

Research Article



Evaluation of the Potential Effects of Nifedipine-induced DNA Damage and Micronucleus Formation in Female Mice and their Embryos

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ABSTRACT

Physiological changes in pregnancy, including changes in body composition and metabolic enzyme activity, can alter drug pharmacokinetics. Nifedipine (Adalat), a dihydropyridine calcium entry blocker, is an effective tocolytic agent with low toxicity and teratogenicity but has potential cardiovascular side effects that may affect the mother as well as the fetus. Therefore, the aim of this study was to assess the genotoxicity effects of maternal Nifedipine administration on micronucleus formation and DNA damage in bone marrow and their embryos. Swiss virgin female mice were allocated in several groups: control, non-pregnant female groups treated with three different doses of Nifedipine (25, 50 and 75mg/kg) and pregnant female mice treated with the same doses of Nifedipine starting from the day 3 to 18 of gestation. The results revealed that micronucleus formation and DNA damaged cells of non-pregnant and pregnant female mice as well as their embryos treated with low dose of Nifedipine (25 mg/kg) relatively similar to those in control cells. In contrast, the results showed that micronucleus formation and DNA damaged cells of non-pregnant and pregnant female mice as well as their embryos treated with medium and high doses of Nifedipine was higher 2 and 3 times compared with control and those treated with low dose of Nifedipine. The results suggest that induction of genetic toxicity by Nifedipine treatment was dose dependent manner. Nifedipine seems to be safe at the low dose but by increasing its concentration caused genetic toxicity due to imbalance in intracellular calcium level which increases of the ROS production.

Keywords: Comet assay, Embryos, Genotoxicity, Micronucleus test, Mice, Nifedipine.

INTRODUCTION

The results of database surveys in developed countries show that 44–93% of pregnant women take at least one prescription medication other than vitamins and iron.¹ Pregnancy leads to a number of physiologic changes that alter drug metabolism and disposition. Despite the impact of pregnancy on pharmacokinetics, this population is underrepresented in and often excluded from clinical trials.^{2,3} Therefore, for many drugs, the pharmacokinetics and effects in pregnant women are largely unknown. One means of overcoming the ethical and practical constraints associated with studying the effects of individual drugs in pregnant women is to use Pharmacometrics models to predict drug disposition at various stages of pregnancy. Traditionally, physiology-based pharmacokinetic (PBPK) models incorporate physiologic parameters (such as blood flow and tissue volumes) with drug-specific parameters (such as physicochemical properties and *in vitro* metabolism data) to predict the concentration of drugs in various tissues. Such PBPK models of pregnancy have historically been used by toxicologists to predict the concentration of toxicants, including drugs, in various tissues.^{4,5} More recently, pharmacokinetic modeling has come to be accepted by regulatory agencies and pharmaceutical scientists as an important component of clinical pharmacology studies that address drug efficacy and drug–drug interactions.⁶

Preterm delivery remains one of the top three causes of perinatal morbidity worldwide. Various pharmacological

agents have been used to stop preterm uterine contractions in an attempt to prevent preterm delivery. When using tocolytic agents the maternal and fetal side-effect profiles are important considerations. Following the most recent Cochrane database review the preferential use of calcium channel blockers over other tocolytic agents is likely to increase.⁷ Nifedipine (Adalat), a dihydropyridine calcium entry blocker, is an effective tocolytic agent with low toxicity and teratogenicity but has potential cardiovascular side effects that may affect the mother as well as the fetus.^{8,9} Animal studies suggest that administration of calcium channel blockers may result in impaired uterine blood flow, potentially resulting in fetal hypoxemia and academia.^{10,11} However, studies in human pregnancies did not confirm significant alterations in uterine blood flow.^{12–14} Fetal cardiovascular responses to Nifedipine (other than those observed in the placental circulation) have not been reported in detail. The cerebral circulation in the human fetus is capable of active auto regulation and a decrease in blood flow resistance with perceived hypoxemia ('brain sparing').¹⁵ In addition, differential changes in the placental and cerebral blood flow resistances may affect the cerebro placental Doppler ratio and the overall distribution of cardiac output.¹⁶ In addition, cardiac effects of Nifedipine may affect the contractility and diastolic cardiac function.

