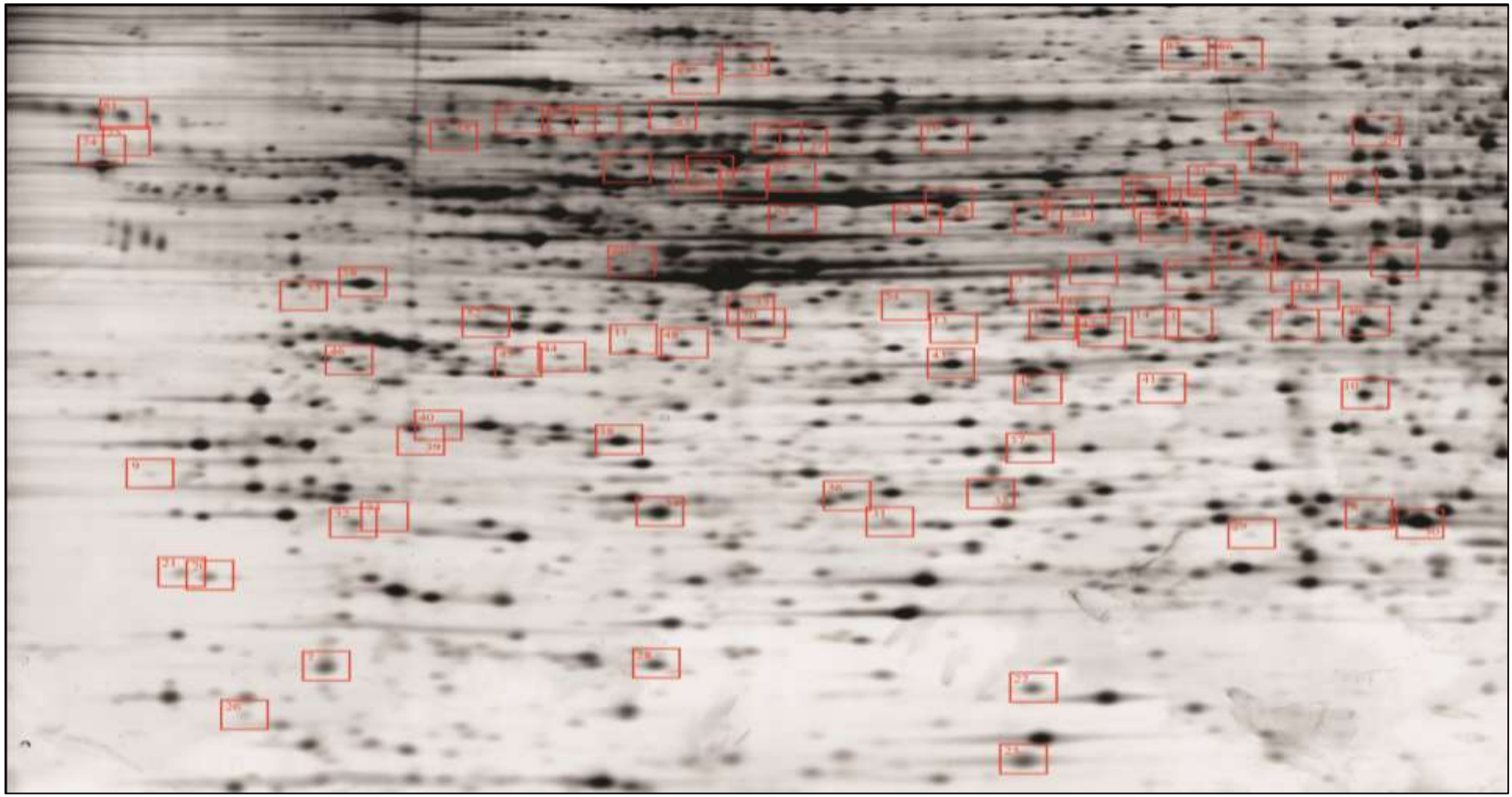


Figure 4.13: Deregulated proteins with at least two fold changes following different treatments at IC_{50} values for Ca Ski cells. Statistical analysis was performed using ANOVA at $p<0.001$ (n=7). The deregulated proteins and fold change are shown in Table 4.3.

Table 4.3: Image of deregulated proteins following different treatments in Ca Ski cells

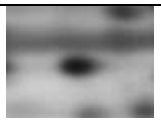
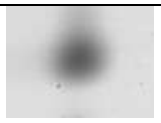
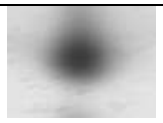
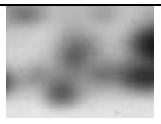
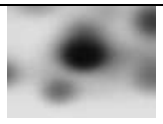
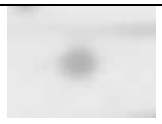
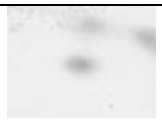
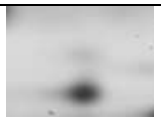
Spot #	Control	SF30	SF60	SF90	Doxorubicin
1		 -2.38	---	 -2.23	---
2		 -3.37	---	 -3.77	---
3		 -2.53	---	---	---
4		 -5.74	---	---	---
5		 3.70	 4.26	 5.03	---
6		 -2.31	---	---	---
7		---	 2.08	---	-3.14
8		---	 4.06	---	---
9		---	 4.83	 4.88	---
10		---	 -2.60	 -2.55	 -2.92
11		---	 2.75	---	---

Table 4.3, continued

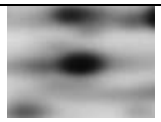
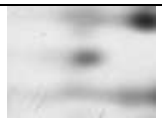
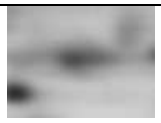
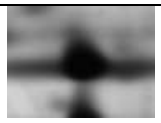
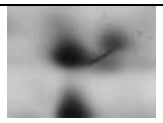
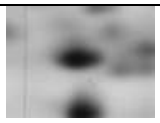
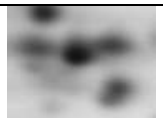
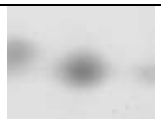
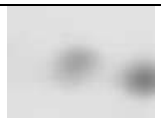
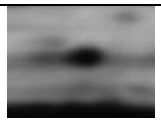
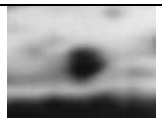
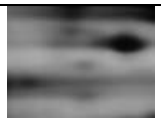
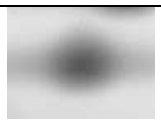
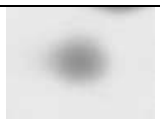
12		---	 -2.16	 -2.32	 -2.20
13		---	 -2.01	---	---
14		---	 -3.15	---	---
15		---	 -4.57	---	---
16		---	 -2.04	---	 -3.22
17		---	 -2.37	---	 -4.13
18		---	 2.91	---	---
19		---	 2.46	---	---
20		---	---	 2.76	---
21		---	---	 3.45	---
22		---	---	 2.54	---
23		---	---	 2.73	---

