CHAPTER IV

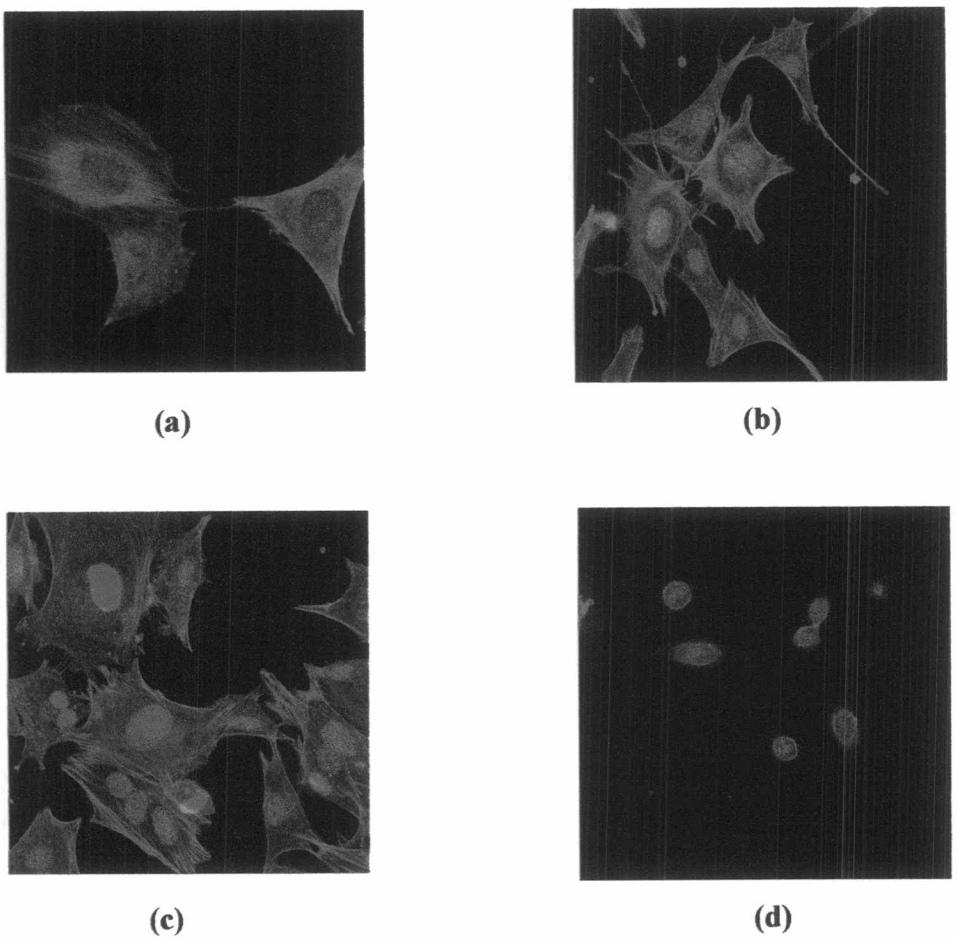
RESULTS

In Vitro Results

Immunofluorescence Microscopy

The Dose-Response Relationship of Sodium Arsenite Effects on Cytoskeleton, Focal Adhesions and Mitochondrial Localization

Actin cytoskeleton of mouse fibroblasts was disrupted by sodium arsenite. At 25 μM sodium arsenite caused a severe loss of F-actin and most cells became rounded as shown in Figure 5.



(a) **(b)**

(c) **(d)**

Figure 5 Actin cytoskeleton of mouse fibroblasts exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 5 μM (c) $NaAsO_2$ 10 μM and (d) $NaAsO_2$ 25 μM

Microtubule of mouse fibroblasts was disrupted by sodium arsenite. At 25 μM sodium arsenite caused a severe loss microtubule and most cells became rounded as shown in Figure 6.

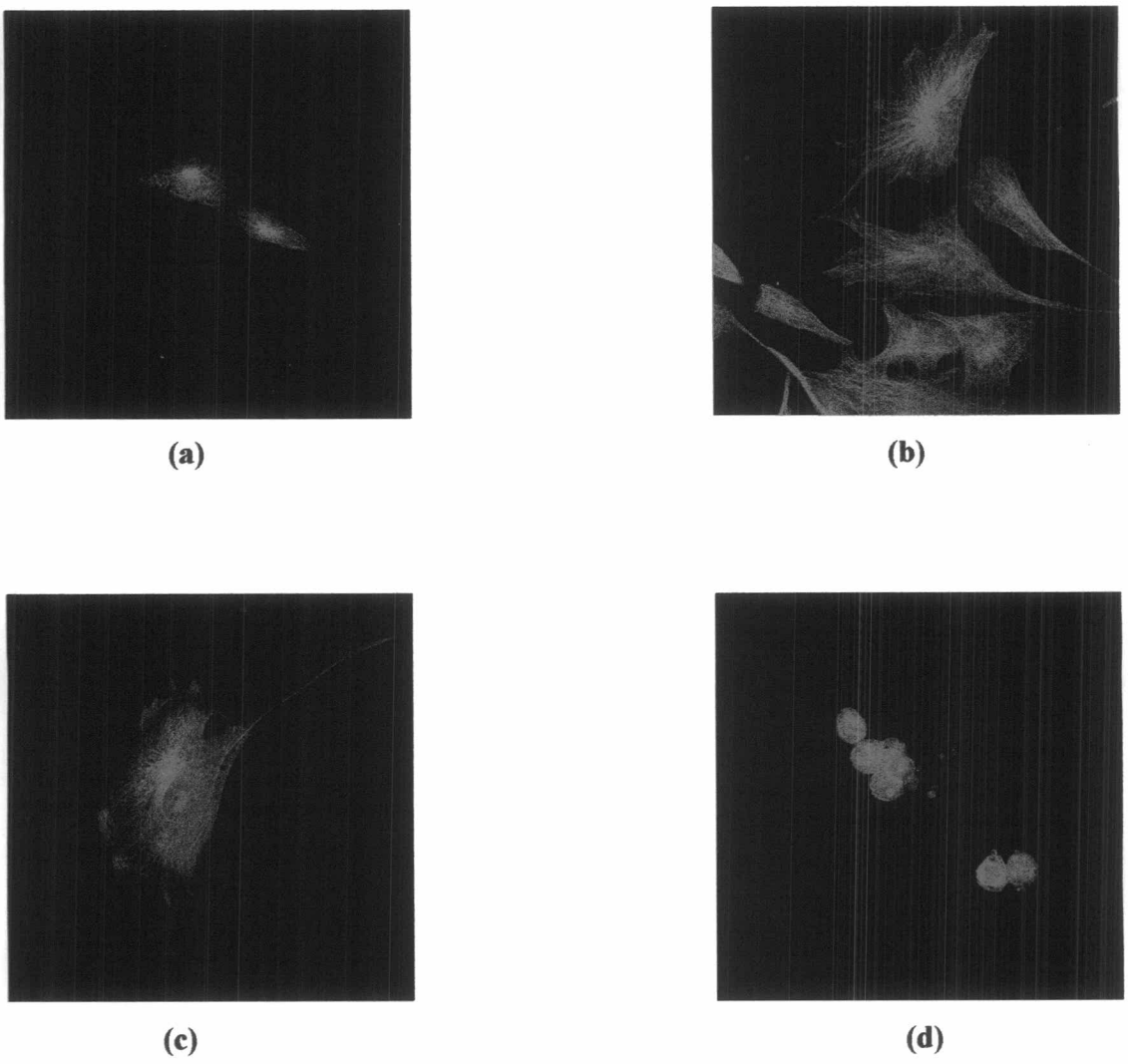


Figure 6 Microtubule of mouse fibroblasts exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 5 μM (c) $NaAsO_2$ 10 μM and (d) $NaAsO_2$ 25 μM

Vinculin of mouse fibroblasts was disrupted by sodium arsenite. Lamellipodia were formed when fibroblasts were exposed to sodium arsenite at 5 μM. At 25 μM sodium arsenite caused a severe loss of vinculin and most cells became rounded as shown in Figure 7.

