Laboratory Cell Biological Technic(2018)

Original Research Article

Study on Teratogenic Effect of Nitrobenzene on Vicia faba Root Tip Cells

Xiumei Tang,Xiaojing Gu,Ye Wen

School of Chemistry and Bioengineering, Jilin Medical University, Jilin, China

ABSTRACT

The mitotic index, micronucleus rate and chromosome aberration of Vicia faba root tip cells were determined by using micronucleus test and chromosome aberration test method of Vicia faba root tip cells with different concentrations of nitrobenzene as mutagens rate. The results showed that nitrobenzene could induce the higher frequency of micronucleus, and the micronucleus rate increased with the increase of nitrobenzene concentration at 4 and 24 h, but decreased after a certain concentration. Different concentrations of nitrobenzene at different treatment time to make Vicia faba root tip cells produce higher frequency chromosome aberration, and produce a variety of types of chromosomal aberrations. Therefore, nitrobenzene has obvious teratogenic effect on Vicia faba root tip cells.

KEYWORDS: nitrobenzene broad bean mitotic micronucleus rate chromosome aberration rate

1. Introduction

Toxicology is a science that studies the nature and mechanism of the effects of chemicals on biological agents and the quantitative assessment of the severity and frequency of these toxic effects. It has evolved from a simple study of toxicology to a modern, comprehensive discipline (Ji Yunjing, 1991). This science has aroused people's attention. This is due to the development of industrial and agricultural production and the progress of science and technology, people in their daily lives exposed to more and more chemical substances, there are many environmental chemicals urgently need to assess the toxicity to clarify its impact on the health of the body, The development of environmental health standards, the assessment of environmental quality, to take preventive measures to provide a scientific basis. Environmental toxicology is the use of toxicological methods to study the living environment (air, water, soil, public places and household chemicals) have been or will enter the toxic chemicals and their products in the environment of the harmful effects of human health And its role in a law of science, it is not only an important part of environmental hygiene, but also a branch of toxicology disciplines. In the past, toxicology research was mainly based on the combination of animal testing and human observation. It was still an important and necessary means for a considerable period of time. With the application of molecular biology theory and methodology and toxicology, the toxicity assessment of chemicals developed into a new model of in vitro cellular and molecular toxicity testing combined with human volunteer trials (Elespuru RKA, 1996).

Nitrobenzene as organic synthesis materials for the production of aniline dyes. In the organic synthesis industry such as dyestuffs, spices and explosives, it is possible to leak and pollute the natural environment during storage, use, handling and transportation, and produce corresponding poisoning to environmental organisms (Yang Jianzhou, Zhang Changhui, 2005). The micronucleus test technology of Vicia faba root tip cells has been widely used in the detection of environmental pollutants by our country as 'biological detection technology' (Quyi et al., 2001). Vicia faba root tip micronucleus test method and chromosome aberration test method in recent years gradually applied to the detection of environmental mutagens and mutagenic research. The plant detection method is considered to be a good test system for mutagenicity analysis. In this paper, the effects of different concentrations of nitrobenzene and different treatment time on the mitotic index, micronucleus rate and chromosome aberration rate of Vicia faba root tip cells were studied in order to explore the teratogenic effect of nitrobenzene on plant cells.

2. Materials and methods

2.1. Research materials

Vicia faba L. is produced in Zhejiang Dongtou. Nitrobenzene is produced by a Shenyang Pharmaceutical Factory, batch number 89063 purchased in Mudanjiang City Chemical Reagent Store, for the analysis of pure. Nitrobenzene was mixed with 5 mg • L-1, 10 mg •L-1, 20 mg •L-1, 30 mg •L-1, 40 mg •L-1, 100 mg •L-1 6 Different concentrations.

