

6. Cytotoxicity Studies

The natural and synthetic compounds, which were found to be hepatoprotective, were subjected to cytotoxicity assay. The cytotoxic activity of compounds was studied by using human neutrophils. An *in vitro* spectrophotometric method was developed for this study which measures the cell viability (%) after the incubation of test compounds with human neutrophils. The assay is based on the reduction of tetrazolium salt WST-1 by mitochondrial dehydrogenases of viable cells to yellow-orange formazan dye, which can be measured spectrophotometrically (Berridge *et al.*, 1996; Hong *et al.*, 2001).

6.1. Cytotoxicity Studies on Natural Antioxidants

The cytotoxicity of compounds 2, 11, 8, 9, 27, 28, 33 and 34 was studied by measuring the effect of different concentrations ranges from 25-200 $\mu\text{g}/\text{mL}$ of each compound on the viability of cells. The % cell viability at 25 $\mu\text{g}/\text{mL}$ concentration of all natural products, except compound 11, was found to be 99.4-65.27%. Compound 11 was found to be the most toxic in the series as it allowed only 35.48% of cell viability at 25 $\mu\text{g}/\text{mL}$. Even at a lower concentration (12.5 $\mu\text{g}/\text{mL}$) it exhibited only 43.61 % cell viability. On the other hand, compound 34 was found to be non-toxic as inferred from the 100% cell viability in all four concentrations. The effect of different concentrations of compounds on cell viability is presented in Figure 6.1, while the ED_{50} values (effective dose of compound at which 50% cells are viable) are presented in Table 6.1.

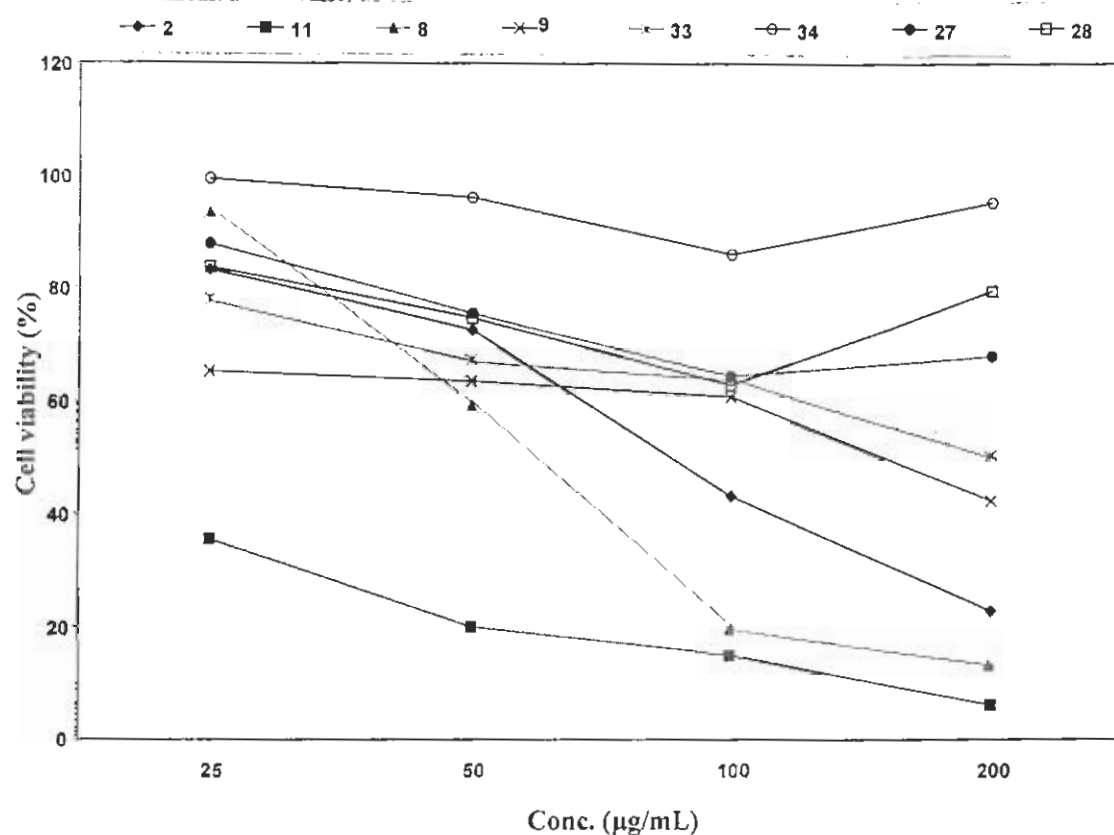


Figure 6.1: Effects of various concentrations of natural compounds 2, 8, 9, 11, 27, 28, 33 and 34, on the viability of human neutrophils after 3 hrs of incubation. All values are the mean of four replicates.