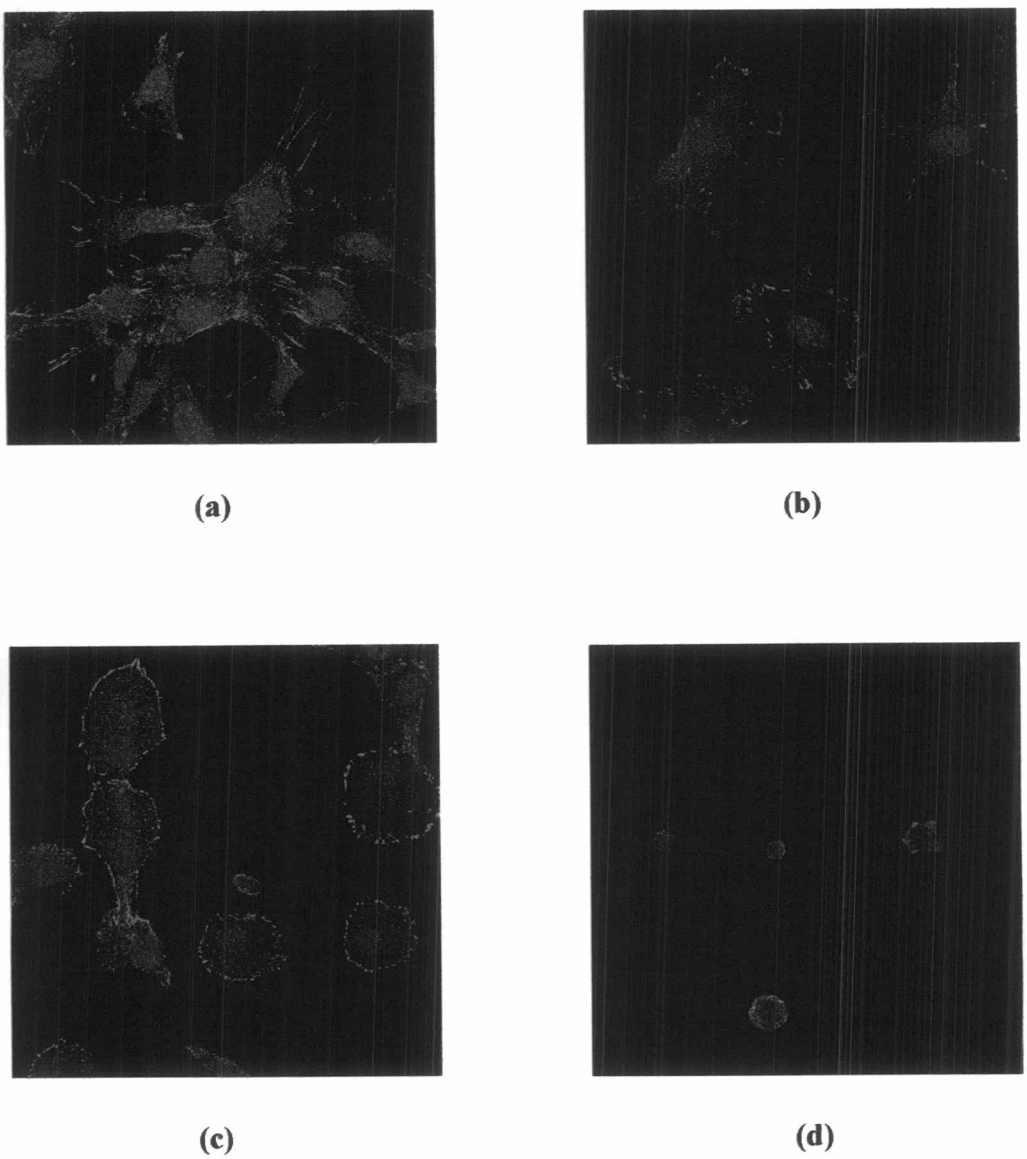


Figure 7 Vinculin of mouse fibroblasts exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 5 μM (c) $NaAsO_2$ 10 μM and (d) $NaAsO_2$ 25 μM

α-Actinin of mouse fibroblasts was disrupted by sodium arsenite. Lamellipodia were formed when fibroblasts were exposed to sodium arsenite at 5 μM and 10 μM. At 25 μM sodium arsenite caused a severe loss α-actinin and most cells became rounded as shown in Figure 8.

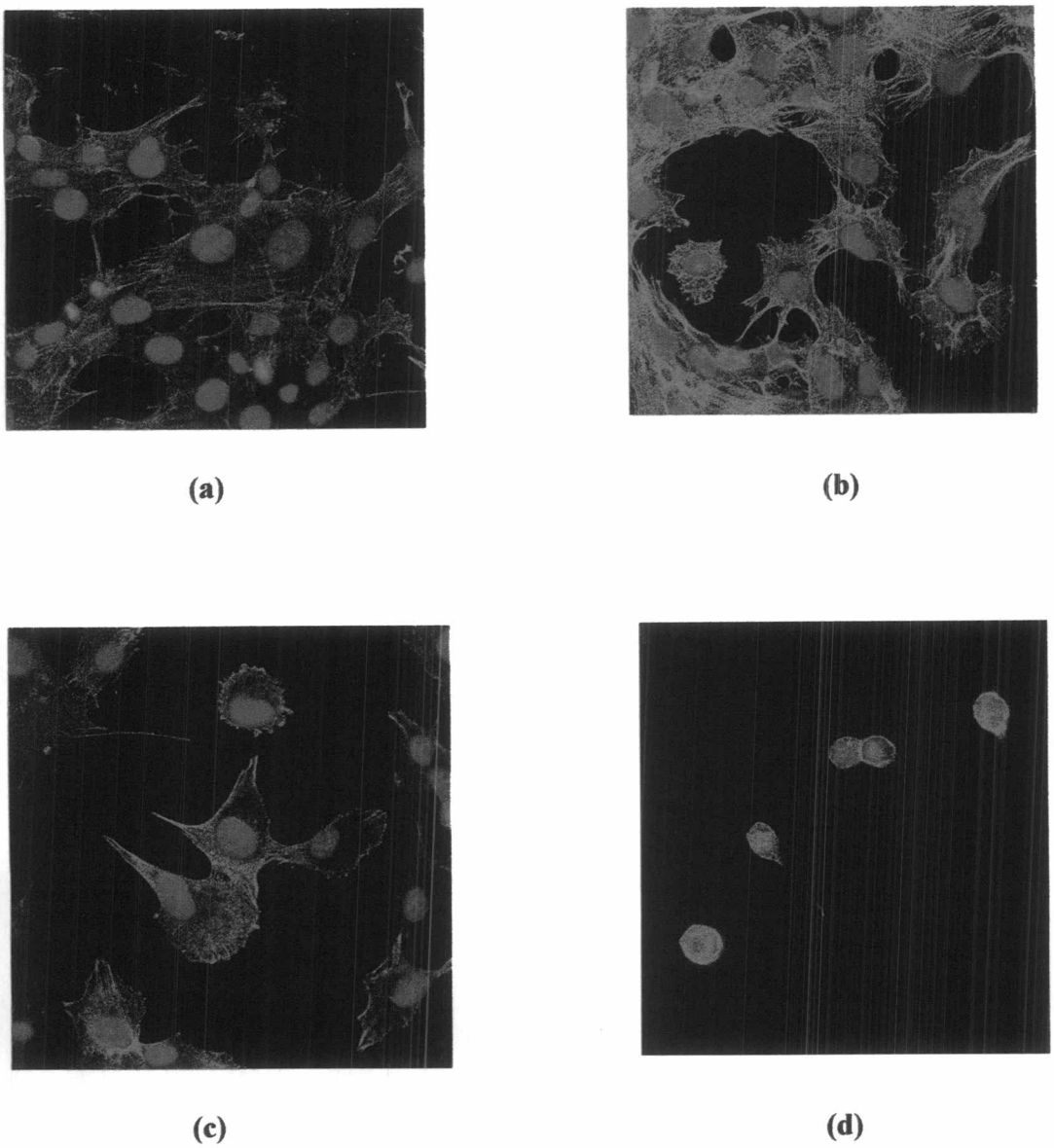
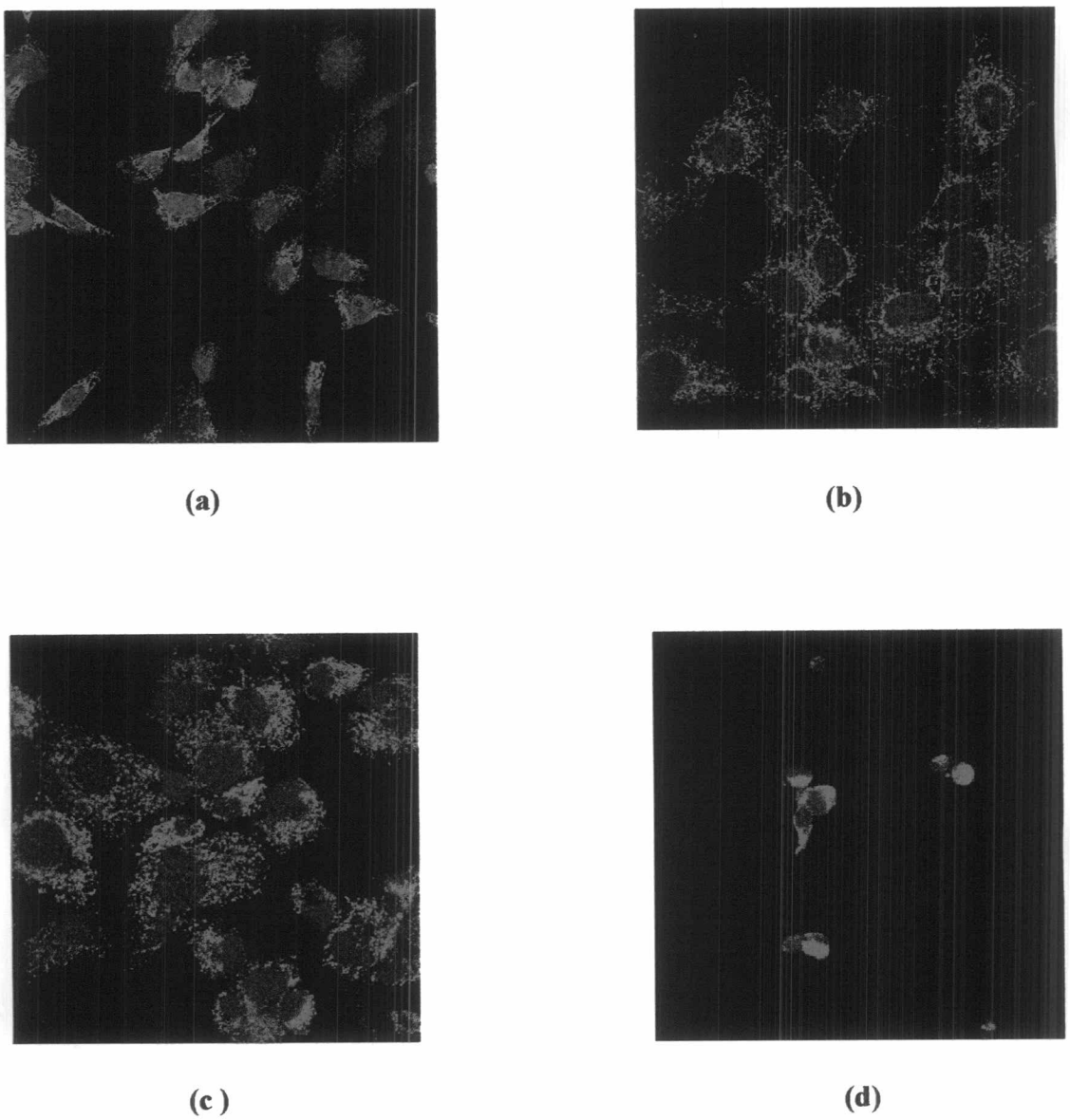


