

## **CLASTOGENIC EFFECT IN FISH AND MICE GENOMES INDUCED BY Na- METAMIZOLE**

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### **ABSTRACT**

This investigation was planned to disclose the capability of the well-known drug Na- metamizole in inducing clastogenic effect (s).

To achieve such a purpose four doses correspond to one, two, five; and ten folds of the daily therapeutic dose were tested. Three different organisms i.e. mice (*Mus musculus*, 2n= 40); (*Tilapia zilli*, 2n= 42); and catfish (*Clarias gariepinus*, 2n= 56) were used employing the following genotoxic bioassays: 1- Analysis of chromosomal abnormalities in mice bone-marrow cells; 2- Micronucleus test of micronucleated polychromatic erythrocytes in mice bone- marrow cells; and 3- Analysis of micronucleated erythrocytes in two different genera of fish. Indoxane (cyclophosphamide, CP) was used as positive control.

The results revealed that Na- metamizole was proven to be highly positive clastogenic agent at all tested doses upon mice as well as fish genomes.

The work recommends that this drug should be omitted from use in Egypt.

### **INTRODUCTION**

Metamizole sodium is a non- steroidal anti- inflammatory drug commonly used in the past as a powerful painkiller and fever reducer. It is better known under the names Dipyron, Analgin and Novalgin.

Metamizole was first synthesized by German company Hoechst AG in 1920, and its mass production started in 1922. It remained freely available worldwide until the 1970s, when it was discovered that the drug carries a small risk of causing agranulocytosis – a very dangerous and potentially fatal condition. Recent studies estimate that the incidence rate of metamizole-induced agranulocytosis is between 0.2 and 2 cases per million person days of use, with approximately 7% of all cases fatal (provided that all patients have access to medical care). In other words, one should expect 50 to 500 deaths annually due to metamizole in a country of 300 million, assuming that every citizen takes the drug once a month. This is not a very high rate compared to other drugs - for example, the prescription drug clozapine is known to be at least 50 times more to trigger agranulocytosis. However, at the time the risk was assumed to be much greater (WHO, 2002) and, as such, excessive for an over- the- counter analgesic, especially considering the existence of safer alternatives (aspirin and ibuprofen).

Metamizole was banned in Sweden in 1974, in the United States in 1977; more than 30 countries, including Japan, Australia, Iran and part of the European Union, have followed suit. In these countries metamizole is still

occasionally used as a veterinary drug. In Germany it became a prescription drug. In these countries metamizole – containing drugs and market them in some countries. In Sweden, the ban was lifted in 1995 and re-introduced in 1999. In other parts of the world (notably in Spain, Mexico, India, Brazil, Russia, Bulgaria, Romania, Israel and Third world countries) metamizole is still freely available over-the-counter, remains one of the most popular analgesics, and plays an important role in self-medication. For example, metamizole and metamizole-containing drugs account for 80% of OTC analgesic market in Russia, whereas ibuprofen accounts for 2.5%. In Brazil, metamizole (*Novalgina*) products, although over-the-counter, carry warning to avoid usage by those under 19 years old, and have several information about early detection and treatment of agranulocytosis. Although the Brazilian government did not push for a ban on the drug, its use has seen a huge decline on the past years as pharmaceutical companies and doctors pushed aspirin, paracetamol and ibuprofen based products as replacement. The most widely available metamizole-containing product still in use in Brazil is Buscopan Plus (under the name of *Buscopan Composto*).

Metamizole received brief period of attention by American media in 2002 (Bonkowski *et al*, 2002), when a Latino immigrant boy was admitted into a Salt Lake City clinic with symptoms of agranulocytosis. It was discovered that the drug remained freely available in Latino shops and highly popular among Mexican immigrants, despite the ban. The ongoing "LATIN" study, a multicenter international case-control study, is examining the incidence of agranulocytosis in Latin American and the role of metamizole.

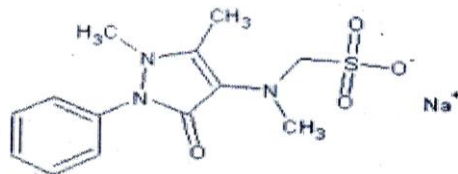
Fish are excellent subjects for the study of the mutagenic and/or carcinogenic potential of contaminants present in water samples since they can metabolize, concentrate and store waterborne pollutants. Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. The main application for model system using fish is to determine the distribution and effects of chemical contaminants in the aquatic environment (Al-Sabti and Metcalfe, 1995; Ali *et al*, 2006 and El-Shehawi *et al*, 2007). In Brazil, a few native species have been used in acute or chronic toxicity assays. Mutagenic studies with native fish species represent an important effort to determine the potential effects of toxic agents on the ichthyofauna.