Table 4.3, continued

24		---	---	 -2.26	---
25		---	---	---	 -2.59
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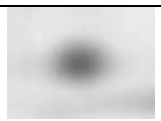
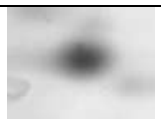
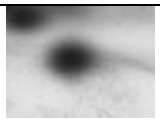
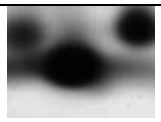
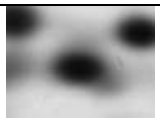
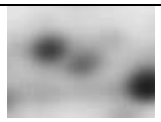
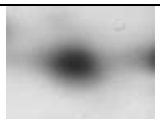
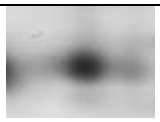
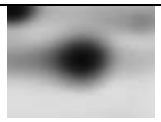
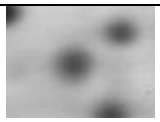
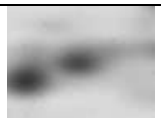
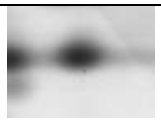
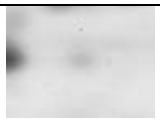
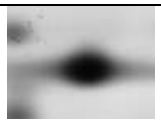
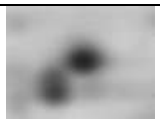
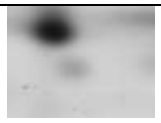
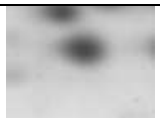
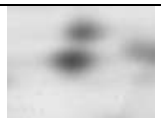
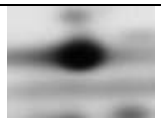
27		---	---	---	-8.88
28		---	---	---	 -6.40
29		---	---	---	 22.00
30		---	---	---	 -2.57
31		---	---	---	 -2.73
32		---	---	---	 3.40
33		---	---	---	 4.08
34		---	---	---	 -2.78
35		---	---	---	 -2.81

Table 4.3, continued

36		---	---	---	 3.36
37		---	---	---	 -4.54
38		---	---	---	 -2.17
39		---	---	---	 6.04
40		---	---	---	 4.01
41		---	---	---	 -3.69
42		---	---	---	 -2.72
43		---	---	---	 -3.02
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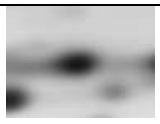
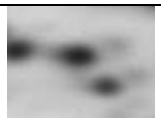
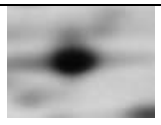
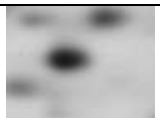
45		---	---	---	 8.13
46		---	---	---	 -2.34
47		---	---	---	 -2.27

Table 4.3, continued

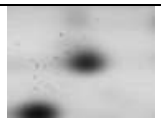
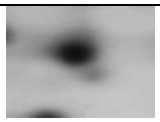
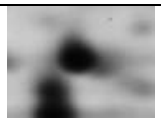
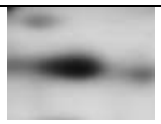
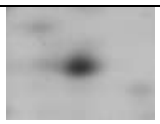
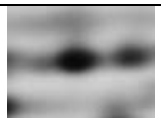
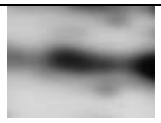
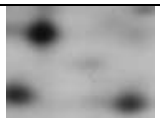
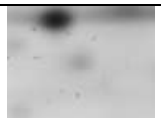
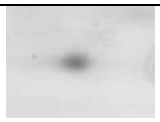
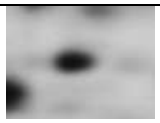
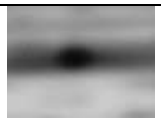
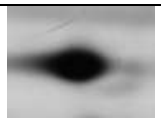
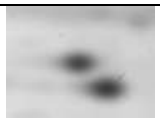
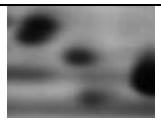
48		---	---	---	 2.15
49		---	---	---	 -3.17
50		---	---	---	 -2.90
51		---	---	---	 -2.72
52		---	---	---	 -7.06
53		---	---	---	 -2.75
54		---	---	---	 -2.31
55		---	---	---	 2.98
56		---	---	---	 5.92
57		---	---	---	 -2.63
58		---	---	---	 -4.79
59		---	---	---	 -3.48

Table 4.3, continued

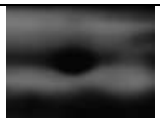
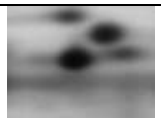
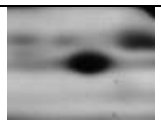
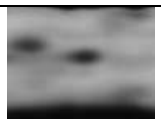
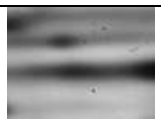
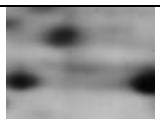
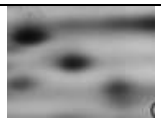
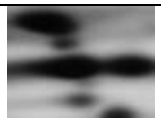
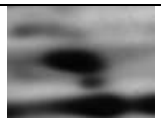
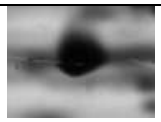
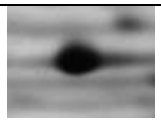
60		---	---	---	 11.38
61		---	---	---	 -3.81
62		---	---	---	 -2.41
63		---	---	---	 2.72
64		---	---	---	 -2.41
65		---	---	---	 2.55
66		---	---	---	 -3.27
67		---	---	---	 -4.78
68		---	---	---	 -2.21
69		---	---	---	 -2.07
70		---	---	---	 -2.23
71		---	---	---	 -5.11

Table 4.3, continued

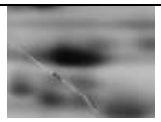
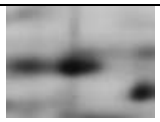
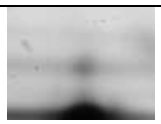
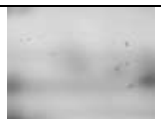
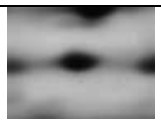
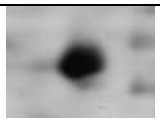
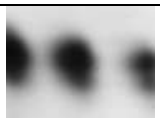
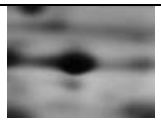
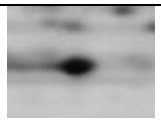
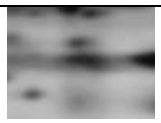
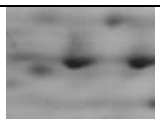
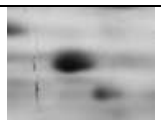
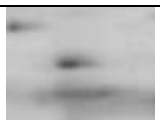
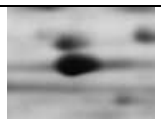
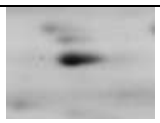
72		---	---	---	 3.33
73		---	---	---	 -3.25
74		---	---	---	 2.93
75		---	---	---	 2.95
76		---	---	---	 -3.09
77		---	---	---	 -4.97
78		---	---	---	 -5.47
79		---	---	---	 -4.47
80		---	---	---	 -2.01
81		---	---	---	 3.61
82		---	---	---	 -7.11
83		---	---	---	 -5.29

Table 4.3, continued

84		---	---	---	 -3.16
85		---	---	---	 2.13
86		---	---	---	 -3.85
87		---	---	---	 -3.26

Positive numbers indicate up-regulation while negative numbers indicate down-regulation.