Figure 8 α-Actinin of mouse fibroblasts exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 5 μM (c) $NaAsO_2$ 10 μM and (d) $NaAsO_2$ 25 μM

Mitochondrial localization of mouse fibroblasts was disrupted by sodium arsenite. At 25 μM sodium arsenite, most cells became rounded as shown in Figure 9.



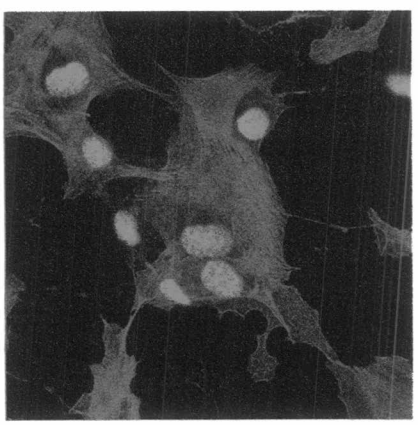
(a) **(b)**

(c) **(d)**

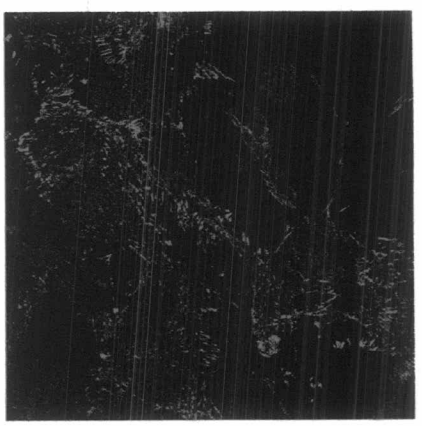
Figure 9 Mitochondrial localization of mouse fibroblasts exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 5 μM (c) $NaAsO_2$ 10 μM and (d) $NaAsO_2$ 25 μM

A Non-Specific Tyrosine Kinase Inhibitor (Genistein) Can Block the Toxic Effects Induced by Sodium Arsenite

Actin cytoskeleton, vinculin, and mitochondrial localization of mouse fibroblasts were disrupted by sodium arsenite but these toxic effects can be blocked by genistein as shown in Figure 10.



(a)



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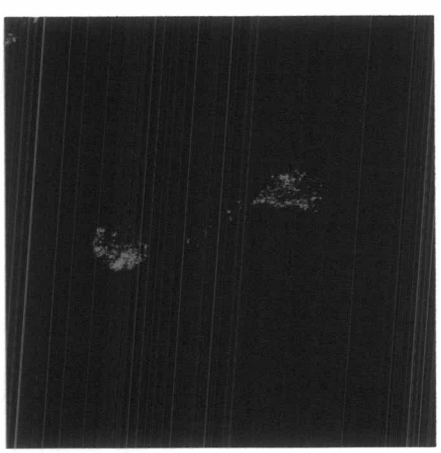
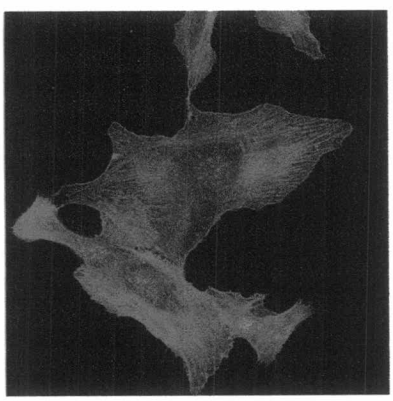


(c)

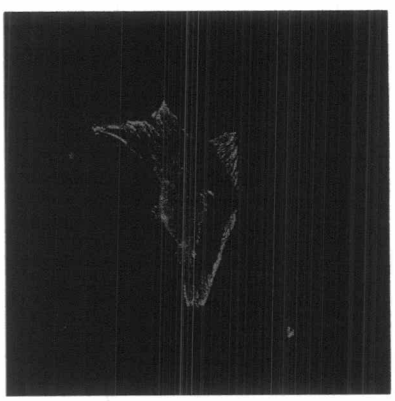
Figure 10 Mouse fibroblasts were exposed to $NaAsO_2$ 25 μM and genistein 40 μg/ml (a) actin cytoskeleton (b) vinculin (c) mitochondrial localization

Non-Specific Serine/Threonine Kinase Inhibitor (Staurosporine) Blocks the Toxic Effects Induced by Sodium Arsenite

Actin cytoskeleton, vinculin, and mitochondrial localization of mouse fibroblasts were disrupted by sodium arsenite but these toxic effects can be blocked by staurosporine as shown in Figure 11.