The present work aims at disclosing the capability of Na-metamizole in inducing clastogenic effect upon mice and fish genomes.

## **MATERIALS AND METHODS**

The drug was locally purchased and used, in two formulated forms i.e., the first form was tablets (each contains 500 mg), the second form was ampoule (2ml contains 1g), this drug is manufactured by the German company Hoechst (this time is Aventis).

Na-metamizole tested in this work is given in (Figure, 1)



Metamizole

**Figure (1): Structure and formula of the tested drug**

**1- Mice:**

Each animal had orally received daily the proper dose (four doses i.e., 5, 10, 25, 50 mg /kg. b. wt. that correspond to 1; 2; 5; and 10 therapeutic dose were tested). Treatment was performed for a week and animals were killed 24 hr. after the last dose, for each treatment four animals were used. Animals of the control group had received equivalent amount of deionized water. Three hours prior to killing, the animals were injected with 0.6 mg/ kg of colchicine. Cell harvest staining and analysis were done according to Adler (1984).

**Micronucleus test:**

Four mice were used for each dose. Experimental design was carried out as that described by Brusick (1986). Bone- marrow smears were made according to Schmid (1975). Staining was carried out according to the method described by Gollapudi and Kamara (1979). The data obtained were analyzed according to Hart and Pederson (1983).

**2- Fish:**

North African catfish (*Clarias gariepinus*) and (*Tilapia zilli*) were collected from natural local area, transferred and kept in glass aquaria measured 120 L for adaptation to laboratory conditions for one month. Dechlorinated tap water was used by leaving water in open container for 48 hr. before use. A total number of 10 fish per dose was used.

Each fish was treated by injection (in trunk muscle) each day for a week.

**Micronucleus Test:**

The peripheral blood smears were obtained through the gills, neck vein and kidney blood by means of a medial- kidney imprint following dissection.

Blood was smeared on clean slides; air- dried for 12 hrs., fixed in absolute methanol for 10 min., followed by 5% Giemsa staining (Ali and El-Shehawi, 2006). A total number of 1000 red cells for each fish were examined, (Grisolia and Strling, 2001).

**RESULTS**

**Chromosomal aberrations in mice:**

Table (1) shows the data obtained from the cytological examination of mice bone- marrow after treatment with Na-metamizole. Different types of aberrations were observed. These types were stickiness, fragments, Robertsonian Centric Fusion (RCF), gap, and polyploidy. Total aberrant metaphases were shown to be 5% in the negative control group. They were increased after treatment with 25 mg cyclophosphamid/ kg. b. wt. to be 34%. Treatment with Na- metamizole gave a percent of 47, 43, 55, and 73 for the tested doses 5, 10, 25, and 50 mg (correspond to 1,2,5, and 10 folds of the daily therapeutic dose). A dose- response relationship was achieved, giving a strong evidence that Na- metamizole is a positive clastogen upon mice genome.

**Micronucleus Test:**

Analysis of micronucleated polychromatic erythrocytes in mice bone- marrow cells after treatment with the tested drug is shown in (Table,2).

Percent of micronucleus was found to be 0.4% in the bone- marrow cells of the negative control. It was increased to be 5.61% after treatment with 25 mg cyclophosphamide. It was found to be 2.6, 5.15, 7, and 8.55% after treatment with 5, 10, 25, and 50 mg/ kg.b.wt respectively. Statistical analysis showed that all tested doses were proven to be different significantly compared with the negative control. However, a dose- response relationship was achieved, giving the second evidence that Na- metamizole is a strong clastogenic agent upon mice genome. Figures (2& 3) illustrate the effect of Na- metamizole upon mice genome.

**Fish:**

**1- Catfish**

Table (3) shows the effect of the tested drug upon the induction of micronucleated red cells. The percent of micronucleus was proven to be 0.41 % in the blood of the negative control. It was 8.06 % for the positive control, and it was 2.04; 3.22; 4.60; and 6.80 after treatment with 5, 10, 25, and 50 mg/ kg. b. wt. all tested doses as statistical analysis showed were found to induce significant increases of micronucleus, and a dose- response relationship was obtained, giving an evidence that Na- metamizole has strong clastogenic activity upon catfish genome.

**2- *Tilapia zilli***

Table (4) illustrates the effect of the tested doses upon the induction of micronucleus in red cells of *Tilapia*. The percent of micronucleus in the blood cells of the negative control was shown to be 0.62 %.