2.2. Research methods

Select the full, uniform size of the broad bean seeds soaked in distilled water for 1 d, let it bulge, and then spread in the pad with wet filter paper culture dish, covered with wet gauze, incubator in the incubator at 23 constant temperature culture, 12 h for Water once. To be about 1 cm, respectively, with distilled water and 5 mg •L-1, 10 mg •L-1, 20 mg •L-1, 30 mg •L-1, 40 mg •L-1, L-1 6 different concentrations of nitrobenzene were treated for 4 h and 24 h respectively. After 24 h, the root tips were cut and fixed with Carnos fixed solution (anhydrous ethanol: glacial acetic acid = 3: 1 v / v) for 24 h, Set 70% ethanol in 4refrigerator to save. Take the apical meristem, conventional shoots. With modified carbolic acid magenta dye (Qian Xiaowei, 1998; Qian Xiaowei et al., 2003). The mitotic index, micronucleus fractionation rate, chromosome aberration rate were observed, and the cells with distortion were photographed. (Test group and treatment see Table 1)

2.3. Statistical methods

Using spss10.0 data processing system for analysis of variance.

3. Results

3.1. Effects of Different Nitrobenzene Concentration and Treatment Time on Micronucleus Rate in Vicia faba Root Tip Cells

From Table 1, nitrobenzene was able to induce higher frequencies of micronuclei and chromosomal aberrations. The micronucleus rate of the 12 test groups was significantly higher than that of the control group ($p < 0.05$ or $p < 0.001$). In the two groups, the concentration of nitrobenzene increased with the increase of nitrobenzene concentration. When the concentration of nitrobenzene was 20 mg •L-1 in group (treatment 24 h) at 10 mg •L-1, the micronucleus rate was the highest. With the nitrobenzene concentration increased gradually and gradually decreased. At different times, nitrobenzene treatment, micronucleus rate b> b, c> c. Starting from 20 mg • L-1,d> d, e> e, f> f, g> g.d> d e> ef> fg> g

Table 1. The effects of Nitrobenzene on the rate of chromosome aberration of Vicia faba root tip cells

	Concentration ($mg \cdot L^{-1}$)			interphase cells/root tip	rate($\bar{x} \pm S$)
Ⅰa(对照)	0	4	10	1 2 2 1 1 0 2 2 1 2	1.4±0.70
Ⅰb	5	4	10	4 3 4 5 2 4 6 5 5 4	4.2±1.14***
Ⅰc	10	4	10	7 8 10 8 9 8 8 9 10 10	8.7±1.06***
Ⅰd	20	4	10	16 15 15 16 15 15 14 16 17 15	15.4±0.84***
Ⅰe	30	4	10	12 10 10 11 11 10 11 9 10 9	10.3±0.95***
Ⅰf	40	4	10	6 8 6 7 7 5 6 7 8 6	6.6±0.97***
Ⅰg	100	4	10	3 2 3 3 4 4 3 4 3 2	3.1±0.74***
Ⅱa(对照)	0	24	10	1 1 2 1 1 0 0 1 1 0	0.8±0.63
Ⅱb	5	24	10	5 6 3 4 5 5 6 4 4 5	4.7±0.95***
Ⅱc	10	24	10	9 10 9 9 11 8 8 9 9 10	9.2±0.92***
Ⅱd	20	24	10	6 5 6 7 6 8 6 6 5 5	6.0±0.82***
Ⅱe	30	24	10	5 5 6 4 5 3 5 4 4 3	4.4±0.97***
Ⅱf	40	24	10	2 2 3 3 2 3 4 5 2 3	2.9±0.99***
Ⅱg	100	24	10	1 2 2 2 1 1 2 2 0 2	1.5±0.71***

Note: each group comarison with the control. *expressing P<0.1, ** expressing P <0.05. * expressing P <0.001.**

3.2. Effects of Different Nitrobenzene Concentration and Treatment Time on Chromosome Aberration Rate of Vicia faba Root Tip Cells

From Table 2, it can be seen that nitrobenzene can induce higher frequency distortion, and the distortion rate increases with the increase of concentration, and peaks at c and b are both 10 mg • L-1, While the concentration of group was 5 mg • L-1. And then with the increase in concentration and distortion rate decreased. While the abundance of nitrobenzene was lower than that of the control group, both I g <a, e, f,g were less than a. At the same nitrobenzene concentration, different treatment time, a> a, b> I b Starting from 10 mg • L-1, with the concentration.