(a)



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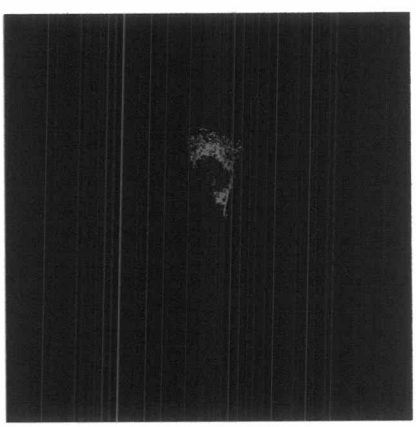
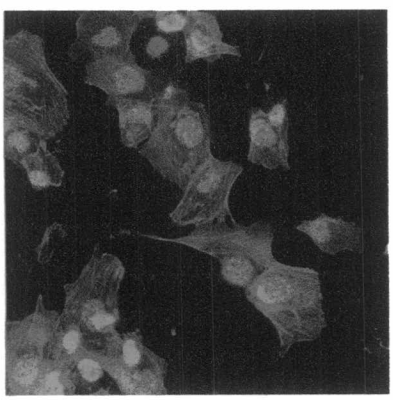


(c)

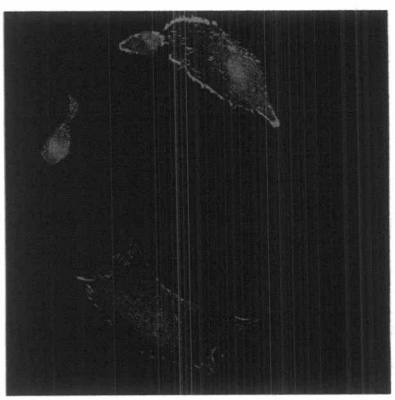
Figure 11 Mouse fibroblasts were exposed to $NaAsO_2$ 25 μM and staurosporine 1nM (a) actin cytoskeleton (b) vinculin (c) mitochondrial localization

The Epidermal Growth Factor Receptor (EGFR) Inhibitor (4,5-Dianilinophthalimide) Can Block the Toxic Effects Induced by Sodium Arsenite

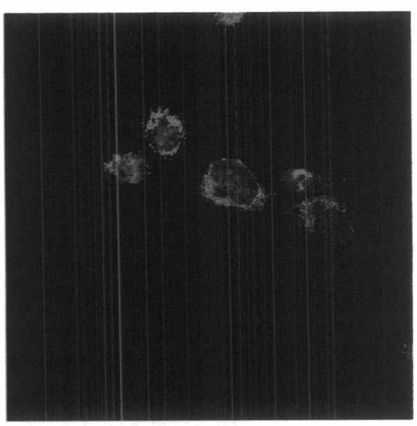
Actin cytoskeleton, vinculin, and mitochondrial localization of mouse fibroblasts were disrupted by sodium arsenite but these toxic effects can be blocked by 4,5-dianilinophthalimide as shown in Figure 12.



(a)



(b)

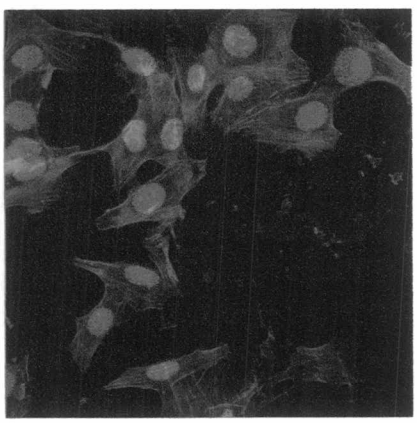


(c)

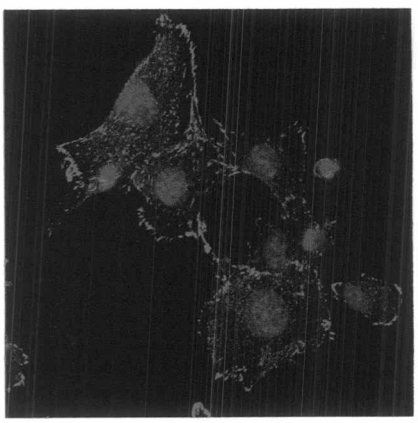
Figure 12 Mouse fibroblasts were exposed to $NaAsO_2$ 25 μM and 4,5-dianilinophthalimide 1 μM (a) actin cytoskeleton (b) vinculin (c) mitochondrial localization

The Phosphatidylinositol 3-Kinase (PI3K) Inhibitor (Wortmannain) Can Block the Toxic Effects Induced by Sodium Arsenite

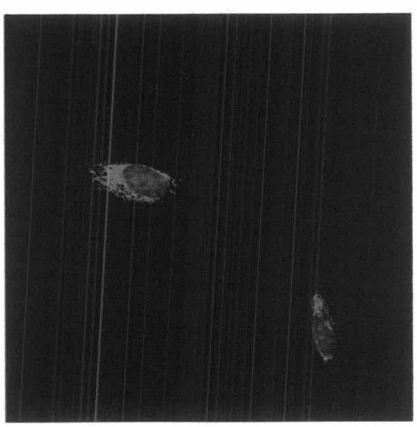
Actin cytoskeleton, vinculin, and mitochondrial localization of mouse fibroblasts were disrupted by sodium arsenite but these toxic effects can be blocked by wortmannin as shown in Figure 13.



(a)



(b)

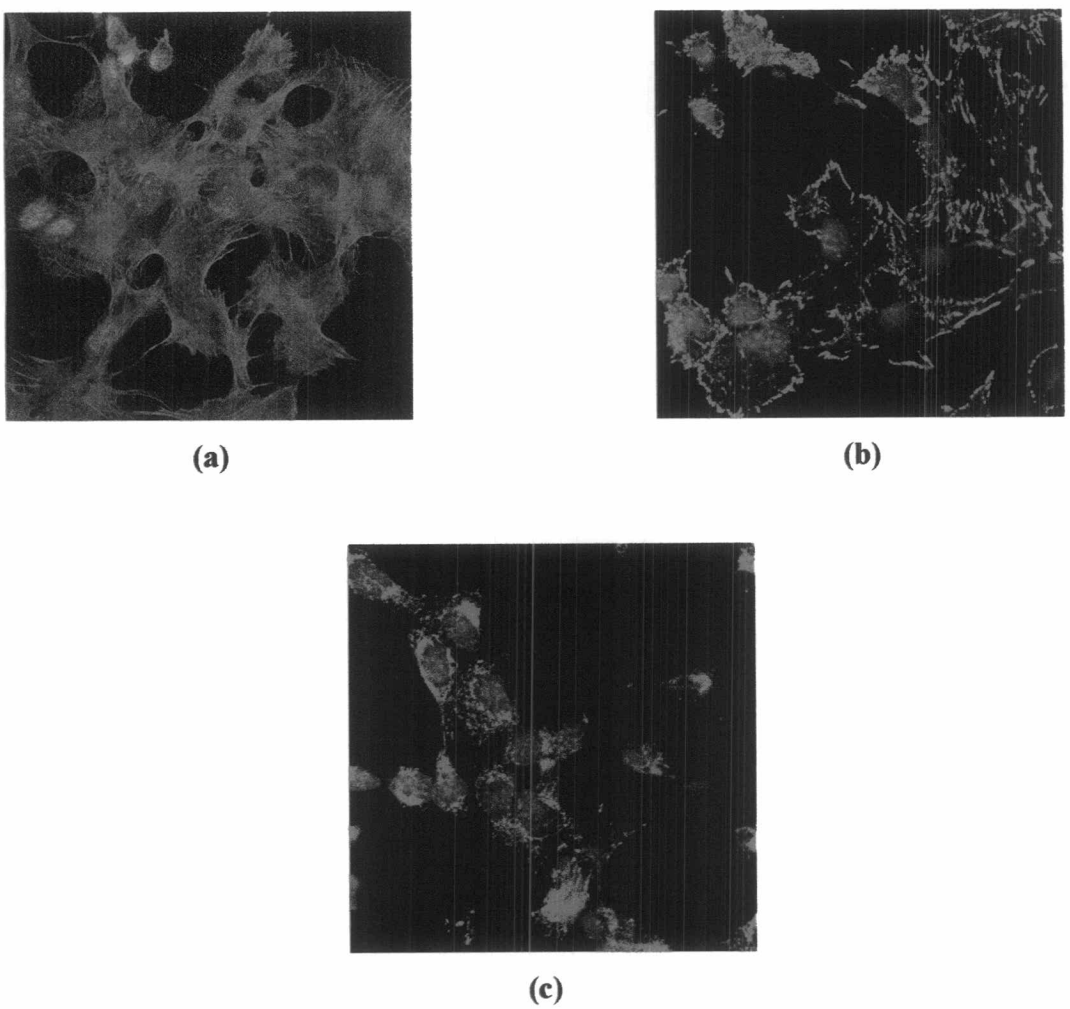


(c)

Figure 13 Mouse fibroblasts were exposed to $NaAsO_2$ 25 μM and wortmannin 200 nM (a) actin cytoskeleton (b) vinculin (c) mitochondrial localization

The RNA Synthesis Inhibitor (Actinomycin D) Can Block the Toxic Effects Induced by Sodium Arsenite

Actin cytoskeleton, vinculin, and mitochondrial localization of mouse fibroblasts were disrupted by sodium arsenite but these toxic effects can be blocked by actinomycin D as shown in Figure 14.