Figure 4.14: Deregulated proteins with at least two fold changes following different treatments at IC_{50} values for Chang Liver cells. Statistical analysis was performed using ANOVA at $p<0.001$ (n=7). The deregulated proteins and fold change are shown in Table 4.4.

Table 4.4: Image of deregulated proteins following different treatments in Chang Liver cells

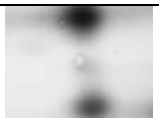
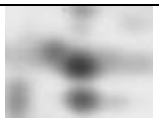
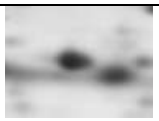
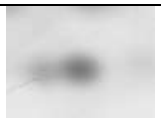
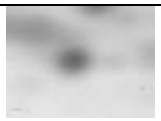
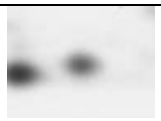
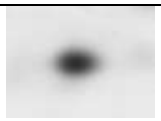
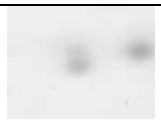
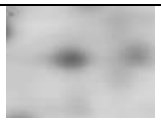
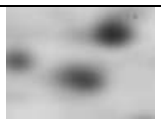
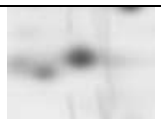
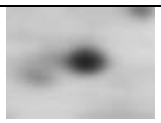
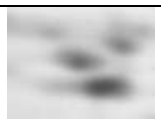
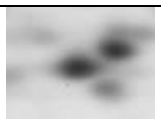
Spot #	Control	SF30	SF60	SF90	Doxorubicin
1		 3.02	---	---	---
2		 3.55	---	---	---
3		-3.09	---	---	---
4		 2.74	---	---	---
5		 -3.01	---	---	---
6		---	 4.20	---	---
7		---	 5.02	---	---
8		---	 2.75	---	---
9		---	 4.29	---	---
10		---	 4.00	---	---
11		---	 3.77	---	---

Table 4.4, continued

12		---	 4.39	---	---
13		---	 2.05	---	---
14		---	 2.78	---	---
15		---	 2.58	---	---
16		---	 3.32	---	---
17		---	 2.31	---	---
18		---	 5.49	---	---
19		---	 2.01	---	-4.68
20		---	 2.44	---	---
21		---	 3.03	---	---
22		---	 3.25	---	---
23		---	 2.28	---	---

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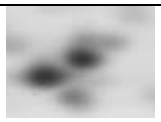
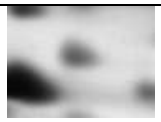
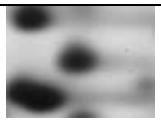
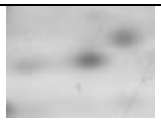
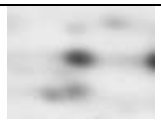
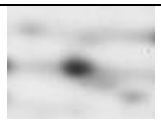
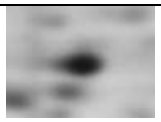
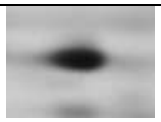
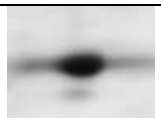
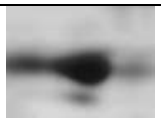
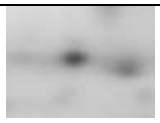
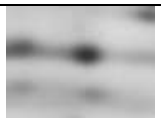
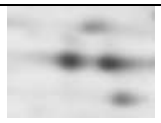
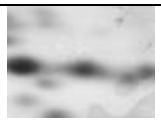
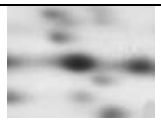
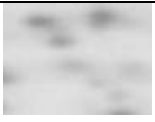
24		---	 2.43	---	---
25		---	 3.08	---	---
26		---	 2.52	---	---
27		---	 -2.88	---	---
28		---	 -2.27	---	---
29		---	 2.57	---	---
30		---	 2.83	---	---
31		---	 3.25	---	 -4.43
32		---	 3.18	---	---
33		---	 -3.06	---	---
34		---	 -5.85	---	 -2.27
35		---	 -7.11	---	---

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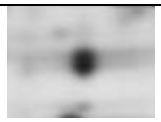
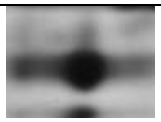
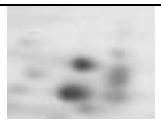
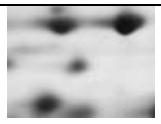
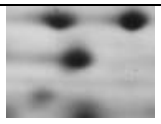
36		---	 -2.22	---	---
37		---	 3.10	---	---
38		---	 -2.23	---	---
39		---	 -2.85	---	---
40		---	 2.76	---	---
41		---	 -2.18	---	---
42		---	 3.38	---	---
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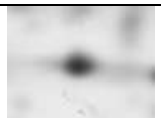
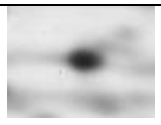
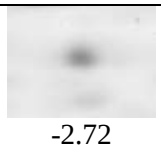
44		---	---	 -2.20	---
45		---	---	 -2.72	---
46		---	---	 -2.54	---
47		---	---	 -2.09	---

Table 4.4, continued

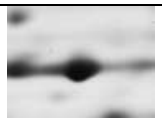
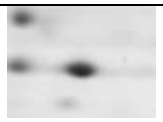
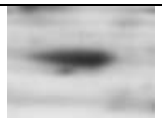
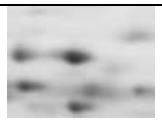
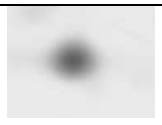
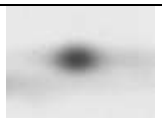
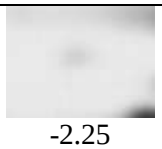
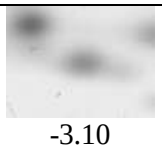
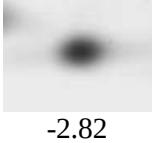
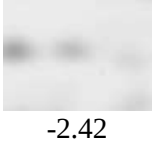
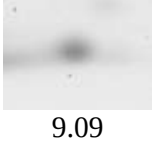
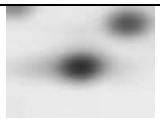
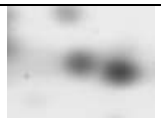
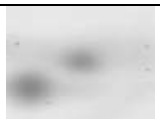
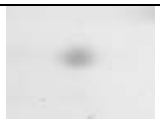
48		---	---	 -2.10	---
49		---	---	 -2.82	---
50		---	---	 -3.61	---
51		---	---	---	-2.29
52		---	---	---	 2.28
53		---	---	---	 -6.15
54		---	---	---	 3.41
55		---	---	---	 -2.25
56		---	---	---	 -3.10
57		---	---	---	 -2.82
58		---	---	---	 -2.42
59		---	---	---	 9.09