Till date there is no information about the potential effect of Nifedipine in induction genetic toxicity either on pregnant female or the embryos. Therefore, the aim of this study was to assess the genotoxicity effects of maternal Nifedipine administration on micronucleus

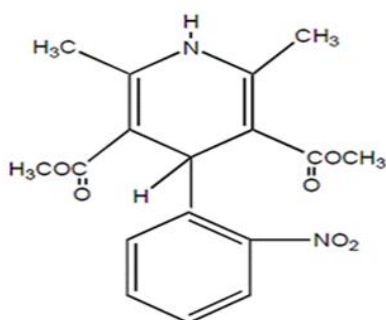


formation and DNA damage in bone marrow and their embryos.

MATERIAL AND METHODS

Test drug

Adalat (Nifedipine) tablets produced by (Bayer health care pharmaceuticals corp) is an anti anginal drug belonging to a class of pharmacological agents, the calcium channel blockers. Nifedipine is 3,5 pyridine dicarboxylic acid 1,4 dihydro-2,6-dimethyl-4-(2-nitrophenyl)-dimethyl ester, the molecular weight is 364.3 and the molecular formula is $C_{17}H_{18}O_6$ and structural formation of Nifedipine is.



Nifedipine is a yellow crystalline substance, practically insoluble in water but soluble in ethanol. Adalat (Nifedipine) tablets contain either 30 or 60 mg of Nifedipine for once-a day oral administration.

Experimental animal and design

Swiss virgin female mice weighting 25-30 g were required in pathogen-free well-ventilation room in order to enable the animals to acclimatize to their environment. Drinking water and food supplied *ad libitum*. Females were divided into two groups the first group was used for studding the effects of the treatments (in non-pregnant females) and the second group was used for studding the effects of the treatments (in pregnant females and their embryos) as following:

The first group was divided into four subgroups. The first subgroup of three virgin females was administrated orally with a dose of (25 mg/kg) once daily.

The second subgroup of three virgin females was administrated orally with a dose of (50 mg/kg) once daily.

The third subgroup of three virgin females was administrated orally with a dose (75 mg/kg) once daily.

Additionally a fourth subgroup of three virgin females served as control.

The second group were housed with adult males by ratio 3:1 after one day of mating the females which exhibiting a vaginal plug were considered as pregnant the day of the appearance of vaginal plug was considered as the first day of pregnancy.

The pregnant females were weighted and caged individually and divided into four parts.

The first subgroup of three pregnant females was administrated orally with a dose of (25 mg/kg) once daily.

The second subgroup of three pregnant females was administrated orally with a dose of (50 mg/kg) once daily.

The third subgroup of three pregnant females was administrated orally with a dose of (75 mg/kg) once daily and the fourth subgroup of three pregnant females served as control.

Nifedipine was dissolved in ethyl alcohol and administrated once daily for 15 consecutive days in virgin female mice and pregnant female mice from day (3) to day (18) of gestation. These doses of Nifedipine are the recommended low, medium and high for human after modified to suit the small weight of albino mice (25g) according to Gaafa et al.¹⁷

After treatment with Nifedipine the virgin and pregnant females were killed, the bone marrow and the liver of females were collected and the embryos and their livers were randomly selected from each subgroup of pregnant females to investigate the genotoxic effects (micronuclei formation and DNA damage) of Nifedipine on females and embryos

Comet assay

Liver tissues collected from female mice and their embryos were isolated by homogenization and centrifugation (15min, 280 g) in a density gradient of Gradisol L (Aqua Medica, Lodz, Poland). The concentration of the cells was adjusted to $(1-3) \times 10^5$ cells/ ml by adding RPMI 1640 without glutamine to the single cell suspension. A freshly prepared suspension of cells in 0.75% low melting point agarose (Sigma Chemicals) dissolved in phosphate buffer saline (PBS; sigma chemicals) was cast onto microscope slides precoated with 0.5% normal melting agarose. The cells were then lysed for 1h at 4°C in a buffer consisting of 2.5M NaCl, 100 mM EDTA, 1% Triton X-100, 10mM Tris, pH10. After the lysis, DNA was allowed to unwind for 40 min in electrophoretic solution consisting of 300mM NaOH, 1mM EDTA, pH>13. Electrophoresis was conducted at 4°C for 30 min at electric field strength 0.73 V/cm (30mA). The slides were then neutralized with 0.4M Tris, pH 7.5, stained with 2ug/ml ethidium. bromide (Sigma Chemicals) and covered with cover slips. The slides were examined at 200 x magnification fluorescence microscope (Nikon Tokyo, Japan) to a COHU 4910 video camera (Cohu, Inc., San Diego, CA, USA) equipped with a UV filter block consist an excitation filter (359nm) and barrier filter (461nm) and connected to a personal computer –based image analysis system, Lucia-Comet v.4.51. Hundred images were randomly selected from each sample and the comet tial DNA was measured.¹⁸ Endogenous DNA damage measured as the mean comet tail DNA of liver tissues of female mice groups. The number of cells scored for each animal was 100.¹⁸