Table 2. The effects of Nitrobenzene on the rate of chromosome aberration of Vicia faba root tip cells

Grpup	Nitrobenzene Concentration (mg · L^{-1})	Process time	No. of root tip No.of1000piece interphase cells/root tip	Chromosome aberration	the rate of chromosome aberration ‰ ($\bar{x} \pm S$)
Ⅰa(对照)	0	4	10	3 2 0 1 1 2 2 1 3 3	1.8±1.03
Ⅰb	5	4	10	8 10 9 9 10 7 9 8 10 11	9.1±1.20 ***
Ⅰc	10	4	10	17 16 18 18 19 19 18 19 20 19	18.3±1.16***
Ⅰd	20	4	10	10 11 11 12 13 12 12 10 14 11	11.6±1.26***
Ⅰe	30	4	10	4 4 3 5 6 5 5 5 4 3	4.4±0.97***
Ⅰf	40	4	10	1 1 0 4 4 2 3 2 2 2	2.1±1.29***
Ⅰg	100	4	10	0 0 1 1 3 0 2 0 1 1	0.9±0.99**
Ⅱa(对照)	0	24	10	4 3 5 3 3 2 2 3 4 3	3.2±0.92
Ⅱb	5	24	10	10 13 13 12 12 11 12 11 10 9	11.3±1.34***
Ⅱc	10	24	10	7 6 7 6 6 8 8 7 8 7	7.0±0.82***
Ⅱd	20	24	10	3 4 4 5 5 5 4 6 2 5	4.3±1.17***
Ⅱe	30	24	10	5 3 4 2 1 2 2 0 1 1	2.1±0.74***
Ⅱf	40	24	10	0 3 0 0 1 2 1 2 2 2	1.3±1.01*
Ⅱg	100	24	10	1 0 0 1 1 1 0 1 2 1	0.8±0.63*

Note: each group comarison with the control. *expressing P<0.1, ** expressing P <0.05. * expressing P <0.001.**

3.3. Effects of Different Nitrobenzene Concentration and Treatment Time on the Split Index of Vicia faba Root Tip Cells

Table 3 shows that, compared with the control group, the two groups with different treatment time with nitrobenzene concentration increased, split index increased. When the concentration of I is 10 mg • L-1, the concentration of is 5 mg • L-1. Then, as the concentration increases, the split index decreases, and the split index increases with the increase of the processing time at the same treatment concentration.

Table 3. The effect of Nitrobenzene on mitotic index of Vicia faba root tip cell

Group	Mitotic index	Average of mitotic index
Ⅰa (对照)	3 2 2 1 2 4 2 1 3 2	2.2±0.92
Ⅰb	6 4 1 5 3 3 2 4 3 3	3.4±1.43**
Ⅰc	5 6 6 7 6 4 8 6 7 3	5.8±1.48***
Ⅰd	1 5 2 1 3 3 3 1 4 4	2.7±1.42***
Ⅰe	2 3 1 2 2 1 1 4 0 2	1.8±1.14*
Ⅰf	1 0 0 1 3 2 1 1 1 1	1.1± 0.88*
Ⅰg	0 0 1 2 1 1 0 0 1 1	0.7±0.67*
Ⅱa(对照)	3 0 3 2 1 2 1 1 1 1	1.5±0.97
Ⅱb	10 9 9 11 6 10 10 8 12 11	9.6±1.71***
Ⅱc	4 6 6 3 6 5 7 9 7 6	5.9±1.66***
Ⅱd	5 4 4 2 4 3 7 4 5 3	4.1±1.37***
Ⅱe	2 2 0 2 3 4 3 3 5 1	2.5±1.43**
Ⅱf	2 3 2 2 1 1 0 3 1 1	1.6±0.97*
Ⅱg	1 1 2 1 0 0 2 1 0 1	0.9±0.74*

Note: each group comarison with the control. *expressing P<0.1, ** expressing P <0.05. * expressing P <0.001.**

3.4. Effects of different nitrobenzene concentrations and treatment time on mitosis of meristem cells in Vicia faba root apricot