Table 6.1: Cytotoxicity of natural compounds on the viability of human neutrophils after 3 hrs of incubation.

Compound	ED ₅₀ (µg/mL)*
2	90.35 ± 1.71
8	63.24 ± 2.63
9	148.56 ± 1.02
11	<12.50
27	>200
28	>200
33	200 ± 4.40
34	>200

* ED₅₀ values are the mean ± standard error mean (SEM) of four assays.

6.2. Cytotoxicity Studies on Synthetic Antioxidants

Synthetic compounds, 45, 49, 51-56, 58, 60, 61, 63, 66, 68, 69, 86, 87, 97 and 102, studied for their hepatoprotective effect, were subjected to cytotoxicity towards human neutrophils. These compounds belong to four different classes i.e. β -*N*-substituted hydrazide, 1,3,4-oxadiazole-2-(3*H*)-thione and coumarin and benzothiazepine.

The effect of various concentrations of β -*N*-substituted hydrazide derivatives 45, 49, 51-56 and 58, on cell viability is presented in Figure 6.2. Compounds 49, 54 and 55 were found to be the most cytotoxic as inferred from their low ED₅₀ values (36.51, <12.50 and 15.30 $\mu\text{g}/\text{mL}$, respectively). However, compounds 45, 51-53, 56 and 58 showed cell viability in the range of 74.54-56.40% even at a higher concentration of 200 $\mu\text{g}/\text{mL}$ representing their non-toxic behavior.

The compounds 68 and 69, belonging to 1,3,4-oxadiazole-2-(3*H*)-thione class, were found to be less toxic as they showed 75% cells viability at a concentration of 200 $\mu\text{g}/\text{mL}$. However, compound 61 was found to be toxic with a low ED₅₀ value <12.50 $\mu\text{g}/\text{mL}$. The effect of different concentrations of compounds 60, 61, 63, 66, 68 and 69 on cell viability is presented in Figure 6.3.

The results of the cytotoxicity assay on compounds 86-87 and 97, 102, which belong to coumarin and benzothiazapine classes respectively, showed that compounds 87 and 97 were toxic indicated by their ED₅₀ values of 112.72 and 93.43 $\mu\text{g}/\text{mL}$, respectively. The effect of different concentrations of compounds 86-87, 97, and 102 on cell viability is presented in Figure 6.4. The ED₅₀ values of all synthetic compounds are presented in Table 6.2.