Table 4.4, continued

60		---	---	---	 5.52
61		---	---	---	 -2.69
62		---	---	---	 -2.17
63		---	---	---	 2.08
64		---	---	---	 -2.37
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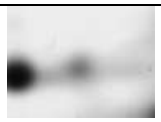
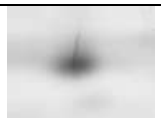
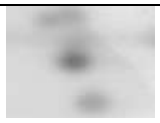
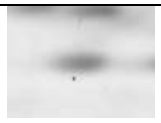
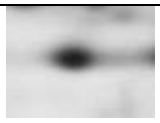
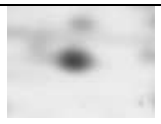
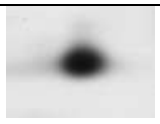
66		---	---	---	 2.01
67		---	---	---	 -2.01
68		---	---	---	 -2.82
69		---	---	---	 2.37
70		---	---	---	 2.32
71		---	---	---	 -3.26

Table 4.4, continued

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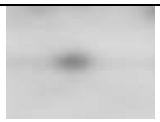
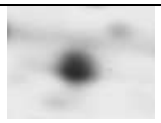
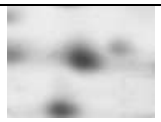
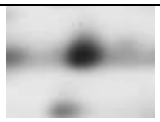
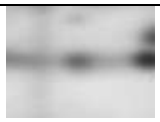
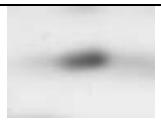
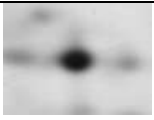
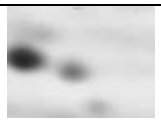
74		---	---	---	 -2.38
75		---	---	---	 3.44
76		---	---	---	 -2.23
77		---	---	---	 2.31
78		---	---	---	 -2.17
79		---	---	---	 -3.77
80		---	---	---	 -2.15
81		---	---	---	 4.88
82		---	---	---	 -2.22
83		---	---	---	 -2.27

Table 4.4, continued

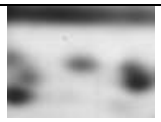
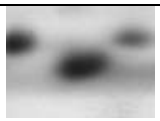
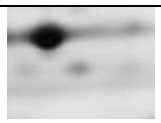
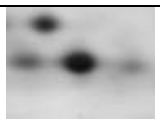
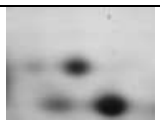
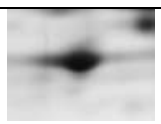
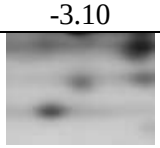
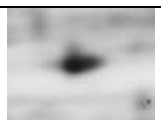
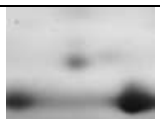
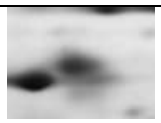
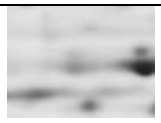
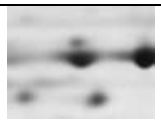
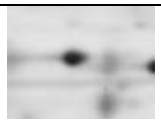
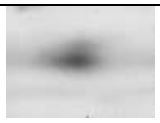
84		---	---	---	 -2.87
85		---	---	---	 2.28
86		---	---	---	 3.72
87		---	---	---	 -2.44
88		---	---	---	 -3.10
89		---	---	---	 -4.28
90		---	---	---	 -3.28
91		---	---	---	 -2.01
92		---	---	---	 2.16
93		---	---	---	 -3.18
94		---	---	---	 -2.40
95		---	---	---	 2.77

Table 4.4, continued

96		---	---	---	 2.83
97		---	---	---	 3.07
98		---	---	---	 2.38
99		---	---	---	 -2.02
100		---	---	---	 -2.35
101		---	---	---	 2.56

Positive numbers indicate up-regulation while negative numbers indicate down-regulation.

4.7 MALDI

Proteins which were significantly deregulated were identified by using MALDI mass spectrometry analysis. The identities of these proteins are shown in the following tables: Table 4.5 (Hep G2 cells), Table 4.6 (Ca Ski cells) and Table 4.7 (Chang Liver cells) respectively.

Table 4.5: Identified proteins in Hep G2 cells

(a) HepG2 cells following SF30 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
2	Apolipoprotein A-I precursor	P02467	69	0.0023	30759	5.56	33511	5.50	5	2
3	Cathepsin D precursor (EC 4.3.23.5)	P07339	806	4e-077	44524	6.10	43315	5.50	37	24
4	Cathepsin D precursor (EC 4.3.23.5)	P07339	584	6.4e-055	44524	6.10	43875	5.26	41	23
5	Transitional endoplasmic reticulum ATPase	P55072	348	2.5e-031	89266	5.14	51158	5.81	13	15
7	Heat shock protein HSP-90 beta	P08238	158	2.5e-012	83212	4.97	55640	5.52	13	12
9	Hemoglobin subunit beta	P68871	60	0.015	15988	6.75	60682	5.69	29	7
10	Elongation factor 2	P13639	60	0.015	95277	6.41	61242	6.49	19	15
12	Heat shock cognate 71kDa protein	P11142	247	3.2e-021	70854	5.37	63483	5.02	15	11
13	Cytokeratin-9	P35527	60	0.016	62092	5.19	65163	6.54	9	6
14	Protein NDRG1	P92597	187	3.2e-015	42808	5.49	66284	5.79	30	12
19	Stress-70 protein	P38646	371	1.3e-033	73635	5.87	79449	5.49	27	21

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(b) HepG2 cells following SF60 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
2	Apolipoprotein A-I precursor	P02467	69	0.0023	30759	5.56	33511	5.50	5	2
4	Cathepsin D precursor (EC 4.3.23.5)	P07339	584	6.4e-055	44524	6.10	43875	5.26	41	23
13	Cytokeratin-9	P35527	60	0.016	62092	5.19	65163	6.54	9	6
28	Proliferating cell nuclear antigen (PCNA)	P12004	149	2e-011	28750	4.57	52278	5.91	42	11
29	Actin	P63261	95	4.7e-006	41766	5.31	54519	6.49	18	8
31	Proliferating cell nuclear antigen (PCNA)	P12004	509	2e-047	28750	4.57	53679	4.63	54	19
35	60S acidic ribosomal protein P0	P05388	66	0.004	34252	5.71	54519	6.24	20	6
36	Anamorsin	Q6FI81	59	0.018	33561	5.44	56480	5.66	17	6
44	78kDa glucose-regulated protein precursor (GRP78)	P11021	752	1e-071	72288	5.07	80289	5.2	25	22
45	Pyruvate carboxylase (EC 6.4.1.1)	P11498	237	3.2e-020	129551	6.37	80570	5.85	22	25