The results of microscopic examination showed that nitrobenzene had the effect of mitosis on the apical meristem cells of Vicia faba, and found abnormalities in the interphase period, early stage, middle stage, late stage and late stage of cell cycle. It can be seen from Table 4 that the chromosomal aberration rate increased with the increase of concentration in the group of different treatment time, when the concentration of Ib was 5 mg • L-1 and b was 5 mg •L-1 Peak. And then decreases with increasing concentration. And I b <b, c> c, d> d, e> e, f> f, g> g were compared with the two groups with different treatment time. In the medium group, the chromosome aberration rate increased with the increase of concentration, and the peak concentration was 10 mg • L-1 when I c concentration was 10 mg •L-1. And then decreases with increasing concentration. And I b <b, c> c, d> d, e> e, f> f, g> g were compared with the two groups with different treatment time. In the later stage, the chromosome aberration rate increased with the increase of concentration, and the peak concentration was 10 mg • L-1 when I c concentration was 5 mg •L-1. And then decreases with increasing concentration. And I b <b, c> c,d> d, e>e,f>f,g>g were compared with the two groups with different treatment time. At the end of the treatment, the chromosome aberration rate increased with the increase of the concentration, and the peak was when the concentration of I c was 10 mg • L-1 and the concentration of b was 5 mg • L-1. And then decreases with increasing concentration. And I b <b, c> c, d> d, e> e, f> f, g> g were compared with the two groups with different treatment time.

3.4.1 Interval

Intercellular abnormalities of the cell division mainly appear micronuclei (Figure 1, 2, 4), double micronucleus (Figure 2), four micronuclei (Figure 3).

3.4.2 Pre-period

Early abnormal rate is not high, abnormal performance mainly pre-micronucleus (Figure 5). Early micronuclei were formed by chromosomal damage or abnormal chromosomal activity during the last mitosis (Qian Xiaowei, 2004)

3.4.3 Medium term

Mid-term chromosomal abnormalities are mainly fragments (Figure 6.7), chromosomal abnormalities (Figure 8) mid-term fragments of the mitosis process of chromosome damage or chromosome activity abnormalities formed.

3.4.4 Late

(Figure Figure 10.16), broken (Figure 10.12.14), adhesion (Figure 9.10.11.12.13), the bridge (Figure 16), double bridge (Figure 9), the bridge, , Multipolar distribution (Figure 11.15) and so on.

3.4.5 End

(Figure 21.23), the syncytial body (Figure 24), the bridge (Figure 22.23), the bridge (Figure 17.18), lag (as shown in Figure 18.19.20.21), the helix is not synchronized (Figure 21.23), the syncytia (Figure 24)

The appearance of micronuclei: may be the last mitotic process of chromosome damage caused by fragments or chromosomal activity caused by abnormal.

Chromosomal hysteresis: the vast majority of chromosomes normally move toward the poles, only individual chromosomes or fragments remain between the poles. This reflects the difference in the speed and process of individual chromosomes moving toward the poles.

Chromosome adhesion: due to the damage of chromosomes and spindle wire damage caused by the late two groups of chromosomes cannot be completely separated, some sub-chromosome fusion phenomenon.

The formation of chromosome bridges: chromosome bridge formation is one of the main features of cell division abnormality and chromosome aberration. The formation of the bridge is due to chromosome breakage re-fused to form double centromere and without centromere chromosome fragments of the results. So, the formation of chromosome bridge is often accompanied by the emergence of chromosome fragments.

Chromosome multipolar distribution: the normal mitotic chromosome in the spindle under the traction, equally divided into the opposite poles, while the nitrobenzene-treated Vicia faba root tip cells have a late mitotic distribution of uneven triangular anomalies Split phase.

Chromosomal dissociation is not synchronized: the mitotic end is actually a nucleon remodeling process, and when the chromosome reaches the poles, it begins to spin. Due to individual chromosomal activity and the emergence of the

solution is not synchronized spiral, that is, most of the chromosomes have been resolved to form a spiral chromosome, the individual unsolved spiral is still in the chromosome state.

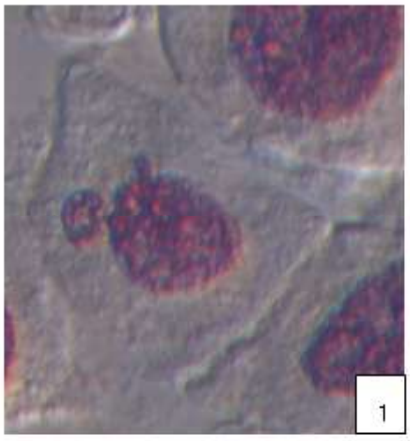
The appearance of syncytia: due to the late emergence of unequal multipolar abnormal distribution, as well as individual chromosomal damage or chromosomal activity abnormalities, leading to syncytia formation.