Micronucleus test

The bone marrow cells of non-pregnant and pregnant female mice as well as liver tissues of their embryos resuspended in a small volume of fetal calf serum on a glass slide were used for smear preparation. The smear of bone marrow and liver cells was prepared from each mouse and embryo. After air-drying, the slide was fixed in methyl alcohol for 10 min and stained with 5% Giemsa stain for 10 min. Three slides were prepared for each animal and were coded before observation and one was selected for scoring. From each coded slide, 2,000 polychromatic erythrocytes (PCEs) were scored for the presence or micronuclei under oil immersion at high power magnification. In addition, the percentage of micronucleated polychromatic erythrocytes (%MnPCEs) was calculated on the basis of the ratio of MnPCEs to PCEs.¹⁹

RESULTS

Comet assay

The results of the comet assay for DNA strand break in nuclei from individual liver cells of non-pregnant and pregnant female mice as well as their embryos exposed to Nifedipine (Adalat) showed considerable DNA damage (Table 1-3). These results showed that DNA damaged cells of non-pregnant and pregnant female mice as well as their embryos treated with low dose of Nifedipine relatively similar to those in control cells (Tables 1-3).

In contrary, these results showed that DNA damaged cells of non-pregnant and pregnant female mice as well as their embryos treated with medium and high doses of Nifedipine was higher 2 and 3 times compared with control and those treated with low dose of Nifedipine (Tables 1-3).

In addition, the DNA damaged cells categorized in class 3, which is defined as the highest category of DNA damage having long tail of damage, were also higher in non-pregnant and pregnant female mice as well as their embryos treated with medium and high doses of Nifedipine compared with control and those treated with low dose of Nifedipine (Tables 1-3).

Micronucleus Test

The effect of Nifedipine (Adalat) on MnPCEs formation in the bone marrow cells of non-pregnant and pregnant female mice as well as their embryos is summarized in Figure 1-3 and Tables 4-6. The results revealed that MnPCEs formation in control of non-pregnant and pregnant female mice as well as their embryos was lower than those in all treated groups. Treatment of non-pregnant and pregnant female mice as well as their embryos with low dose of Nifedipine increased slightly the formation of MnPCEs compared with the control group (Figures 1-3 and Tables 4-6). However, the increase rates of MnPCEs formation in non-pregnant female mice and their embryos with low dose of Nifedipine was not significantly different except in pregnant female mice compared with control group.

On the other hand, treatment of non-pregnant and pregnant female mice as well as their embryos with medium and high doses of Nifedipine increased significantly the incidence of MnPCEs compared with control group. However, the rates of MnPCEs formation in non-pregnant and pregnant female mice as well as their embryos due to treatment with the high dose of Nifedipine was significantly higher than those treated with medium dose Nifedipine (Figure 1-3 and Tables 4-6).

Table 1: Visual score of DNA damage in liver tissues of non-pregnant female mice exposed to several concentrations of Nifedipine (Adalat) using comet assay.

Mice	Treatment	Number of animals	No. of cells		Class ^y of comet				DNA damaged cells (%)
			Analyzed (*)	Total comets	0	1	2	3	
Non-pregnant	Control	3	300	20	280	17	3	0	6.7
	Adalat L	3	300	25	275	17	8	0	8.3
	Adalat M	3	300	33	267	19	6	5	11.0
	Adalat H	3	300	51	249	13	18	20	17.0

^y: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus. (*): No of cells analyzed were 100 per an animal.

Table 2: Visual score of DNA damage in liver tissues of pregnant female mice exposed to several concentrations of Nifedipine (Adalat) using comet assay.

Mice	Treatment	Number of animals	No. of cells		Class ^y of comet				DNA damaged cells (%)
			Analyzed (*)	Total comets	0	1	2	3	
Pregnant	Control	3	300	20	280	17	3	0	6.7
	Adalat L	3	300	28	272	15	10	3	9.3
	Adalat M	3	300	42	258	21	13	8	14.0
	Adalat H	3	300	65	235	19	24	22	21.7

^y: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus. (*): No of cells analyzed were 100 per an animal.