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(c) HepG2 cells following SF90 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
2	Apolipoprotein A-I precursor	P02467	69	0.0023	30759	5.56	33511	5.50	5	2
3	Cathepsin D precursor (EC 4.3.23.5)	P07339	806	4e-077	44524	6.10	43315	5.50	37	24
4	Cathepsin D precursor (EC 4.3.23.5)	P07339	584	6.4e-055	44524	6.10	43875	5.26	41	23
29	Actin	P63261	95	4.7e-006	41766	5.31	54519	6.49	18	8
31	Proliferating cell nuclear antigen (PCNA)	P12004	509	2e-047	28750	4.57	53679	4.63	54	19
47	Ferritin light chain	P02792	146	4e-011	20007	5.51	22306	5.73	28	7
48	Chromobox protein homolog 3	Q13185	191	1.3e-015	20798	5.23	23147	2.35	20	6
49	Lysosomal protective protein precursor (EC 3.4.16.5)	P10619	175	5.1e-014	54431	6.16	43315	4.51	9	7
50	Proliferating cell nuclear antigen (PCNA)	P12004	117	3.2e-008	28750	4.57	51158	4.56	35	11
52	Ubiquinone biosynthesis protein COQ9	O75208	250	1.6e-021	35487	5.61	52278	5.49	15	7

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(d) HepG2 cells following doxorubicin treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
2	Apolipoprotein A-I precursor	P02467	69	0.0023	30759	5.56	33511	5.50	5	2
7	Heat shock protein HSP 90-beta	P08238	158	2.5e-012	83212	4.97	55640	5.52	13	12
19	Stress-70 protein	P38646	371	1.3e-033	73635	5.87	79449	5.49	27	21
31	Proliferating cell nuclear antigen (PCNA)	P12004	509	2e-047	28750	4.57	53679	4.63	54	19
35	60S acidic ribosomal protein P0	P05388	66	0.004	34252	5.71	54519	6.24	20	6
47	Ferritin light chain	P02792	146	4e-011	20007	5.51	22306	5.73	28	7
53	Nucleoside diphosphate kinase A (EC 2.7.4.6)	P15531	466	4e-043	17138	5.83	18105	6.09	89	19
54	ATP synthase D chain (EC 3.6.3.14)	O75947	409	2e-037	18479	5.21	27628	5.61	61	15
55	Peroxiredoxin-2 (EC 1.11.1.15)	P32119	510	1.6e-047	21878	5.66	28469	5.76	47	17
56	Ubiquitin-conjugating enzyme E2-25kDa (EC 6.3.2.19)	P61086	387	3.2e-035	22393	5.33	32670	5.48	39	12
57	Proteasome subunit beta type 4 precursor (EC 4.3.25.1)	P28070	485	5.1e-045	29185	5.72	35471	5.86	42	16
58	Rho GDP-dissociation inhibitor 1	P52565	516	4e-048	23193	5.02	36592	5.36	39	17
59	Heat-shock protein beta-1	P04792	93	8.4e-006	22768	5.98	37712	5.95	13	4
60	Peroxiredoxin-6 (EC 1.11.1.15)	P30041	738	2.5e-070	25019	6.00	38273	6.51	70	26
61	Proteasome subunit alpha type 6 (EC 3.4.15.1)	P60900	367	3.2e-033	27382	6.34	39393	6.61	40	18
62	Proteasome subunit alpha type 5 (EC 3.4.15.1)	P28066	199	2e-016	26394	4.74	39113	4.68	56	13

Table 4.5 (d), continued

60	Peroxiredoxin-6 (EC 1.11.1.15)	P30041	738	2.5e-070	25019	6.00	38273	6.51	70	26
61	Proteasome subunit alpha type 6 (EC 3.4.15.1)	P60900	367	3.2e-033	27382	6.34	39393	6.61	40	18
62	Proteasome subunit alpha type 5 (EC 3.4.15.1)	P28066	199	2e-016	26394	4.74	39113	4.68	56	13
63	Ran-specific GTPase-activating protein	P43487	303	8e-027	23296	5.19	39673	5.59	40	10
64	Prolyl 4-hydroxylase subunit alpha-1 precursor (EC 1.14.11.2)	P13674	68	0.0023	61011	5.70	40794	6.60	9	5
65	Cathepsin D precursor (EC 4.3.23.5)	P07339	675	5.1e-064	44524	6.10	43035	5.9	42	28
66	Proteasome activator complex subunit 2	Q15129	657	3.2e-062	27344	5.44	43875	5.59	47	20
67	Cathepsin B precursor (EC 3.4.22.1)	P07858	195	5.1e-016	37797	5.88	45275	5.41	23	11
69	Chloride intracellular channel protein 1	Q15089	1100	1.6e-106	26096	5.09	44995	5.56	77	27
70	Sulfotransferase 1A1 (EC 2.8.2.1)	P50225	433	8e-040	34175	6.16	45836	6.48	55	23
72	Copper chaperone for superoxide dismutase	O14618	81	0.00012	29022	5.32	46676	5.49	15	7
73	Microtubule-associated protein RP/EB family member 1	Q15691	476	4e-044	29980	5.02	47516	5.28	48	19
74	Uncharacterized protein C17orf25	Q9HC38	338	2.5e-030	34771	5.40	49477	5.61	34	13
75	ADP-sugar pyrophosphate (EC 3.6.1.13)	Q9UKK9	532	1e-049	24312	4.87	48077	4.91	48	16
76	Nicotinate-nucleotide pyrophosphate (EC 2.4.2.19)	Q15274	301	1.3e-026	30796	5.81	46106	6.11	19	13
77	Thioredoxin-like protein 1	O43396	191	1.3e-015	32231	4.84	49136	5.11	27	11
78	60S acidic ribosomal protein P0	P05388	620	1.6e-058	34252	5.71	50954	5.76	53	21
79	Transaldolase (EC 2.2.1.2)	P37837	225	5.1e-019	37516	6.36	51257	5.93	27	12

Table 4.5 (d), continued

80	Transaldolase (EC 2.2.1.2)	P37837	506	4e-047	37516	6.36	51257	6.18	33	20
81	Nucleophosmin	P06748	534	6.4e-050	32555	4.64	53772	4.97	31	16
83	Thioredoxin-like protein 2	O76003	177	3.2e-014	37408	5.31	53772	5.50	20	9
84	Uroporphyrinogen decarboxylase (EC 4.1.1.37)	P06132	523	8e-049	40761	5.77	56409	6.08	42	25
85	Short/branched chain specific acyl-CoA dehydrogenase (EC 1.3.99.-)	P45954	251	1.3e-021	47455	6.53	57015	6.16	22	16
86	Galactokinase (EC 2.7.1.6)	P51570	168	2.5e-013	42246	6.04	57015	6.38	39	19
87	Cytokeratin-18	P05783	809	2e-077	48029	5.34	62166	5.71	52	35
88	Proliferation-associated protein 2G4	O43846	630	1.6e-059	43759	6.13	64893	6.55	45	30
89	Alpha-enolase (EC 4.2.1.11)	P06733	478	2.5e-044	47139	7.01	66106	6.51	49	24
90	S-adenosylmethionine synthase isoform type-1 (EC 2.5.1.6)	Q00266	177	3.2e-014	43620	5.86	66106	6.39	14	8
91	Protein disulfide-isomerase A6 precursor (EC 5.3.4.1)	Q15084	855	5.1e-082	48091	4.95	67318	5.23	55	29
92	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	P36957	402	1e-036	48609	9.01	67924	6.09	30	19
93	ATP synthase subunit beta (EC 3.6.3.14)	P06576	875	5.1e-084	56525	5.26	68574	5.09	48	37
94	RuvB-like 1 (EC 3.6.1.-)	P82276	123	8e-009	50196	6.02	69742	6.53	16	6
95	Tubulin alpha-6 chain	Q9BQE3	77	0.00031	49863	4.96	69742	5.20	6	4
96	Aldehyde dehydrogenase X (EC 1.2.1.3)	P30837	1230	1.6e-119	57202	6.36	70348	5.28	56	37
97	Vacuolar ATP synthase subunit B (EC 3.6.3.14)	P21281	423	8e-039	56465	5.57	70651	5.90	48	27