It was found to be 9.42% in the blood cells of the positive control. It was 3.12, 3.62, 5.42, and 7.93% after treatment with the tested doses (Table,4). Statistical analysis revealed that all tested doses were found to be highly significant compared with the negative control.

A dose- response relationship was achieved, giving a strong evidence that Na- metamizole has a strong clastogenic activity upon *Tilapia* genome. Figures (4& 5) illustrate the effect of the tested drug upon micronucleus induction

**Table (1): Chromosomal abnormalities in mice bone marrow cells after treatment with Na- metamizole**

Dose Mg/kg. b. wt.	Type of aberrations					Total aberrant Metaphase
	Stickiness	Fragment	RCF	Poly ploidy	Gap	
Negative control	4	—	—	—	1	5
Positive control	22	3	4	2	3	34
5	12	11	6	8	10	47
10	13	10	5	7	8	43
25	20	13	8	2	12	55
50	29	14	13	6	11	48

- A dose of 25 mg was orally used.

**Table (2): Micronucleated polychromatic erythrocytes in mice bone marrow after treatment with Na- metamizole**

Dose; mg/ kg. b. wt.	No. cells counted	No. micronucleated polychromatic erythrocytes	% MPE
Negative Control	4000	16	0.4
Positive control	4000	228	*5.61
5	4000	104	*2.6
10	4000	206	*5.15
25	4000	280	*7.00
50	4000	342	*8.55

\* Micronucleated polychromatic erythrocytes

**Table (3): Micronucleated erythrocytes in blood red cells of catfish after treatment with Na- metamizole**

<b>Dose; mg/ kg. b. wt.</b>	<b>No. cells counted</b>	<b>No. red cells with micronucleus</b>	<b>%</b>
<b>Negative Control</b>	10.000	41	0.4 ± 0.4
<b>Positive control</b>	10.000	806	*8.06 ± 1.4
<b>5</b>	10.000	204	*2.04 ± 0.6
<b>10</b>	10.000	322	*3.22 ± 0.8
<b>25</b>	10.000	460	*4.60 ± 0.6
<b>50</b>	10.000	680	*6.80 ± 1.1

**Table (4): Micronucleated erythrocytes in blood red cells of catfish after treatment with Na- metamizole**

<b>Dose; mg/ kg. b. wt.</b>	<b>No. cells counted</b>	<b>No. red cells with micronucleus</b>	<b>%</b>
<b>Negative Control</b>	10.000	62	0.62 ± 0.3
<b>Positive control</b>	10.000	942	*9.42 ± 1.2
<b>5</b>	10.000	312	*3.12 ± 0.8
<b>10</b>	10.000	362	*3.62 ± 0.6
<b>25</b>	10.000	542	*5.42 ± 0.4
<b>50</b>	10.000	793	*7.93 ± 1.1

\* 25 mg/ kg of cyclophosphamide

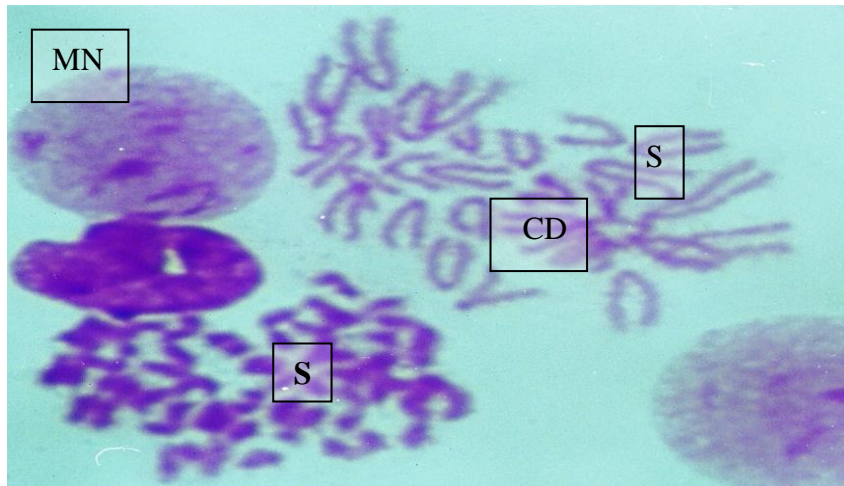


Figure (2): Photomicrograph showing the effect of Na- metamizole upon mice bone- marrow cells.MN :micronucleus.CD:chromatid deletion.R:ring chromosome.S:stickiness.