Table 3: Visual score of DNA damage in liver tissues of embryos of female mice exposed to several concentrations of Nifedipine (Adalat) using comet assay.

Mice	Treatment	Number of animals	No. of cells		Class [‡] of comet				DNA damaged cells (%)
			Analyzed (*)	Total comets	0	1	2	3	
Embryos	Control	3	300	20	280	17	3	0	6.7
	Adalat L	3	300	26	274	15	6	5	8.7
	Adalat M	3	300	37	263	21	9	7	12.3
	Adalat H	3	300	56	244	12	21	23	18.7

‡: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus. (*): No of cells analyzed were 100 per an animal.

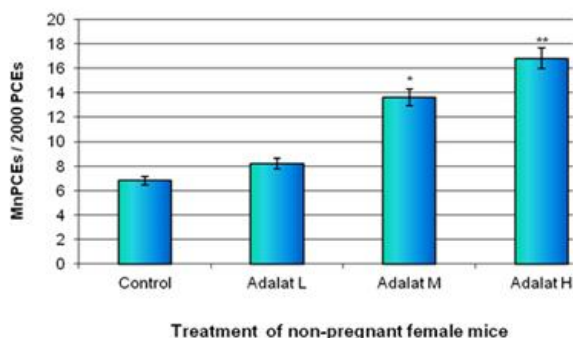


Figure 1: Micro nucleated polychromatic erythrocytes (MnPCEs) of non-pregnant female mice exposed to several concentration of Nifedipine (Adalat) (mean ± SEM)

Table 4: The Micro nucleated polychromatic erythrocytes (MnPCEs) of non-pregnant female mice exposed to several concentration of Nifedipine (Adalat) (mean ± SEM)

Treatment	MnPCEs / 2000 PCEs
Control	6.8±0.11
Adalat L	8.2±0.12
Adalat M	13.6±0.10*
Adalat H	16.8±0.13**

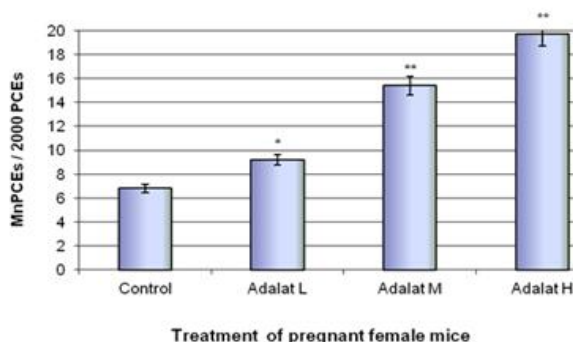


Figure 2: Micro nucleated polychromatic erythrocytes (MnPCEs) of pregnant female mice exposed to several concentration of Nifedipine (Adalat) (mean ± SEM)

DISCUSSION

The present study was performed to evaluate the potential effect of Nifedipine in inducing genetic toxicity

either on pregnant female or the embryos. Therefore, the aim of this study was to assess the genotoxicity effects of maternal Nifedipine administration on micronucleus formation and DNA damage in bone marrow and their embryos.

The results revealed that micronucleus formation and DNA damaged cells of non-pregnant and pregnant female mice as well as their embryos treated with low dose of Nifedipine (25 mg/kg) relatively similar to those in control cells.

The findings were in agreement with the observations reported by Gaafa et al.¹⁷ They found that Nifedipine pretreatments at dose of 25mg/kg were found to safe and induce highly protective effects against the evinced diethyl dithiocarbamate (DDC) hepatotoxicity, manifesting that free radical, cytochrome P450, and calcium-dependent processes contribute to DDC liver toxicity.

Table 5: The Micro nucleated polychromatic erythrocytes (MnPCEs) of pregnant female mice exposed to several concentration of Nifedipine (Adalat) (mean ± SEM)

Treatment	MnPCEs / 2000 PCEs
Control	6.8±0.11
Adalat L	9.2±0.11*
Adalat M	15.4±0.10**
Adalat H	19.7±0.12**

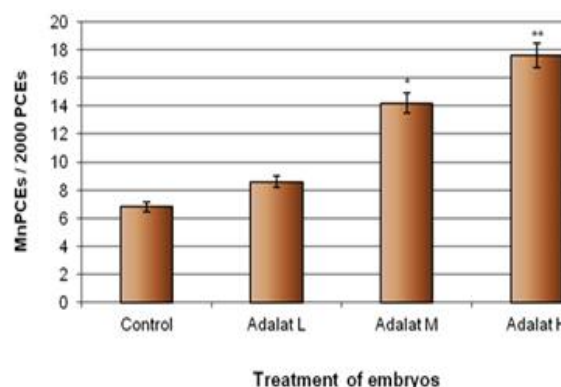


Figure (3): Micronucleated polychromatic erythrocytes (MnPCEs) of mice embryos exposed to several concentration of nifedipine (Adalat) (mean ± SEM)