Table 4.5 (d), continued

98	Heterogenous nuclear ribonucleoprotein K	P61978	366	4e-033	50944	5.39	76409	5.65	33	28
99	Heterogenous nuclear ribonucleoprotein K	P61978	678	2.5e-064	50944	5.39	76409	5.56	44	26
100	Heterogenous nuclear ribonucleoprotein K	P61978	590	1.6e-055	50944	5.39	76409	5.53	41	24
101	Lamin-B1	P20700	552	1e-051	66368	5.11	75196	4.42	38	28
102	Heterogenous nuclear ribonucleoprotein K	P61978	758	2.5e-072	50944	5.39	76409	5.39	44	36
103	Lamin-B1	P20700	207	3.2e-017	66368	5.11	75803	5.15	18	12
104	Bifunctional purine biosynthesis protein PURH	P31939	765	5.1e-073	64575	6.27	76409	6.65	55	38
105	Bifunctional purine biosynthesis protein PURH	P31939	80	0.00016	64575	6.27	76409	6.54	15	10
106	Dihydropyrimidinase-related protein 2	Q16555	875	5.1e-084	62255	5.95	76409	6.51	53	35
107	Lamin-B2	Q03252	245	5.1e-021	67647	5.29	78227	5.10	16	17
108	Copine-1	Q99829	249	2e-021	59022	5.52	78227	6.50	13	13
109	Lamin-B2	Q03252	590	1.6e-055	67647	5.29	78227	5.60	49	47
110	78kDa glucose-regulated protein precursor	P11021	135	5.2e-010	72288	5.07	78227	5.33	18	13
111	Lamin-B1	P20700	132	1e-009	66368	5.11	79439	5.28	22	18
112	Lamin-B1	P20700	1020	1.6e-098	66368	5.11	79439	5.31	53	44
113	Stress-70 protein	P38646	204	6.4e-017	73635	5.87	80651	5.81	9	10
114	Serum albumin precursor	P02768	56	0.041	69321	5.92	78833	6.08	8	8
115	Cytoplasmic dynein 1 intermediate chain 2	Q13409	364	6.4e-033	71412	5.08	86106	5.39	32	18

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

Table 4.6: Identified proteins in Ca Ski cells

(a) Ca Ski cells following SF30 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
4	Leukocyte elastase inhibitor	P30740	242	1e-020	42715	5.90	60651	6.18	22	14

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(b) Ca Ski cells following SF60 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
10	Actin	P63261	95	4.7e-006	41766	5.31	47318	6.49	18	8
16	Cytokeratin-9	P35527	60	0.016	62092	5.19	61863	6.53	9	6

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(c) Ca Ski cells following SF90 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
10	Actin	P63261	95	4.7e-006	41766	5.31	47318	6.49	18	8

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(d) Ca Ski cells following doxorubicin treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
10	Actin	P63261	95	4.7e-006	41766	5.31	47318	6.49	18	8
16	Cytokeratin-9	P35527	60	0.016	62092	5.19	61863	6.53	9	6
28	Chromobox protein homolog 3	Q13185	191	1.3e-015	20798	5.23	23147	2.35	20	6
30	Peroxiredoxin-6 (EC 1.11.1.15)	P30041	738	2.5e-070	25019	6.00	38273	6.51	70	26
34	Ran-specific GTPase-activating protein	P43487	303	8e-027	23296	5.19	39673	5.59	40	10
37	Proteasome activator complex subunit 3	P61289	363	8e-033	29488	5.69	41257	5.96	44	18
38	Microtubule-associated protein RP/EB family member 1	Q15691	476	4e-044	29980	5.02	47516	5.28	48	19
43	60S acidic ribosomal protein P0	P05388	620	1.6e-058	34252	5.71	50954	5.76	53	21
44	Suppressor of G2 allele of SKP1 homolog	Q9Y2Z0	394	6.4e-036	40998	5.07	51560	5.44	25	14
47	Isocitrate dehydrogenase [NAD] subunit alpha (EC 1.1.1.41)	P50213	115	5.1e-008	39566	6.47	54590	6.06	12	7
50	Thioredoxin-like protein 2	O76003	177	3.2e-014	37408	5.31	53772	5.50	20	9
66	Heterogenous nuclear ribonucleoprotein H’	P55795	427	3.2e-039	49232	5.89	68530	6.21	36	17
67	Heterogenous nuclear ribonucleoprotein H	P31943	104	6.4e-007	49198	5.89	68530	6.19	11	6
77	Heterogenous nuclear ribonucleoprotein K	P61978	366	4e-033	50944	5.39	76409	5.65	33	28
78	Heterogenous nuclear ribonucleoprotein K	P61978	590	1.6e-055	50944	5.39	76409	5.53	41	24

Table 4.6(d), continued

79	Dihydropyrimidinase-related protein 2	Q16555	875	5.1e-084	62255	5.95	76409	6.51	53	35
84	Cytoplasmic dynein 1 intermediate chain 2	Q13409	364	6.4e-033	71412	5.08	86106	5.39	32	18

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

Table 4.7: Identified proteins in Chang Liver cells

(a) Chang Liver cells following SF30 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
2	Heterogenous nuclear ribonucleoprotein H	P31943	194	6.4e-016	49198	5.89	40045	5.59	13	8

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(b) Chang Liver cells following SF60 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
10	Macrophage capping protein	P40121	66	0.004	38494	5.88	33984	5.79	8	4
11	Cathepsin D precursor (EC 4.3.23.5)	P07339	806	4e-077	44524	6.10	43315	5.50	37	24
17	Ubiquinone biosynthesis protein COQ9	O75208	250	1.6e-021	35487	5.61	52278	5.49	15	7
20	40S ribosomal protein SA	P08865	58	0.023	32833	4.79	47924	4.70	15	4
21	Heat shock protein HSP 90-beta	P08238	150	1.6e-011	83212	4.97	51560	5.44	9	7
25	Phosphoglycerate kinase 1 (EC 2.7.2.3)	P00558	83	9e-005	44586	8.30	56409	6.53	7	6
27	Tubulin alpha-6 chain	Q9BQE3	72	0.001	49863	4.96	56409	6.08	5	4
28	Macrophage capping protein	P40121	85	4.8e-005	38494	5.88	57015	6.16	9	4
29	Heat shock 70kDa protein 1	P08107	301	1.3e-026	69995	5.48	58833	5.13	14	15
30	Heat shock cognate 71kDa protein	P11142	351	1.3e-031	70854	5.37	60045	5.00	9	10
31	Vimentin	P08670	516	4e-048	53619	5.06	65500	4.86	23	16
32	78kDa glucose-related protein precursor	P11021	364	6.4e-033	72288	5.07	63075	4.84	19	19
33	Dihydrolipollysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	P36957	84	6e-005	48609	9.01	67924	6.09	8	6
34	Heterogenous nuclear ribonucleoprotein H’	P55795	427	3.2e-039	49232	5.89	68530	6.21	36	17
35	Heterogenous nuclear ribonucleoprotein H	P31943	104	6.4e-007	49198	5.89	68530	6.16	11	6
36	T-complex protein 1 subunit beta	P78371	1160	1.6e-112	57452	6.01	70348	6.48	45	33