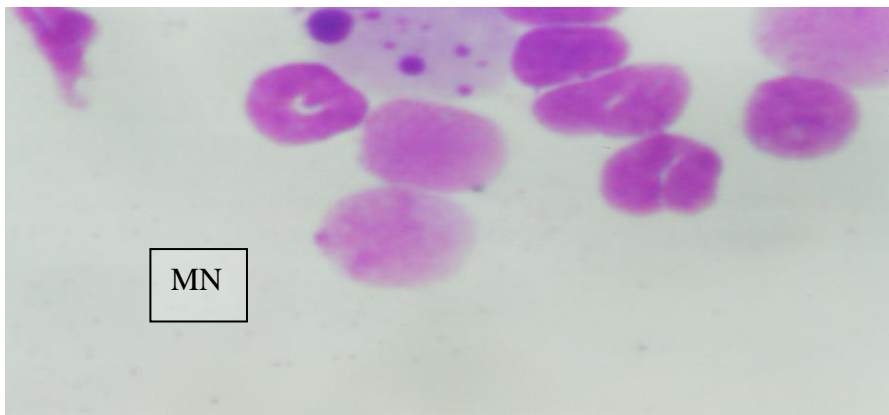
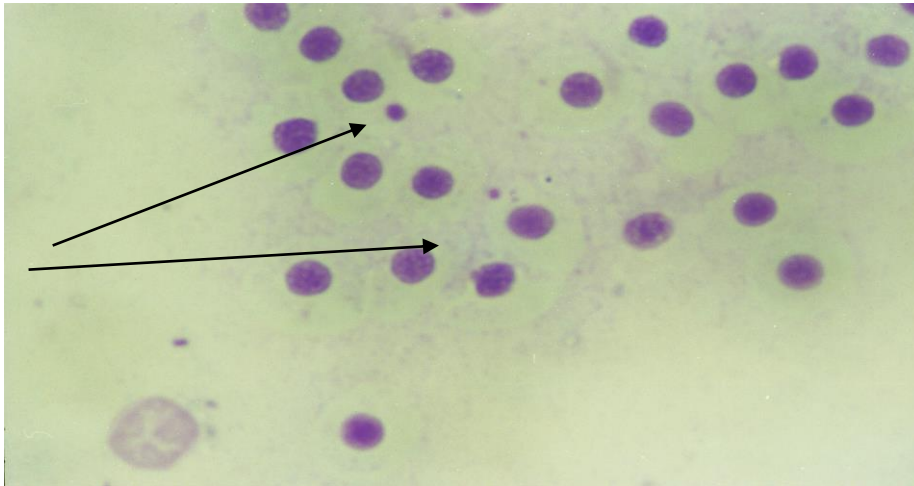
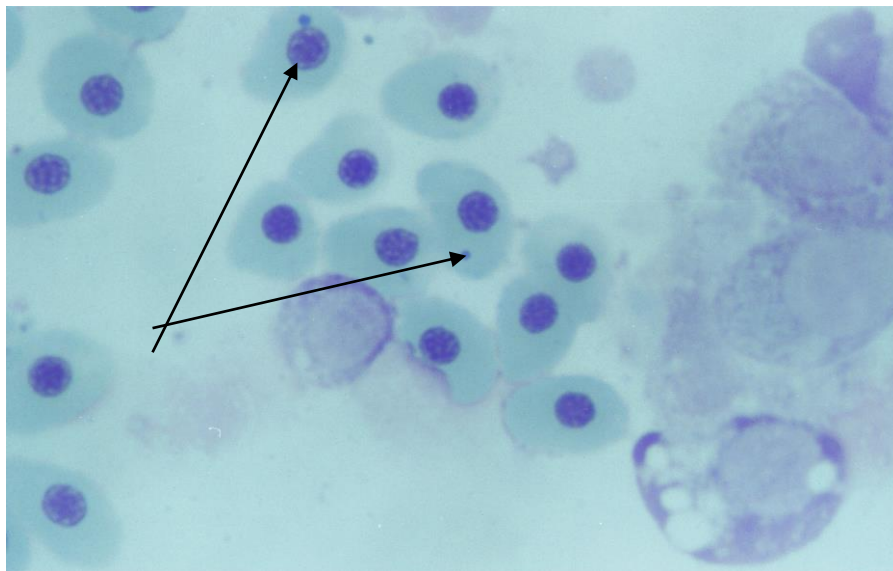


Figure (3): Photomicrograph showing the clastogenic activity of Na- metamizole upon polychromatic erythrocytes. MN :micronucleus.