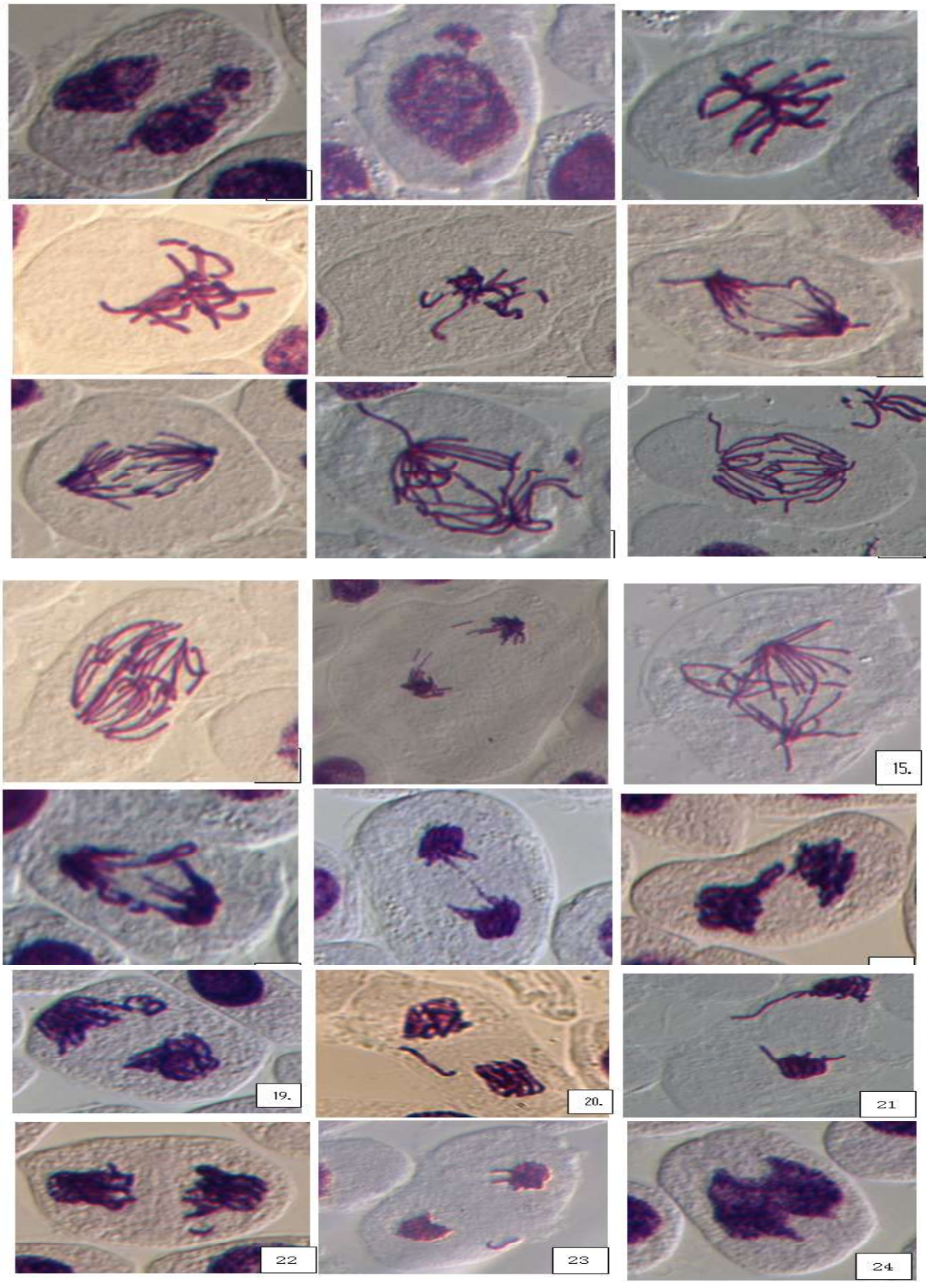
Table 4. Table4 The effect of Nitrobenzene on the rate of chromosome aberration of Vicia faba root tip cell