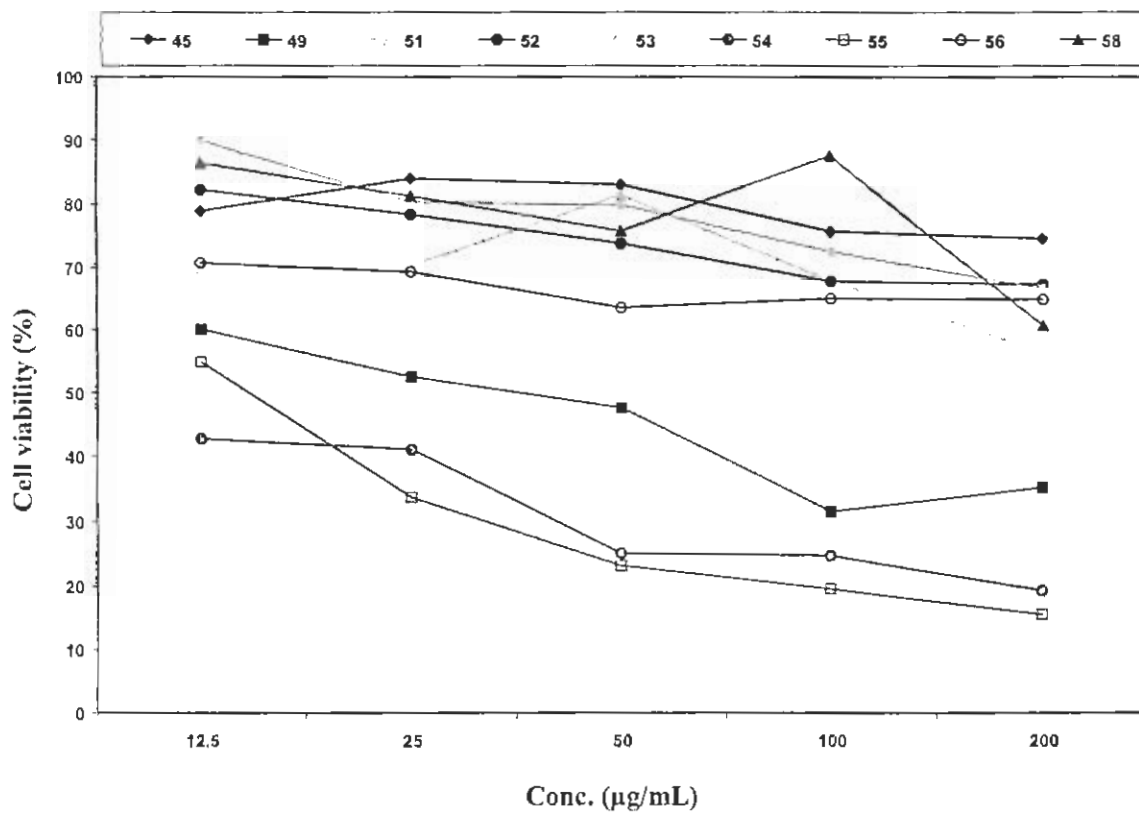


Figure 6.2: Effects of various concentrations of β -*N*-substituted hydrazide derivatives 45, 49, 51-56, and 58 on the viability of human neutrophils after 3 hrs incubation. All values are the mean of four replicates.

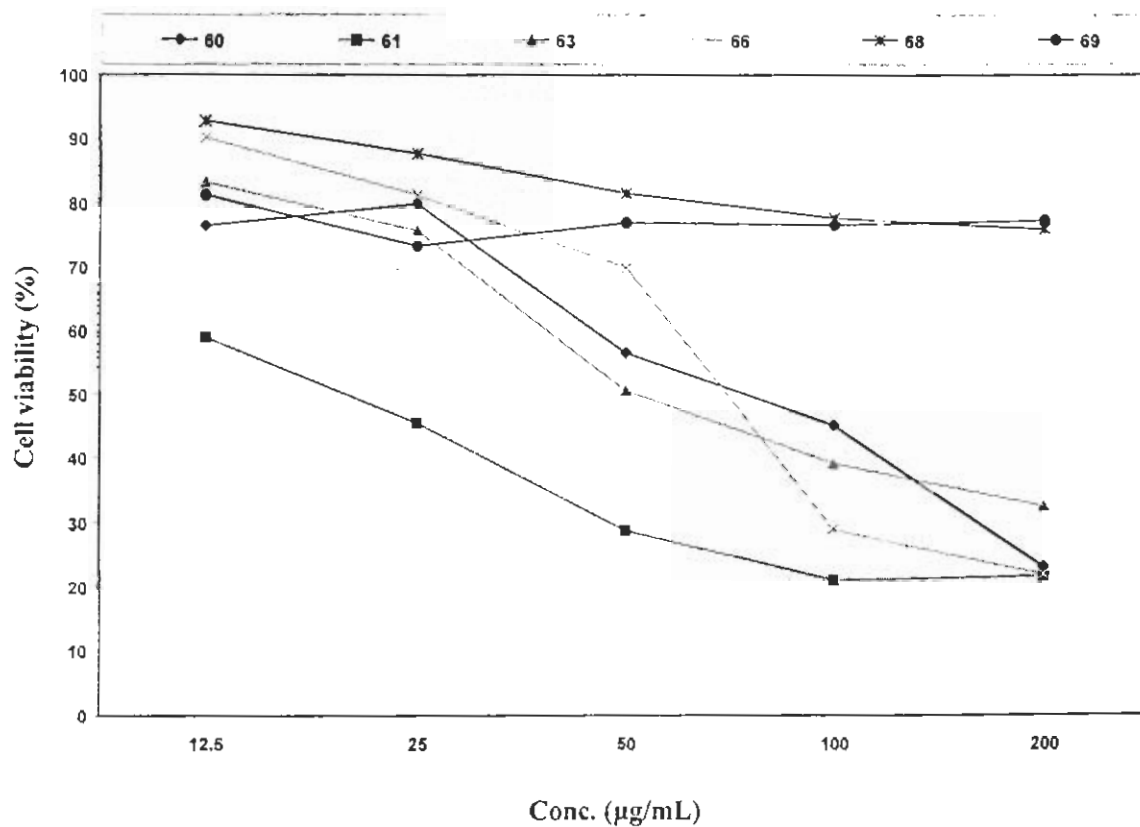


Figure 6.3: Effects of various concentrations of 3,4-oxadiazole-2-(3H)-thione derivatives 60, 61, 63, 66, 68 and 69 on the viability of human neutrophils after 3 hrs incubation. All values are the mean of four replicates.