Table 6: The Micronucleated polychromatic erythrocytes (MnPCEs) of embryos of female mice exposed to several concentration of nifedipine (Adalat) (mean \pm SEM)

Treatment	MnPCEs / 2000 PCEs
Control	6.8 \pm 0.11
Adalat L	8.6 \pm 0.12*
Adalat M	14.2 \pm 0.11**
Adalat H	17.6 \pm 0.14**

The protective mechanism of Nifedipine against agents inducing genetic toxicity is not well understood. It seems to be dose dependent action. Nifedipine and other calcium antagonists were found to inhibit the increase in hepatocellular calcium level induced by different toxic agents in rats²⁰, by acetaminophen in mice²¹, and in isolated rat hepatocytes.^{22,23} In this respect, the restoration of a high intracellular calcium level to normal levels, under the influence of calcium-channel blockers, would prevent the hepatic cell injury caused by a variety of calcium-elevating toxic agents, as reported from *in vivo*^{21,24} and *in vitro* studies.^{22,23}

In contrary, these results showed that micronucleus formation and DNA damaged cells of non-pregnant and pregnant female mice as well as their embryos treated with medium and high doses of Nifedipine was higher 2 and 3 times compared with control and those treated with low dose of Nifedipine.

During acute liver injury, an increase in the level of intracellular calcium, can cause damage to the cell by activation of calcium-dependent degenerative enzymes; these include the stimulation of Ca²⁺-dependent phospholipases, proteases, and endonucleases with resultant membrane digestion, destruction of critical proteins, and DNA fragmentation, respectively.^{25,21} Therefore, we could suggest that increase the genetic toxicity by increasing the dose of Nifedipine could be attributed to imbalance in intracellular calcium level. Popescu et al.²⁶ reported that increasing the genotoxicity associated with caspase activation which was in part correlated to an increase in intracellular calcium levels. In addition, Li and Cao²⁷ reported that Induction of DNA damage in Barrett's cells and Barrett's esophageal adenocarcinoma cells was observed due to increase of intracellular calcium and increases ROS production.

Conclusion: induction of genetic toxicity by Nifedipine treatment is a dose dependent manner. Nifedipine seems to be safe at the low dose but by increasing its concentration causing genetic toxicity due to imbalance in intracellular calcium level which increase of the ROS production.

REFERENCES

- Daw JR, Hanley GE, Greyson DL, Morgan SG, Prescription drug use during pregnancy in developed countries: a systematic review, *Pharmacoepidemiol. Drug Saf.*, 20, 2011, 895–902.
- McCullough LB, Coverdale JH, Chervenak FA, A comprehensive ethical framework for responsibly designing and conducting pharmacologic research that involves pregnant women, *Am. J. Obstet. Gynecol.*, 193, 2005, 901–907.
- Lyerly AD, Little MO, Faden R, The second wave: Toward responsible inclusion of pregnant women in research, *Int. J. Fem. Approaches Bioeth*, 1, 2008, 5–22.
- Rowland M, Balant L, Peck C, Physiologically based pharmacokinetics in drug development and regulatory science: a workshop report (Georgetown University, Washington, DC, May 29-30, 2002). *AAPS PharmSci*, 6, 2004, E6.
- Corley RA, Mast TJ, Carney EW, Rogers JM, Daston GP, Evaluation of physiologically based models of pregnancy and lactation for their application in children's health risk assessments, *Crit. Rev. Toxicol.*, 33, 2003, 137–211.
- Zhao P, et al., Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review, *Clin. Pharmacol. Ther.*, 89, 2011, 259–267.
- King JF, Flenady VJ, Papatsonis DN, Dekker GA, Carbonne B, Calcium channel blockers for inhibiting preterm labour, *The Cochrane Library*, Issue 1, 2003; <http://www.updatesoftware.com> [Accessed 11 July 2003].
- Papatsonis DN, Kok JH, van Geijn HP, Bleker OP, Ader HJ, Dekker GA, Neonatal effects of nifedipine and ritodrine for preterm labor, *Obstet Gynecol*, 95, 2000, 477–481.
- Magee LA, Schick B, Donnenfeld AE, Sage SR, Conover B, Cook L, McElhatton PR, Schmidt MA, Koren G, The safety of calcium channel blockers in human pregnancy: a prospective, multicenter cohort study, *Am J Obstet Gynecol*, 174, 1996, 823–828.
- Harake B, Gilbert RD, Ashwal S, Power GG, Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep, *Am J Obstet Gynecol*, 157, 1987, 1003–1008.
- Ducsay CA, Thompson JS, Wu AT, Novy M, Effects of calcium entry blocker (nifedipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes, *Am J Obstet Gynecol*, 157, 1987, 1482–1486.
- Ulmsten U, Treatment of normotensive and hypertensive patients with preterm labor using oral nifedipine, a calcium antagonist, *Arch Gynecol*, 236, 1984, 69–72.
- Kupferminc M, Lessing JB, Yaron Y, Peyser MR. Nifedipine versus ritodrine for suppression of preterm labour. *Br J Obstet Gynaecol*, 100, 1993, 1090–1094.
- Monga M, Creasy RK, Pharmacologic management of preterm labor, *Semin Perinatol*, 19, 1995, 84–96.
- Wladimiroff JW, Tonge HM, Stewart PA, Doppler ultrasound assessment of cerebral blood flow in the human fetus, *Br J Obstet Gynaecol*, 93, 1986, 471–475.
- Gramellini D, Folli MC, Raboni S, Vadora E, Merialdi A, Cerebral-umbilical Doppler ratio as a predictor of adverse perinatal outcome, *Obstet Gynecol*, 79, 1992, 416–420.
- Gaafa KM, Badawy MM, Hamza AA, The protective effects of ascorbic acid, cimetidine, and nifedipine on