Table 4.7 (b), continued

37	Endoplasmin precursor	P14625	275	5.1e-024	92411	4.76	73378	4.76	9	14
40	Stress-70 protein	P38646	573	8e-054	73635	5.87	80651	5.50	27	25
42	78kDa glucose-related protein precursor	P11021	182	1e-014	72288	5.07	77621	5.34	5	6

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(c) Chang Liver cells following SF90 treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
43	Tubulin beta-1 chain	Q9H4B7	67	0.0036	50295	5.05	55196	6.00	4	4
44	Thioredoxin-like protein 2	O76003	70	0.0016	37408	5.31	55803	5.53	11	6
45	Leukocyte elastase inhibitor	P30740	242	1e-020	42715	5.90	59439	6.29	22	14
46	Protein FAM10A4	Q8IZP2	99	2.2e-006	27390	5.01	66712	5.28	17	8
47	Thioredoxin reductase 1 (EC 1.8.1.9)	Q16881	69	0.002	54672	6.07	73378	6.38	5	6

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

(d) Chang Liver cells following doxorubicin treatment

Spot #	Spot ID	Accession No. (Swiss-prot)	Score (Mascot)	Expect	Theoretical		Actual		SC (%)	Queries matched
					Mass	pI value	Mass	pI value		
31	Vimentin	P08670	516	4e-048	53619	5.06	65500	4.86	23	16
34	Heterogenous nuclear ribonucleoprotein H’	P55795	427	3.2e-039	49232	5.89	68530	6.21	36	17
52	Cytokeratin-18	P05783	159	2e-012	48029	5.34	13378	4.60	9	8
53	Chromobox protein homolog 3	Q13185	191	1.3e-015	20798	5.23	23147	2.35	20	6
55	Heme-binding protein 1	Q9NRV9	229	2e-019	21084	5.71	24287	5.70	30	8
56	Protein DJ1	Q99497	58	0.026	19878	6.33	25500	6.11	7	2
57	Thioredoxin-dependent peroxide reductase (EC 1.11.1.15)	P30048	234	6.4e-020	27675	7.67	26712	6.3	29	9
60	Peroxiredoxin-6 (EC 1.11.1.15)	P30041	76	0.00038	25019	6.00	32166	6.23	7	4
61	Heat shock protein beta-1	P04792	282	1e-024	22768	5.98	32166	5.81	36	13
62	NADH dehydrogenase (EC 1.6.5.3)	O75489	394	6.4e-036	30223	6.99	32772	5.79	26	14
64	Replication protein A 32kDa subunit	P15927	95	5.4e-006	29228	5.75	40651	6.04	11	4
65	Proteasome activator complex subunit 3	P61289	363	8e-033	29488	5.69	41257	5.96	44	18
66	Elongation factor 1-beta	P24534	290	1.6e-026	24748	4.50	44287	4.55	29	11
67	ADP-sugar pyrophosphate (EC 3.6.1.13)	Q9UKK9	532	1e-049	24312	4.87	48077	4.91	48	16
68	Sulfatase-modifying factor 2 precursor	Q8NBJ7	189	2e-015	33836	7.79	46712	6.06	25	14
69	Elongation factor 1-delta	P29692	206	4e-017	31103	4.90	49136	5.00	25	11
70	60S acidic ribosomal protein P0	P05388	66	0.004	34252	5.71	49742	6.25	20	6
71	60S acidic ribosomal protein P0	P05388	805	5.1e-077	34252	5.71	50348	5.84	51	22
72	Transaldolase (EC 2.2.1.2)	P37837	506	4e-047	37516	6.36	50954	6.18	33	20
73	Suppressor of G2 allele of SKP1 homolog	Q9Y2Z0	394	6.4e-036	40998	5.07	51560	5.44	25	14

Table 4.7 (d), continued

76	Isocitrate dehydrogenase [NAD] subunit alpha (EC 1.1.1.41)	P50213	200	1.6e-016	39566	6.47	53984	6.08	8	6
78	Heterogenous nuclear ribonucleoproteins C1/C2	P07910	422	1e-038	33650	4.95	55196	6.31	20	15
79	40S ribosomal protein SA	P08865	94	6.7e-006	32833	4.79	55196	4.96	18	6
80	Galactokinase (EC 2.7.1.6)	P51570	168	2.5e-013	42246	6.04	57015	6.38	39	19
81	Vimentin	P08670	288	2.5e-025	53619	5.06	56409	4.73	31	23
82	Uroporphyrinogen decarboxylase (EC 4.1.1.37)	P06132	523	8e-049	40761	5.77	56409	6.09	42	25
83	Reticulocalbin-1 precursor	Q15293	444	6.4e-041	38866	4.86	62469	4.70	34	17
86	Vimentin	P08670	88	2.3e-005	53619	5.06	63075	4.75	7	8
87	Cytokeratin-18	P05783	562	1e-052	48029	5.34	63075	5.59	38	25
88	Vimentin	P08670	1330	1.6e-129	53619	5.06	63075	4.73	57	45
89	Cytokeratin-17	Q04695	1050	1.6e-101	48076	4.97	64893	5.18	54	42
93	Heterogenous nuclear ribonucleoprotein K	P61978	678	2.5e-064	50944	5.39	76409	5.56	44	26
94	Heterogenous nuclear ribonucleoprotein K	P61978	590	1.6e-055	50944	5.39	76409	5.53	41	24
95	Lamin-B1	P20700	207	3.2e-017	66368	5.11	75803	5.15	18	12
98	Lamin-B1	P20700	132	1e-009	66368	5.11	79439	5.28	22	18
99	Lamin-B1	P20700	1020	1.6e-098	66368	5.11	79439	5.31	53	44

Accession number: the serial number of a protein matched to Swiss-prot.
Score: Mascot score greater than 59 was considered as confidence.
Expect: the number of times the match would be expected to occur purely by chance in a search of the entire database.
SC: sequence coverage in which percentage of identified peptides in the whole protein.
Queries matched: the matched peptides compared to the original protein.

4.8 Real-time RT-PCR

Selected deregulated proteins were subjected to real time RT-PCR for validation of protein expression. The selection of proteins was based on the known function of the proteins in cellular system. The results are shown in Table 4.8 (Hep G2 cells), Table 4.9 (Ca Ski cells) and Table 4.10 (Chang Liver cells). The RQ values represent the expression level or fold change in treated samples compared to untreated samples. Values of higher than one indicate up-regulation of mRNA transcription whereas values of less than one indicate down-regulation.