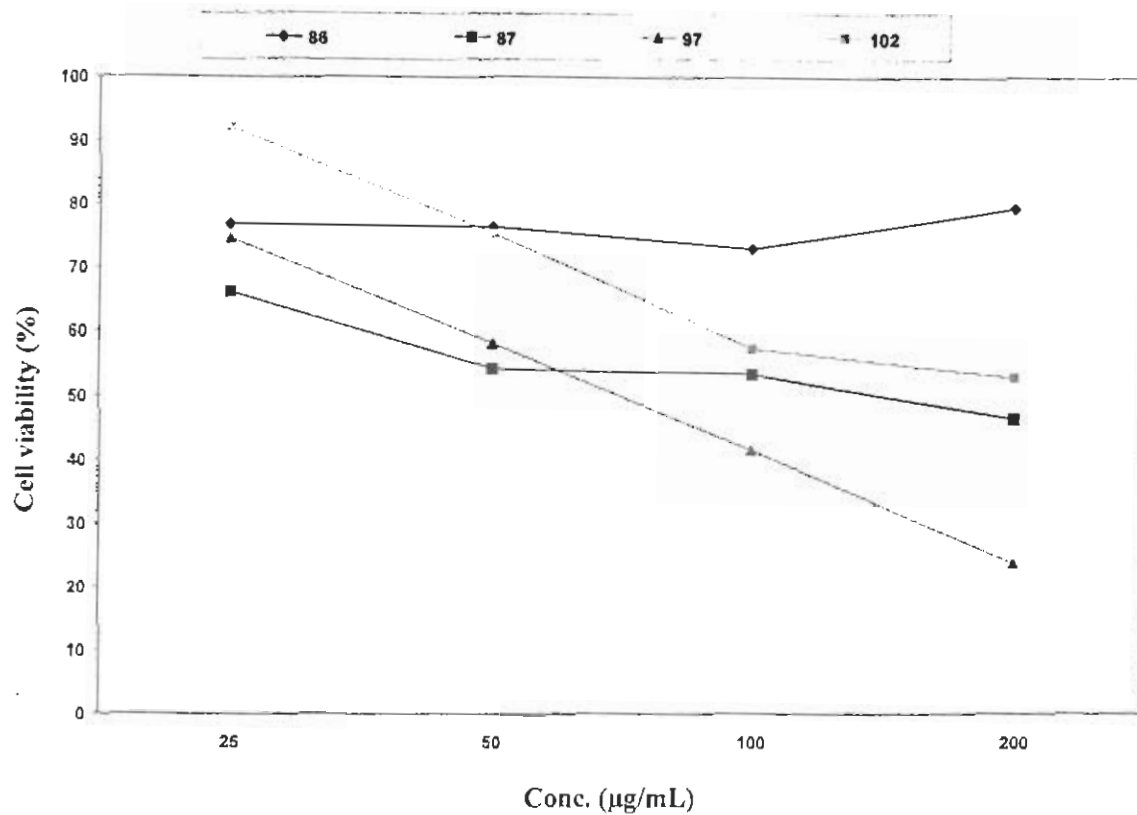


Figure 6.4: Effects of various concentrations of coumarins 86, 87 and 97 and benzothiazepine derivative 102 on the viability of human neutrophils after 3 hrs. incubation. All values are the mean of four replicates.

Table 6.2: Cytotoxicity of synthetic compounds on the viability of human neutrophils after 3 hrs of incubation.

Compound Nos.	ED ₅₀ (µg/mL)*
45	>200
49	36.51 ± 4.47
51	>200
52	>200
53	>200
54	<12.50
55	15.30 ± 2.45
56	>200
58	>200
60	80.30 ± 6.02
61	<12.50
63	64.80 ± 4.21
66	69.92 ± 5.30
68	>200
69	>200
86	>200
87	112.72 ± 3.14
97	93.43 ± 9.31
102	>200

* ED₅₀ values are the mean ± standard error mean (SEM) of four assays.