(%) ($\bar{x} \pm S$)

Group	chromosome aberration rate per phase				The total chromosome aberration rate ($\bar{x} \pm S$)
	前期 Prophase	中期 Metaphase	后期 Anaphase	末期 Telophase	
Ⅰa(对照)	0.14±0.49	0.54±0.72	3.13± 1.21	1.96±0.87	1.8±1.03
Ⅰb	3.22±0.63***	4.95±0.50***	9.66±0.94***	8.25±1.03***	9.1±1.20***
Ⅰc	2.58±0.52**	10.21±0.85***	19.86±1.32***	20.14±1.23***	18.3±1.16***
Ⅰd	1.96±0.84*	6.83±0.74***	10.91±1.05***	10.03±1.09***	11.6±1.26***
Ⅰe	1.01±0.76**	4.14±0.61***	7.64±0.96***	7.97±0.96***	4.4±0.97 ***
Ⅰf	0.69±0.60*	2.56±0.83**	5.02±0.88***	5.42±1.11***	2.1±1.29***
Ⅰg	0.31±0.45*	1.70±0.76**	2.88±1.24***	2.01±0.89***	0.9±0.99***
Ⅱa(对照)	0.16±0.53	0.63±0.84	4.28±1.04	2.34±1.20	3.2±0.92
Ⅱb	3.65±0.72***	4.97±0.73***	13.97±0.95***	12.75±1.13***	11.3±1.34***
Ⅱc	2.13±0.85***	5.86±0.55***	8.63±1.22***	7.99±0.97***	7.0±0.82 ***
Ⅱd	1.87±0.54*	4.42±0.67***	6.74±1.19***	6.82±0.93**	4.3±1.17***
Ⅱe	0.96±0.46***	3.05±0.81***	4.86±0.97***	5.06±1.06***	2.1±0.74***
Ⅱf	0.56±0.77*	2.48±0.62***	3.02±1.20***	3.47±1.10***	1.3±1.01*
Ⅱg	0.21±0.58*	1.01±0.79***	1.31±1.23***	1.89±1.04***	0.8±0.63*

Note: each group comarison with the control, *expressing P<0.1, ** expressing P <0.05. * expressing P <0.001.**




15.
19.
20.
21
22
23
24

1: interphase micronucleus; 2: interphase micronucleus, double micronucleus; 3: interphase four micronuclei; 4: pre-micronucleus, syncytium; 5: pre-micronucleus; 6: intermediate fragment; 7: 8: late fragment, chromosome arrangement abnormalities; 9: late chromosome double bridge, adhesion; 10: late fragments, adhesions; 11: late adhesion, multipolar; 12: late lag, fragments, adhesions; 16: late chromosome bridge; 18: late lag, chromosome bridge; 19: late lag; 20: late lag; 21: late lag , Chromosome unwinding is not synchronized; 22: terminal fragment; 23: terminal fragment, chromosome unwinding step; 24: nuclear abnormality

4. Discussion

This study found that from Table 5 we can see that nitrobenzene can induce higher frequencies of micronuclei and chromosomal aberrations. The micronucleus rate of the 12 test groups was significantly higher than that of the control group ($p < 0.05$ or $p < 0.001$). During the two groups of treatment time, the micronucleus rate increased with the increase of nitrobenzene concentration, which may be due to the increase of the concentration of nitrobenzene to strengthen the DNA damage to the cell, the chromosome aberration rate increased, micronucleus the rate also increases. When the concentration of nitrobenzene in group was 20 mg •L-1 and the concentration of 10 mg •L-1 in group was the highest. But with the nitrobenzene concentration to further increase the micronucleus rate gradually decreased. And with the extension of time, the micronucleus rate II b> b, c> c. Starting from 20 / L, d> d, e> e, f> f, g> g. This may be due to the increase in the concentration of nitrobenzene, the concentration increases, the damage to the cells intensified, not only lead to the generation of intracellular chromosomal aberrations, but also effectively prevent the cell spindle filament tubulin polymerization Cells remain in the division period, and thus the micronucleus rate decreased during the interval. May also be due to the prolongation of nitrobenzene treatment time and the concentration of the cell cycle lead to prolonged, so that some of the damaged cells did not complete the mitosis process, resulting in decreased micronucleus rate. Studies have shown that there are two ways to form micronuclei: one way is because the chromosome fragments produced after the G2 phase of the previous division cannot coordinate with the normal chromosome during the division process, and are excluded from the nucleus And the other is due to various forms of backward chromosomes, which are caused by chromosomes and chromosome groups (Li Hong, 1997).