Table 4.8: RT-PCR (relative quantification) of selected proteins in Hep G2 cells following treatments

No.	Protein ID	Gene	Assay ID	RQ			
				Doxorubicin	SF30	SF60	SF90
1	Cathepsin D precursor	CTSD	Hs00157201_m1*	0.61[a]	0.37	0.43	0.76
2	Heat shock protein HSP-90 beta	HSP90AB1	Hs01546474_g1*	0.24[a]	0.31[a]	-	-
3	Heat shock cognate 71kDa protein	HSPA8	Hs00852842_gH	-	0.90[a]	-	-
4	Protein NDRG1	NDRG1	Hs00608387_m1*	-	1.40[a]	-	-
5	Stress-70 protein	HSPA9	Hs00269818_m1*	0.35	0.40[a]	-	-
6	Proliferating cell nuclear antigen (PCNA)	PCNA	Hs00427214_g1*	0.99[a]	-	0.12[a]	0.09[a]
7	Actin	ACTG1	Hs02340971_gH	-	-	0.69	0.77
8	60S acidic ribosomal protein P0	RPLP0	Hs99999902_m1*	0.25	-	0.35[a]	-
9	Cytokeratin-9	KRT9	Hs00413861_m1*	-	-	1.56[a]	-
10	78kDa glucose-regulated protein precursor (GRP78)	HSPA5	Hs00946084_g1*	0.16	-	0.47	-
11	Ferritin light chain	FTL	Hs00830226_gH*	0.13	-	-	0.65[a]
12	Chromobox protein homolog 3	CBX3	Hs00371848_m1	-	-	-	0.94[a]
13	Lysosomal protective protein precursor	CTSA	Hs00264902_m1*	-	-	-	0.78
14	Ubiquinone biosynthesis protein COQ9	COQ9	Hs00382782_m1*	-	-	-	0.53
15	Peroxiredoxin-2	PRDX2	Hs03044902_g1*	0.34[a]	-	-	-
16	Ubiquitin-conjugating enzyme E2-25kDa	UBE2K	Hs00193507_m1*	0.16[a]	-	-	-
17	Sulfotransferase 1A1	SULT1A1	HS00236900_m1	0.68[a]	-	-	-
18	Uncharacterized protein C17orf25	GLOD4	Hs00274996_m1	0.67[a]	-	-	-
19	Cytokeratin-18	KRT18	Hs01941416_g1*	0.42	-	-	-
20	Proliferation-associated protein 2G4	PA2G4	Hs00854538_g1*	0.84[a]	-	-	-
21	RuvB-like 1	RUVBL1	Hs00186558_m1*	0.33	-	-	-
22	Lamin-B1	LMNB1	Hs01059202_m1*	0.10	-	-	-

The endogenous control was 18S.

[a] indicates that RNA and protein deregulation are similar.

- indicates that the assay was not performed as the protein was not deregulated.

Table 4.9: RT-PCR (relative quantification) of selected proteins in Ca Ski cells following treatments

No.	Protein ID	Gene	Assay ID	RQ			
				Doxorubicin	SF30	SF60	SF90
1	Heat shock cognate 71kDa protein	HSPA8	Hs00852842_gH	-	0.75[a]	-	-
2	Actin	ACTG1	Hs02340971_gH	0.95[a]	-	1.10	1.08
3	60S acidic ribosomal protein P0	RPLP0	Hs99999902_m1*	0.91[a]	-	0.11[a]	-
4	Cytokeratin-9	KRT9	Hs00413861_m1*	2.69	-	-	-
5	Chromobox protein homolog 3	CBX3	Hs00371848_m1	2.39	-	-	-
6	Thioredoxin-like protein 2	GLRX3	Hs01582641_g1*	0.77[a]	-	-	-
7	Proteasome activator complex subunit 3	PSME3	Hs00195072_m1*	1.17	-	-	-
8	Suppressor of G2 allele of SKP1 homolog	SUGT1	Hs00362511_g1*	2.19[a]	-	-	-
9	Peroxiredoxin-6	PRDX5	Hs00201536_m1	0.90[a]	-	-	-
10	Microtubule-associated protein RP/EB family member 1	MAPRE1	Hs01121102_g1*	1.94	-	-	-
11	Ran-specific GTPase-activating protein	RANBP1	Hs01597912_g1*	0.87[a]	-	-	-

The endogenous control was 18S.

[a] indicates that RNA and protein deregulation are similar.

- indicates that the assay was not performed as the protein was not deregulated.

Table 4.10: RT-PCR (relative quantification) of selected proteins in Chang Liver cells following treatments

No.	Protein ID	Gene	Assay ID	RQ			
				Doxorubicin	SF30	SF60	SF90
1	Cathepsin D precursor	CTSD	Hs00157201_m1*	-	-	1.32[a]	-
2	Heat shock cognate 71kDa protein	HSPA8	Hs00852842_gH	-	-	0.92	-
3	Stress-70 protein	HSPA9	Hs00269818_m1*	-	-	1.49[a]	-
4	60S acidic ribosomal protein P0	RPLP0	Hs99999902_m1*	0.56[a]	-	-	-
5	78kDa glucose-regulated protein precursor (GRP78)	HSPA5	Hs00946084_g1*	-	-	1.19[a]	-
6	Chromobox protein homolog 3	CBX3	Hs00371848_m1	0.30[a]	-	-	-
7	Ubiquinone biosynthesis protein COQ9	COQ9	Hs00382782_m1*	-	-	1.10[a]	-
8	Cytokeratin-18	KRT18	Hs01941416_g1*	0.10	-	-	-
9	Lamin-B1	LMNB1	Hs01059202_m1*	0.07	-	-	-
10	Heterogenous nuclear ribonucleoprotein H	HPNPH1	Hs00800662_sH	-	1.76[a]	0.82[a]	-
11	Macrophage capping protein	CAPG	Hs00156249_m1*	-	-	0.80	-
12	40S ribosomal protein SA	RPSA	Hs00399294_g1	0.75[a]	-	0.79	-
13	Vimentin	VIM	Hs00185584_m1*	0.44	-	0.76	-
14	T-complex protein 1 subunit beta	CCT2	Hs00197562_m1*	-	-	0.79[a]	-
15	Thioredoxin-like protein 2	GLRX3	Hs01582641_g1*	-	-	-	0.90[a]
16	Leukocyte elastase inhibitor	SERPINB1	Hs00961948_m1*	-	-	-	1.22
17	Protein FAM10A4	ST13	Hs00832556_sH*	-	-	-	0.80[a]
18	Thioredoxin reductase 1	TXNRD1	Hs00917067_m1*	-	-	-	0.86[a]
19	Protein DJ1	PARK7	Hs00994896_g1*	0.14[a]	-	-	-
20	Proteasome activator complex subunit 3	PSME3	Hs00195072_m1*	0.25[a]	-	-	-
21	Suppressor of G2 allele of SKP1 homolog	SUGT1	Hs00362511_g1*	0.26[a]	-	-	-
22	Cytokeratin-17	KRT17	Hs01588578_m1*	0.54[a]	-	-	-

The endogenous control was 18S.

[a] indicates that RNA transcription and protein deregulation shared a similar pattern.

- indicates that the assay was not performed as the protein was not deregulated.