- diethyldithiocarbamate-induced hepatic toxicity in albino rats, *Drug and Chemical Toxicology*, 2011, 1–15.
18. Blasiak J, Arabski M, Krupa R, Wozniak K, Zadrozny M, Kasznicki J, Zurawska M, Drzewoski J, DNA damage and repair in type 2 diabetes mellitus, *Mutation Research*, 554, 2004, 297-304.
 19. Adler ID, Cytogenetic tests in mammals. In: Venitt S, Parry JM (eds) *Mutagenicity testing (a practical approach)*. IRI Press, Oxford, 1984, 275–306.
 20. Landon E J, Naukam RJ, Rama Sastry BV, Effects of calcium channels blocking agents on calcium and centrilobular necrosis in the liver of rats treated with hepatotoxic agents, *Bioch Pharmacol.*, 35, 1986, 697–705.
 21. Ray SD, Kamendulis LM, Gurule MW, Yorkin RD, Corcoran GB, Ca²⁺ antagonists inhibit DNA fragmentation and toxic cell death induced by acetoaminophen, *FASEB J*, 7, 1993, 453–463.
 22. Thibault N, Peytavin G, Claude JR, Calcium channel blocking agents protects against acetaminophen-induced cytotoxicity in rat hepatocytes, *J Biochem Toxicol.*, 6, 1991, 237–238.
 23. Ellouk-Achard S, Mawet E, Thibault N, Dutertre-Catella, Thevenin M, Claude JR, Protective effect of Nifedipine against cytotoxicity and intracellular calcium alterations induced by acetaminophen in rat hepatocyte cultures, *Drug Chem Toxicol.*, 18, 1995, 105–107.
 24. Tandon SK, Singh S, Jain VK, Prasad S, Chelation in metal intoxication. XXXVIII: effect of structurally different chelating agents in treatment of nickel intoxication in rat, *Fundam Appl Toxicol.*, 31, 1996, 141–148.
 25. Seth SD, Maulik SK, Gupta YK, Calcium and calcium channel blockers: basic and clinical considerations, *Indian J Exp Biol.*, 25, 1987, 729–741.
 26. Popescu R, Heiss EH, Ferk F, Peschel A, Knasmueller S, Dirsch VM, Krupitza G, Kopp B, Ikarugamycin induces DNA damage, intracellular calcium increase, p38 MAP kinase activation and apoptosis in HL-60 human promyelocytic leukemia cells, *Mutat Res.*, 709-710, 2011, 60-66.
 27. Li D, Cao W, Role of intracellular calcium and NADPH oxidase NOX5-S in acid-induced DNA damage in Barrett's cells and Barrett's esophageal adenocarcinoma cells, *Am J Physiol Gastrointest Liver Physiol.*, 306(10), 2014, G863-72.

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