The chromosomal aberration rate of both groups increased with the increase of concentration. When the concentration of nitrobenzene in group was 10 mg • L-1, and the chromosome aberration rate was the highest at mg • L-1. With the further increase in concentration and gradually decreased. At the same treatment concentration, at different times, with the same concentration of nitrobenzene treatment, chromosome aberration rate b> b, starting from 10 mg • L-1, with the concentration of . It is shown that different concentrations of nitrobenzene can induce higher frequency chromosomal aberrations at different treatment times, and the effect is different with the treatment time. The results showed that nitrobenzene could induce multiple chromosomal aberrations: interphase and pre-micronucleus; mid-term fragment; late micronucleus, chromosome retention, chromosome bridge, fragment, adhesive multi-pole. The most prominent of this test is the double bridge and the fragment; the final fragment. Chromosome aberration may be multi-channel. Micronucleus formation, chromosome retention, are likely due to nitrobenzene destroyed the function or formation of the spindle wire, it may interfere with some of their own chromosomal movement of the chromosome cannot reach the equatorial plane, resulting in backward. Chromosome adhesion, fusion and the emergence of the bridge may be due to the direct or indirect role of nitrobenzene in DNA molecules, resulting in DNA breakage damage, resulting in a new re-connection, chromosome adhesion, fusion, chromosome bridge, Rearrangement. Nitrobenzene also leads to chromosome poles, with respect to the structure and polarity of cell division, which is not yet clear. It is generally believed that the structure and determination of cell division poles of different species may be controlled by different mechanisms (Xin Xiangtai, Liang Wanfu, 1997). The structure and polarity of the splitting pole in the mitosis of the broad bean root tip are likely to belong to the 'plate' structure. We know that pre-mitotic, in each stained monomer, contains a special DNA sequence, called centromere DNA. Its position called centromere (centromere). In the later period, at the centromere gradually assembled another protein composite structure, called the kinetochore. The pellets are closely linked to the centromere. Another important event in the transition from the early to the mid-term is the assembly of the spindle. A spindle is a temporary organelle that is directly related to cell division and chromosome movement. Mainly composed of microtubules and their binding proteins. The chromosomes are trapped by the kernels, and the microtubules emitted by the spindle poles are combined with the agglomerates on the chromosome side to form the microtubules and the other side of the microtubules are associated with the other side of the chromosome. There are two proteins in the pellet: Mad protein, Bub protein. They make the pellets sensitized, allowing the microtubules to move in contact with the pellets. Mad2 and Bub1 are associated with the chromosome loading spindle, and as long as the pellets are caught by the microtubules, Mad2 and Bub1 disappear. Do not catch, do not disappear while the chromosome is not trapped by microtubules and cannot enter the late. The globules act before the microtubule, and suppresses the signal and inhibits the cell cycle to move down one stage. Experiments show that nitrobenzene destroys the lagged chromosomes that have not yet been associated with microtubules, allowing the chromosome to lag, but the cells can be transformed downwards. Therefore, the cause of mitotic disorders, in the late emergence of a variety of distortion.

The results showed that different concentrations of nitrobenzene could cause the apical meristem cell division index of the broad bean (Qian Xiaowei, 2004), when the c, b peak. And then with the increase in the concentration of split index decreased. This may be due to the low concentration of nitrobenzene, prolong the time of cell division, shorten the interval between the split interval, so that the entire split cycle shortened, and the split level is higher than the normal level; but with the concentration of rising, the situation with the low concentration Under the opposite (Duan Changqun, Wang Huan school, 1995). Indicating that nitrobenzene at different concentrations on the effect of cell division is different. While the same treatment concentration of different treatment time of the two groups,, indicating that with the extension of time, split index increased. This may be due to the mitotic phase, nitrobenzene to prevent the cell spindle filament tubulin polymerization and make the cells remain in the split, resulting in increased split index.