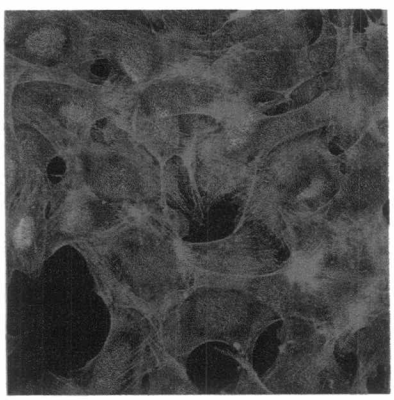
(a) **(b)**

(c)

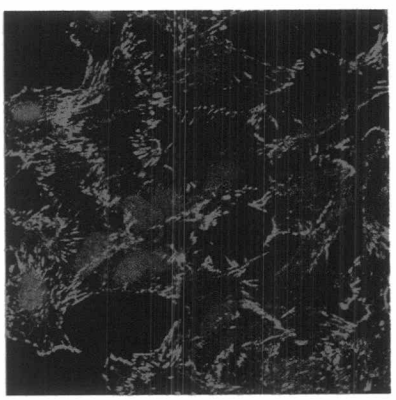
Figure 14 Mouse fibroblasts were exposed to $NaAsO_2$ 25 μM and actinomycin D 10 μg/ml (a) actin cytoskeleton (b) vinculin (c) mitochondrial localization

The Protein Synthesis Inhibitor (Cycloheximide) Can Block the Toxic Effects Induced by Sodium Arsenite

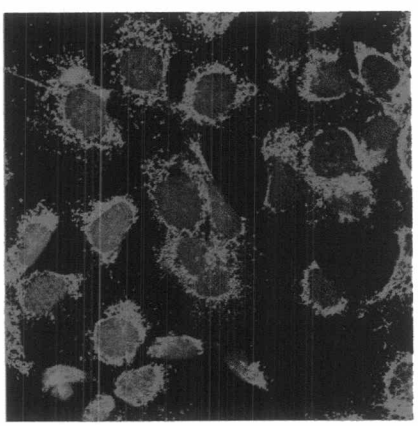
Actin cytoskeleton, vinculin, and mitochondrial localization of mouse fibroblasts were disrupted by sodium arsenite but these toxic effects can be blocked by cycloheximide as shown in Figure 15.



(a)



(b)



(c)

Figure 15 Mouse fibroblasts were exposed to $NaAsO_2$ 25 μM and cycloheximide 5 μg/ml (a) actin cytoskeleton (b) vinculin (c) mitochondrial localization

The Phosphatidylinositol 3-Kinase Inhibitor and MAP Kinase Inhibitor (Apigenin) Can Block the Toxic Effects Induced by Sodium Arsenite

Actin cytoskeleton, vinculin, and mitochondrial localization of mouse fibroblasts were disrupted by sodium arsenite but these toxic effects can be blocked by apigenin as shown in Figure 16.

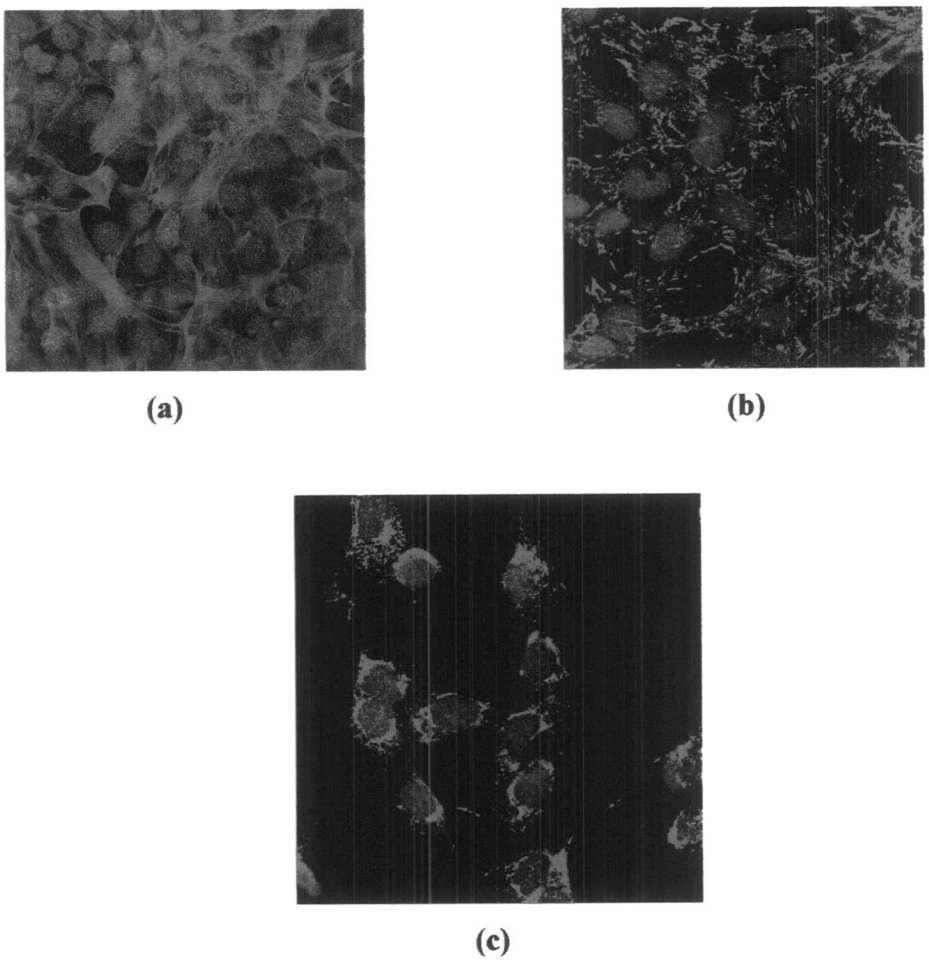
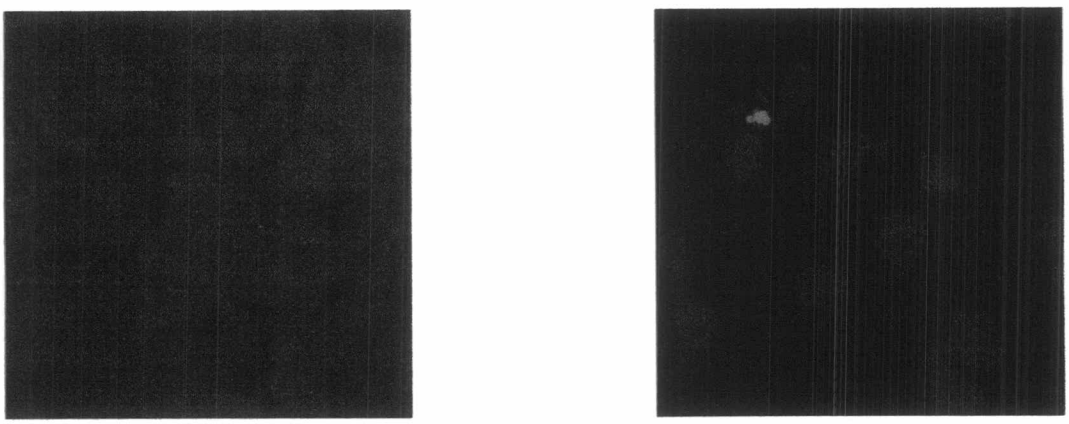


Figure 16 Mouse fibroblasts were exposed to $NaAsO_2$ 25 μM and apigenin 100 μM (a) actin cytoskeleton (b) vinculin (c) mitochondrial localization

Detection of Apoptosis in Sodium Arsenite-Exposed Mouse Fibroblasts by In Situ Cell Death Detection Kit, Fluorescein

There is no apoptosis in sodium arsenite-exposed mouse fibroblasts as shown in Figure 17.