**Figure (4):** Micronucleated red cells in catfish after treatment with Na-metamizole.



**Figure (5):** Micronucleated red cells in *Tilapia zilli* after treatment with Na-metamizole.

## DISCUSSION

DNA damage consists of two broad categories; i.e. visible effects or (macro lesions detectable through cytological analysis of chromosomes and non- visible changes or (micro lesions) occur at the nucleotide level (Seehy and Soliman, 2007) Macro lesions can be subdivided into change in chromosome number (gain or loss of single chromosome or sets of chromosomes) and changes in chromosome structure (break, deletion, rearrangement....etc.).

One mean of detecting *in vivo* genetic activity of an environmental pollutant, is to examine mitotically active cells that have been arrested at metaphase from structural changes and rearrangement of their chromosomes. The occurrence of such aberrations correlates well with the administration of known mutagen to animals and thus may serve as an indicator for possible mutagenic potential of test articles (Adler, 1984; Brusick, 1986, and Seehy and Soliman, 2007).

Micronuclei are cytoplasmic chromatin- containing bodies formed when acentric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. Because genetic damage that results in chromosome breaks, structurally abnormal chromosomes, or spindle abnormalities leads to micronucleus formation, the incidence of micronuclei serves as an index of these types of damage. It has been established that essentially all agents that cause double strand chromosome breaks (clastogens) induce micronuclei. Because enumeration of micronuclei is much faster and less technically demanding than is scoring of chromosomal aberrations, and because micronuclei arise from two important types of genetic damage (clastogenesis and spindle disruption), the micronucleus assay has been widely used to screen for chemicals that cause these types of damage (Seehy & Soliman, 2007).

Scoring of micronuclei can be performed relatively easily and on different cell types relevant for human biomonitoring: lymphocytes, fibroblasts and exfoliated epithelial cells, without extra *in vitro* cultivation step. MN observed in exfoliated cells is not induced when the cells are at the epithelial surface, but when they are in the basal layer.

The analysis of data obtained from mice and fish (catfish & *Tilapia*) revealed that Na-metamizole is a positive clastogen, since highly significant increases in chromosomal abnormalities; micronucleated polychromatic erythrocytes; and micronucleated red cells were observed; and a dose-response relationship was achieved. The regarding the formation of micronucleus in red cells. Results obtained from this work showed that *Tilapia* genome was more sensitive than that of catfish.

Aquatic animals have often been used in bioassays to monitor water quality of effluent and surface water (Brugs *et al*, 1977) the development of biological monitoring techniques based on fish offers the possibility of checking water pollution with fast responses on low concentrations of direct acting toxicants (Badr and El-Dib, 1978 and El-Shehawi *et al*, 2007).

The genetic effect of this drug has been of this drug has been reported by Arkhipchuk *et al* (2004); and Cleuvers (2004).

In conclusion, the present investigation revealed that this drug should be banned and omitted from medical use in Egypt.

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الأثر التفسيري للمادة الوراثية فى السمك والفئران والمستحدث بعقار الصوديوم  
ميثاميزول  
أحمد السيد خالد  
كلية الزراعة - سابا باشا - جامعة الاسكندرية

هدف البحث الحالى لدراسة الأثر التفسيري للمادة الوراثية والمستحدث بواسطة عقار  
الصوديوم ميثاميزول والمستخدم تجارياً فى مصر تحت إسم نوقالجين والذى أوقف إستخدامه فى  
عديد من الدول مثل أمريكا وأوربا .

ولتحقيق هذا الغرض تم الحصول على العقار محلياً فى صورتين الأول هى الأقراص  
والثانية هى الحقن . إستخدمت الأولى لمعاملة الفئران وإستخدمت الثانية للحقن فى عضلة الظهر  
فى الأسماك .

وتم توظيف الفئران المعملية البيضاء وسمك القرموط والبلطى لهذه الدراسة .

هذا وقد تم توظيف الإختبارات التالية : -

١- تحليل الكرموسومات فى نخاع عظام الفئران

٢- تحليل خلايا الدم الحمراء غير الناضجة فى الفئران .

٣- تحليل خلايا الدم الحمراء الناضجة فى كلا جنسى السمك.

هذا وقد أظهرت النتائج أهمية استخدام السمك ككشاف حساس جدا للأثر الضار الناتج عن  
التلوث والملوثات البيئية.

وأظهر تحليل النتائج المتحصل عليها قدرة تكسيرية موجبة وعالية جدا للعقار قيد الأختبار  
وذلك على المادة الوراثية للفئران وكذلك المادة الوراثية للأسماك . وتوصى الدراسة بوقف إستخدام  
العقار كخافض للحرارة ومخفف للألم .