Through the different concentrations, different time treatment can be observed in part of the cell nucleus close to the cell membrane distribution, with the increase in concentration, the root tip of the hydrolysis effect is poor, the degree of cell dispersion is getting lower, aggregation and overlap phenomenon, and the nucleus showed condensation, Deep dyeing. Nuclear deformation irregular, nuclear elongation deformation, the nucleus appears to a number of buds. Therefore, the appropriate concentration of nitrobenzene over time can induce the apical apoptosis of Vicia faba root tip cells, which is similar to the report of Zhang Yuehua (Zhang Yuehua et al., 2007).

Group	Average of mitotic Index % ($\bar{x} \pm S$)	Micronucleus % ($\bar{x} \pm S$)	Rate of chromosome aberration % ($\bar{x} \pm S$)
Ⅰa	2.2±0.92	1.4±0.70	1.8 ±1.03
Ⅰb	3.4±1.43**	4.2±1.14***	9.1±1.20***
Ⅰc	5.8±1.48***	8.7±1.06***	18.3±1.16***
Ⅰd	2.7±1.42***	15.4±0.84***	11.6±1.26***
Ⅰe	1.8±1.14*	10.3±0.95***	4.4±0.97***
Ⅰf	1.1±0.88*	6.6±0.97***	2.1±1.29***
Ⅰg	0.7±0.67*	3.1±0.74***	0.9±0.99**
Ⅱa	1.5±0.97	0.8±0.63	3.2±0.92
Ⅱb	9.6±1.17***	4.7±0.95***	11.3±1.34***
Ⅱc	5.9±1.66***	9.2±0.92***	7.0±0.82***
Ⅱd	4.1±1.37***	6.0±0.82***	4.3±1.17***
Ⅱe	2.5±1.43**	4.4±0.97***	2.1±0.74***
Ⅱf	1.6±0.97*	2.9±0.99***	1.3±1.01*
Ⅱg	0.9±0.74*	1.5±0.71***	0.8±0.63*

Note: each group comarison with the control. *expressing P<0.1, ** expressing P <0.05. * expressing P <0.001.**

Table 5. Comparison with the mitotic index,the micronucleus rate and rate lf chromosome aberration induced by Nitrobenzene

References

1. Duan Changqun, Wang Huan School. The cytotoxic effects of heavy metals on the root tip of Vicia faba bean and the micronucleus treatment of Vicia faba root tips. Chinese Journal of Botany .1995.37 (1): 14-24
2. Ji Yunjing. Practical Toxicology Handbook [M]. Beijing: China Environmental Science Press, 1991.1.
3. Li Hong. Study on the Abnormalities of Wheat Root Tip Cells Induced by Saturated P-dichlorophenyl Chloride. Yuzhou University (Natural Science Edition). 1997.14 (1): 36-42
4. Study on Genetic Toxicity of Rare Earth Complex Fertilizers and Three Rare Earth Elements [J]. Acta Metallurgica Sinica, 2001, 23 (3): 243-246 (in Chinese with English abstract)
5. Qian Xiaowei. Improvement of micronucleus test method for Vicia faba root tip cells. Journal of Wenzhou Normal College. 1998.06 (6): 64-65 Qian Xiaowei et al. China Public Health .2003.19: 1335
6. Qian Xiaowei. Genetics. 2004.26: 337
7. Qian Xiaowei. Journal of Cell Biology. 2004.26: 171
8. XIN Xun-tai, LIANG Wan-fu.Study on the effect of raw lacquer on mitosis of apical meristem cells in onion. Acta Agronomica Sinica. 1997.04 (1): 50-53
9. Yang Jianzhou, Zhang Changhui. Fine chemical raw materials and intermediates manual - synthetic dyes and pigments. Beijing: Chemical Industry Press .2005.
10. Zhang Yuehua, Ran Chao, Shang Yanyan, Qiao Zhenzhen, Li Shengchang, Fan Zhaifei. Effects of Nitrobenzene on Apoptosis of Vicia faba Root Tip Cells. Biology Bulletin. 2007.42 (1) 47-48
11. Elespuru R K A. Future approaches to genetie toxicology risk zssessmene.Moutation Research / Reviews in Genetie Toxicology, 1996.365 (1-3): 191-204