(a) (b)

Figure 17 Mouse fibroblasts were exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 25 μM

Cervical Cancer Cell (HeLa) Morphology Change Induced by Sodium Arsenite

For F-actin staining, the HeLa cells became rounded when cells were exposed to sodium arsenite as shown in Figure 18.

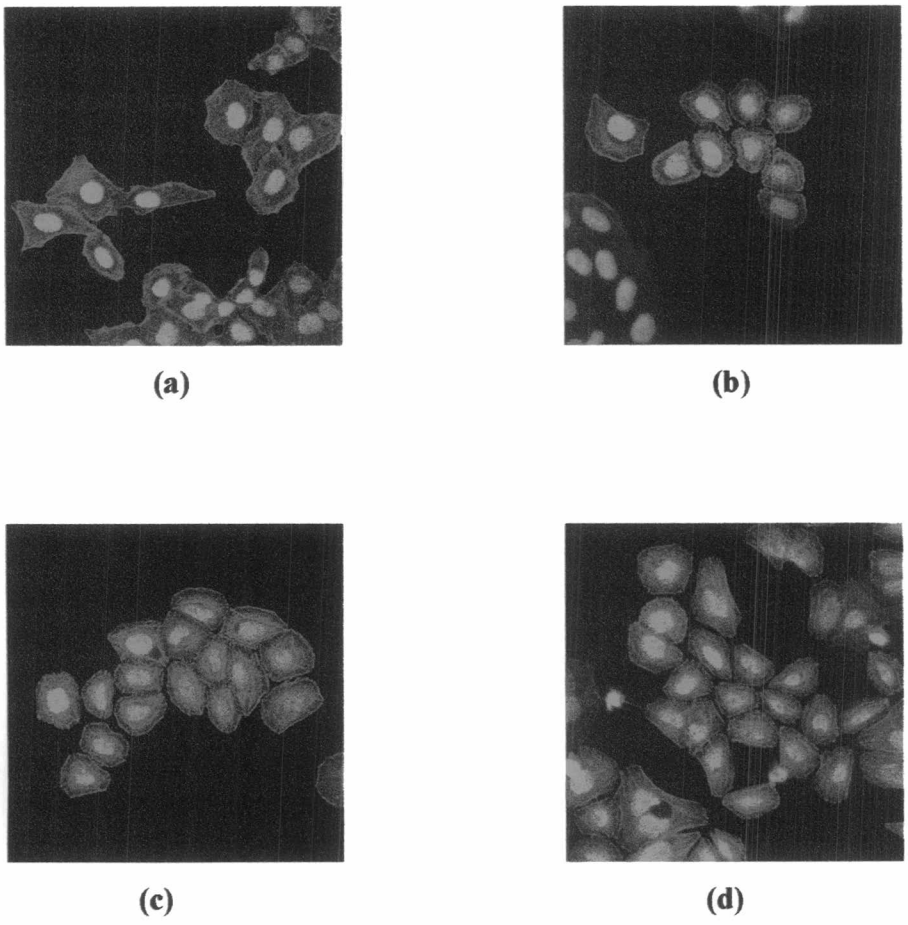
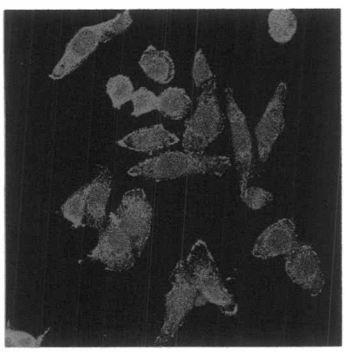
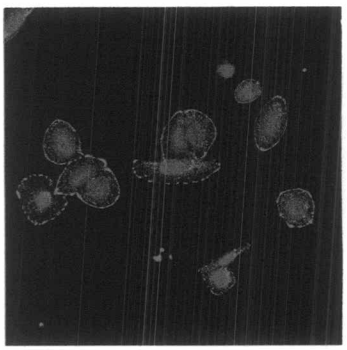


Figure 18 Actin cytoskeleton of HeLa cells exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 5 μM (c) $NaAsO_2$ 10 μM (d) $NaAsO_2$ 25 μM

For vinculin staining, the HeLa cells became rounded when cells were exposed to sodium arsenite as shown in Figure 19.

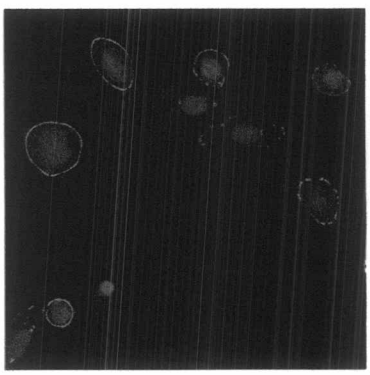


(a)



(b)

(c)



(d)

Figure 19 Vinculin of HeLa cells exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 5 μM (c) $NaAsO_2$ 10 μM (d) $NaAsO_2$ 25 μM

For mitochondrial staining, the HeLa cells became rounded when cells were exposed to sodium arsenite as shown in Figure 20.

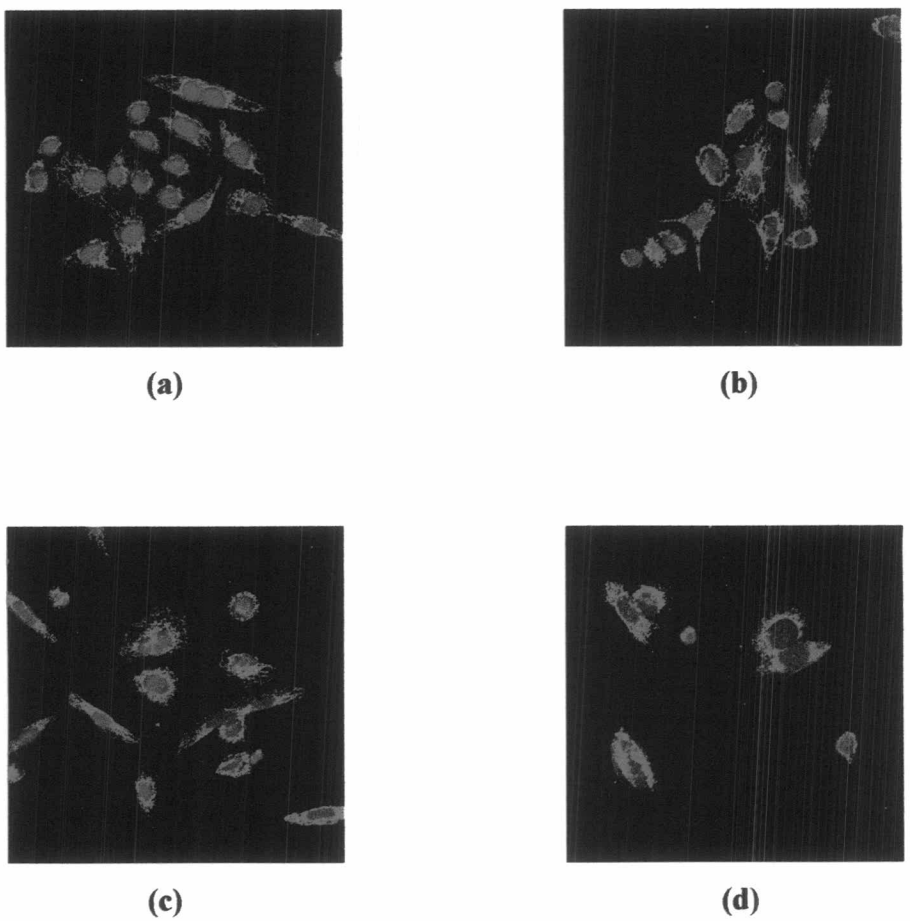


Figure 20 Mitochondrial localization of HeLa cells exposed to (a) $NaAsO_2$ 0 μM (b) $NaAsO_2$ 5 μM (c) $NaAsO_2$ 10 μM (d) $NaAsO_2$ 25 μM

Cell Area Was Measured by Laser Scanning Confocal Microscopy

Cell area of mouse fibroblasts stained with anti-F-actin was reduced when cell were exposed to sodium arsenite. This effect can be blocked by all inhibitors as shown in Table 2.

Table 2 Cell area of mouse fibroblasts stained with anti-F-actin

F-Actin Staining	Cell Area (μm x μm, N = 10, Mean ± S.E.)
$NaAsO_2$ 0 μM	1200 ± 120
$NaAsO_2$ 25 μM	200 ± 20*
$NaAsO_2$ 25 μM + Genistein 40 μg/ml	1200 ± 120
$NaAsO_2$ 25 μM + 4,5-Dianilinophthalimide 1μM	1100 ± 90
$NaAsO_2$ 25 μM + Wortmannin 200 nM	1300 ± 100
$NaAsO_2$ 25 μM + Cycloheximide 5 μg/ml	1100 ± 40
$NaAsO_2$ 25 μM + Actinomycin D 10 μg/ml	1000 ± 80
$NaAsO_2$ 25 μM + Apigenin 100 μM	1000 ± 70

*p value < 0.05 as a significant when compared with the control

Cell area of mouse fibroblasts stained with anti-vinculin was reduced when cell were exposed to sodium arsenite. This effect can be blocked by all inhibitors as shown in Table 3.

Table 3 Cell area of mouse fibroblasts stained with anti-vinculin

Vinculin Staining	Cell Area (μm x μm, N = 10, Mean ± S.E.)
$NaAsO_2$ 0 μM	1100 ± 50
$NaAsO_2$ 25 μM	150 ± 20*
$NaAsO_2$ 25 μM + Genistein 40 $\mu g/ml$	1200 ± 140
$NaAsO_2$ 25 μM + 4,5-Dianilinophthalimide 1 μM	1300 ± 100
$NaAsO_2$ 25 μM + Wortmannin 200 nM	1300 ± 60
$NaAsO_2$ 25 μM + Cycloheximide 5 $\mu g/ml$	1200 ± 70
$NaAsO_2$ 25 μM + Actinomycin D 10 $\mu g/ml$	1100 ± 60
$NaAsO_2$ 25 μM + Apigenin 100 μM	1100 ± 80

*p value < 0.05 as a significant when compared with the control

Cell area of mouse fibroblasts stained with anti-mitochondrial HSP70 was reduced when cell were exposed to sodium arsenite. This effect can be blocked by all inhibitors as shown in Table 4.

Table 4 Cell area of mouse fibroblasts stained with anti-mitochondrial HSP70

Mitochondrial Staining	Cell Area (μm x μm, N = 10, Mean ± S.E.)
$NaAsO_2$ 0 μM	960 ± 60
$NaAsO_2$ 25 μM	140 ± 8*
$NaAsO_2$ 25 μM + Genistein 40 $\mu g/ml$	1200 ± 130
$NaAsO_2$ 25 μM + 4,5-Dianilinophthalimide 1μM	870 ± 60
$NaAsO_2$ 25 μM + Wortmannin 200 nM	860 ± 50
$NaAsO_2$ 25 μM + Cycloheximide 5 $\mu g/ml$	1000 ± 60
$NaAsO_2$ 25 μM + Actinomycin D 10 $\mu g/ml$	900 ± 40
$NaAsO_2$ 25 μM + Apigenin 100 μM	1200 ± 100

*p value < 0.05 as a significant when compared with the control

Cell Fluorescence Intensity Was Measured by Laser Scanning Confocal Microscopy

Cell fluorescence intensity of mouse fibroblasts stained with anti-F-actin was reduced when cell were exposed to sodium arsenite. This effect can be blocked by all inhibitors as shown in Table 5.

Table 5 Cell fluorescence intensity of mouse fibroblasts stained with anti-F-actin

F-Actin Staining	Cell Fluorescence Intensity (arbitrary unit, N = 10, Mean ± S.E.)
$NaAsO_2$ 0 μM	69000 ± 3700
$NaAsO_2$ 25 μM	19600 ± 1900*
$NaAsO_2$ 25 μM + Genistein 40 μg/ml	69000 ± 5600
$NaAsO_2$ 25 μM + 4,5-Dianilinophthalimide 1μM	66700 ± 4800
$NaAsO_2$ 25 μM + Wortmannin 200 nM	67000 ± 5700
$NaAsO_2$ 25 μM + Cycloheximide 5 μg/ml	70200 ± 5400
$NaAsO_2$ 25 μM + Actinomycin D 10 μg/ml	56500 ± 5100
$NaAsO_2$ 25 μM + Apigenin 100 μM	72000 ± 6100

*p value < 0.05 as a significant when compared with the control

Cell fluorescence intensity of mouse fibroblasts stained with anti-vinculin was reduced when cell were exposed to sodium arsenite. This effect can be blocked by all inhibitors as shown in Table 6.

Table 6 Cell fluorescence intensity of mouse fibroblasts stained with anti-vinculin

Vinculin Staining	Cell Fluorescence Intensity (arbitrary unit, N = 10, Mean ± S.E.)
$NaAsO_2$ 0 μM	45100 ± 3200
$NaAsO_2$ 25 μM	9700 ± 1200*
$NaAsO_2$ 25 μM + Genistein 40 μg/ml	57300 ± 5000
$NaAsO_2$ 25 μM + 4,5-Dianilinophthalimide 1μM	48300 ± 5400
$NaAsO_2$ 25 μM + Wortmannin 200 nM	49000 ± 4400
$NaAsO_2$ 25 μM + Cycloheximide 5 μg/ml	44000 ± 4000
$NaAsO_2$ 25 μM + Actinomycin D 10 μg/ml	44000 ± 3400
$NaAsO_2$ 25 μM + Apigenin 100 μM	43000 ± 2600

*p value < 0.05 as a significant when compared with the control

Cell fluorescence intensity of mouse fibroblasts stained with anti-mitochondrial HSP70 was reduced when cell were exposed to sodium arsenite. This effect can be blocked by all inhibitors as shown in Table 7.

Table 7 Cell fluorescence intensity of mouse fibroblasts stained with anti-mitochondrial HSP70

Mitochondrial Staining	Cell Fluorescence Intensity (arbitrary unit, N = 10, Mean ± S.E.)
$NaAsO_2$ 0 μM	78200 ± 4600
$NaAsO_2$ 25 μM	8200 ± 730*
$NaAsO_2$ 25 μM + Genistein 40 μg/ml	70000 ± 3100
$NaAsO_2$ 25 μM + 4,5-Dianilinophthalimide 1μM	72800 ± 7900
$NaAsO_2$ 25 μM + Wortmannin 200 nM	63500 ± 8000
$NaAsO_2$ 25 μM + Cycloheximide 5 μg/ml	70500 ± 2300
$NaAsO_2$ 25 μM + Actinomycin D 10 μg/ml	67000 ± 4200
$NaAsO_2$ 25 μM + Apigenin 100 μM	69000 ± 3700

*p value < 0.05 as a significant when compared with the control

Immunoblotting

SAPK/JNK Expression

All inhibitors can not block SAPK/JNK expression in sodium arsenite-exposed mouse fibroblasts as shown in Figure 21.

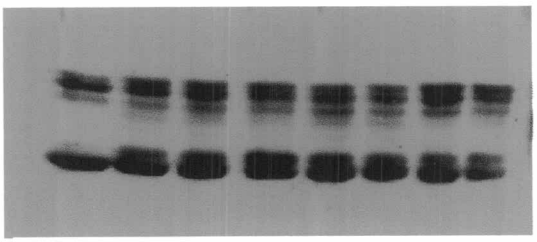


Figure 21 SAPK/JNK expression of mouse fibroblasts exposed to $NaAsO_2$ 0 μM (lane 1), $NaAsO_2$ 25 μM (lane 2), $NaAsO_2$ 25 μM + wortmannin 200 nM (lane 3), $NaAsO_2$ 25 μM + genistein 40 μg/ml (lane 4), $NaAsO_2$ 25 μM + 4,5-dianilinopthalimide 1 μM (lane 5), $NaAsO_2$ 25 μM + PP1 10 μM (lane 6), $NaAsO_2$ 25 μM + cycloheximide 5 μg/ml (lane 7), and $NaAsO_2$ 25 μM + actinomycin D 10 μg/ml (lane 8).

Phospho-SAPK/JNK Expression

All inhibitors can not block phospho-SAPK/JNK expression in sodium arsenite-exposed mouse fibroblasts as shown in Figure 22.

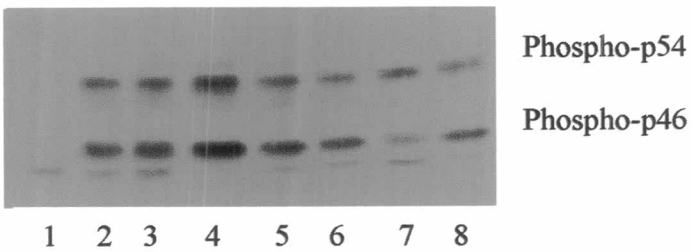


Figure 22 Phospho-SAPK/JNK expression of mouse fibroblasts exposed to $NaAsO_2$ 0 μM (lane 1), $NaAsO_2$ 25 μM (lane 2), $NaAsO_2$ 25 μM + wortmannin 200 nM (lane 3), $NaAsO_2$ 25 μM + genistein 40 μg/ml (lane 4), $NaAsO_2$ 25 μM + 4,5-dianilinopthalimide 1 μM (lane 5), $NaAsO_2$ 25 μM + PP1 10 μM (lane 6), $NaAsO_2$ 25 μM + cycloheximide 5 μg/ml (lane 7), and $NaAsO_2$ 25 μM + actinomycin D 10 μg/ml (lane 8).

p38 MAP Kinase Expression

All inhibitors can not block p38 MAP kinase expression in sodium arsenite-exposed mouse fibroblasts as shown in Figure 23.

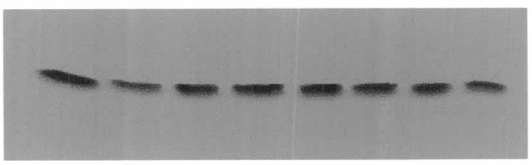


Figure 23 p38 MAP kinase expression of mouse fibroblasts exposed to $NaAsO_2$ 0 μM (lane 1), $NaAsO_2$ 25 μM (lane 2), $NaAsO_2$ 25 μM + wortmannin 200 nM (lane 3), $NaAsO_2$ 25 μM + genistein 40 μg/ml (lane 4), $NaAsO_2$ 25 μM + 4,5-dianilinopthalimide 1 μM (lane 5), $NaAsO_2$ 25 μM + PP1 10 μM (lane 6), $NaAsO_2$ 25 μM + cycloheximide 5 μg/ml (lane 7), and $NaAsO_2$ 25 μM + actinomycin D 10 μg/ml (lane 8).

Phospho-p38 MAP Kinase Expression

All inhibitors can not block phospho-p38 MAP kinase expression in sodium arsenite-exposed mouse fibroblasts as shown in Figure 24.

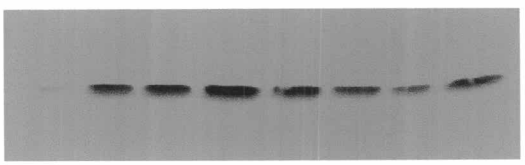


Figure 24 Phospho-p38 MAP kinase expression of mouse fibroblasts exposed to $NaAsO_2$ 0 μM (lane 1), $NaAsO_2$ 25 μM (lane 2), $NaAsO_2$ 25 μM + wortmannin 200 nM (lane 3), $NaAsO_2$ 25 μM + genistein 40 μg/ml (lane 4), $NaAsO_2$ 25 μM + 4,5-dianilinopthalimide 1 μM (lane 5), $NaAsO_2$ 25 μM + PP1 10 μM (lane 6), $NaAsO_2$ 25 μM + cycloheximide 5 μg/ml (lane 7), and $NaAsO_2$ 25 μM + actinomycin D 10 μg/ml (lane 8).

PAK Expression

All inhibitors can not block PAK expression in sodium arsenite-exposed mouse fibroblasts as shown in Figure 25.

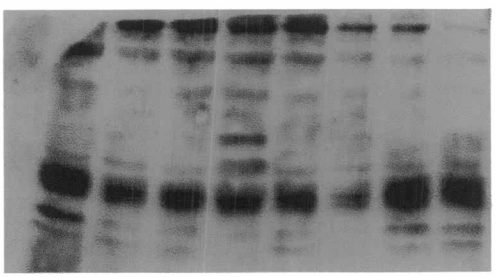


Figure 25 PAK expression of mouse fibroblasts exposed to $NaAsO_2$ 0 μM (lane 1), $NaAsO_2$ 25 μM (lane 2), $NaAsO_2$ 25 μM + wortmannin 200 nM (lane 3), $NaAsO_2$ 25 μM + genistein 40 μg/ml (lane 4), $NaAsO_2$ 25 μM + 4,5-dianilinopthalimide 1 μM (lane 5), $NaAsO_2$ 25 μM + PP1 10 μM (lane 6), $NaAsO_2$ 25 μM + cycloheximide 5 μg/ml (lane 7), and $NaAsO_2$ 25 μM + actinomycin D 10 μg/ml (lane 8).

In Vivo Results

Interleukin-6 production was reduced in sodium arsenite-exposed rats but this effect can be blocked by apigenin as shown in Table 8 and Figure 26.

Table 8 The plasma IL-6 levels (Mean ± S.E.) in sodium arsenite-exposed rats

Group	IL-6 Level (pg/ml)
Control	8.12 ± 3.53
$NaAsO_2$ 2.5 mg/kg, p.o.	5.83 ± 2.71*
$NaAsO_2$ 5 mg/kg, p.o.	3.33 ± 3.33*
$NaAsO_2$ 10 mg/kg, p.o.	2.50 ± 1.71*
Pretreatment with genistein 30 mg/kg, p.o. for 1 hour and then $NaAsO_2$ 10 mg/kg, p.o.	3.33 ± 1.67*
Pretreatment with apigenin 30 mg/kg, p.o. for 1 hour and then $NaAsO_2$ 10 mg/kg, p.o.	10.00 ± 4.45#

* p value < 0.05 as significant when compared with the control group

p value < 0.05 as significant when compared with the sodium arsenite 10 mg/kg-exposed group

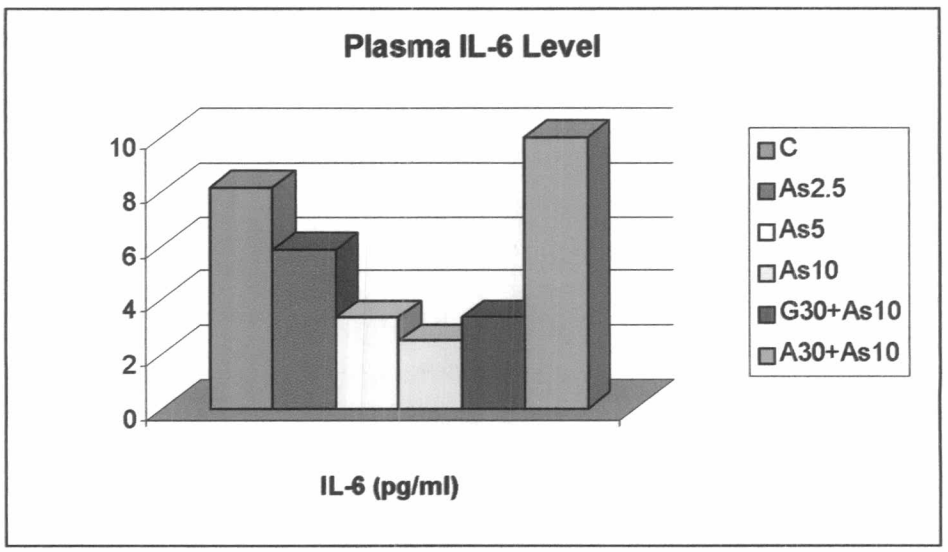


Figure 26 The plasma IL-6 levels in sodium arsenite-exposed rats