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EVALUATION OF THE CYTOGENETIC EFFECT OF CYCLOOXYGENASE INHIBITOR; FLUNIXIN MEGLUMINE (FINADYNE)® IN RATS

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ABSTRACT

A study was carried out to evaluate the effect of Flunixin meglumine(Finadyne)®, a cyclo-oxygenase inhibitor, on different cytogenetic parameters including mitotic index, chromosomal aberrations, quantitative estimation of nucleic acids (DNA and RNA) and detection of genetic diversity using Random Amplified Polymorphic DNA "RAPD" technique in rats. The experiment was conducted on 30 female albino rats divided into three equal groups each of ten as follows: The first group was the control group. The second group was intramuscularly injected with finadyne; 2.5 mg /kg body weight once daily for 7 successive days. The third group was intramuscularly injected with the same dose of finadyne for 30 days "prolonged administration". Analysis of the obtained results indicated that, finadyne elicited a significant decrease in the mitotic index when intramuscularly injected to rats for 7 days and 30 days compared with the control .Only prolonged administration of finadyne for 30 days evoked a significant increase in the mean values of the total structural chromosomal aberrations. Quantitative estimation of nucleic acids (DNA and RNA) revealed significant decrease in DNA and RNA contents of rats given finadyne for 30 days.Analysis of genetic identity and diversity using Random Amplified Polymorphic DNA (RAPD) technique by the means of random oligodeoxyribonucleotide primers showed that the numbers of produced bands were found to be variable among groups using different primers. DNA extracted from blood of rats injected with finadyne for 30 days showed the lowest numbers of polymorphic with unique bands compared with the control. The dendrogram based on the correlation matrix between different groups showed high similarity between amplified polymorphic bands of DNA of the control and those of rats treated with finadyne for 7 days while there was significant genetic diversity between DNA of rats treated with finadyne for 30 days compared with those of the control. Taken together, our findings are not in favor of the unjustified prolonged administration of flunixin meglumin due to the detectable hazard of cytogenetic influences.

INTRODUCTION

Cyclo-Oxygenase (COX) is an enzyme that is responsible for formation of important biological mediators called prostanoids (including prostaglandins, prostacyclin and thromboxane). Currently three COX isoenzymes are known-COX-1, COX-2 and COX-3. COX-1 is considered a constitutive enzyme, being found in most mammalian cells. More recently it has been shown to be upregulated in various carcinomas and to have a central role in tumorigenesis. COX-2, on the other hand, is undetectable in most normal tissues. It is an inducible enzyme, becoming abundant in activated macrophages and other cells at sites of inflammation while COX-3 is a splice variant of COX-1 which retains intron one and has a frameshift mutation, thus some prefer the name COX-1b or COX-1 variant (COX-1v)[1].

The most significant difference between the isoenzymes, which allows for selective inhibition, is the substitution of isoleucine at position 523 in COX-1 with valine in COX-2. The relatively smaller Va_{l523} residue in COX-2 allows access to a hydrophobic side-pocket in the enzyme (which isoleucine523 sterically hinders). Coxibs; new Non-Steroidal Anti-Inflammatory Drug (NSAID) , bind to this alternative site and are considered to be selective inhibitors of COX-2[2].

Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain; this is the mechanism of action of classical non-steroidal anti-inflammatory drugs, the main non selective COX inhibitors such as the well-known drugs: aspirin and ibuprofen[3].

Several studies have reported an inverse relationship between cancer incidence and regular use of cyclooxygenase inhibitors, including aspirin[4, 5]. The dietary administration of cyclooxygenase inhibitors, such as ibuprofen and celecoxib, significantly reduced the incidence, multiplicity and volume of tumors in female Spragne-Dawley rats with dimethylbenzathracene-induced mammary carcinomas[6, 7]. The inhibition of Cox-2 function by the use of a Cox-2 inhibitor or Cox-2-null cells markedly increased DNA damage-induced apoptosis[8].

On the other hand, some studies have documented a strong relationship between cyclooxygenase inhibitors and induction of DNA strand breaks and cell death by apoptosis "cell cycle arrest"[9, 10]. Sulfasalazine or SASP;NSAID induced Sister Chromatid Exchanges (SCE) and Micronuclei (MN) frequencies in the peripheral blood erythrocytes of male and female mice administered 675, 1350 or 2700 mg/kg SASP by gavage for 90 days. Weak but significant dose-related increases in MN were also recorded in the bone marrow cells of male mice administered 500, 1000 and 2000 mg/kg SASP for 3 days[11].

In the same line, Ibrulj et al.[12] recorded that paracetamol concentration of 200 microg/ml expressed certain genotoxic effects in human peripheral blood lymphocytes. The cellular damage induced by Paracetamol leads to expression in chromosome aberrations such as gaps, chromatid breaks, acentric fragments and polyploid metaphases[13].

Stemmed from the previous outlines, the present study was performed to evaluate the effect of Flunixin meglumine (Finadyne)®, a cyclo-oxygenase inhibitor and a potent analgesic, antipyretic, and anti-inflammatory drug acting by inhibiting the cell's production of prostaglandins and other chemicals that stimulate the body's inflammatory response, on different cytogenetic parameters including mitotic index, chromosomal aberrations, quantitative estimation of nucleic acids

(DNA and RNA) and detection of genetic diversity using random amplified polymorphic DNA "RAPD" technique in rats.

MATERIALS AND METHODS

Drug: Flunixine meglumine (Finadyne)®, Schering-Plough Veterinaire Co., 50 ml injectable solution vials- each ml contains 50 mg flunixine meglumine.

Rats: Thirty albino rats were obtained from the Animal House, Faculty of Veterinary Medicine, Zagazig University. The animals were kept under hygienic conditions, housed in metal cages and bedded with wood shavings, fed on a balanced ration and watered *ad-libitum*. They were accommodated to the laboratory conditions for two weeks before being experimented with.

Experimental design :

Rats were divided into three equal groups each of 10 as follows:

The first group received no-treatment and was considered as a control group. The second group was intramuscularly injected with finadyne; 2.5 mg /kg body weight once daily for 7 successive days[14]. The third group was intramuscularly injected with finadyne; 2.5 mg/kg body weight once daily for 30 days "prolonged administration".

Cytogenetic evaluation:

I. Chromosomal aberrations and Mitotic Index (MI):

Bone marrow extraction and preparation :

It was carried out according to Yosida and Aman[15]. Colchicine solution; 0.5% was intramuscularly injected into rat groups at a dose of 0.25 ml/100 gm body weight to stop the mitotic division.

After ninety minutes of colchicine treatment, rats was sacrificed and both femurs were dissected and opened at both ends of cutting epiphysis.

One ml of physiological saline solution was injected from one end while the cell suspension was collected at the other one in a centrifuge tube using one tube for each femur.

In order to facilitate the hypotonic treatment, the cells were suspended in five ml of 0.9 NaCl then centrifuged for 10 minutes at 1000 rpm. and the supernatant was discarded.

Hypotonic solution treatment was carried out in order to increase the cell volume. The cells were suspended in two ml of 0.56% KCl and incubated for 20 minutes at 37°C then the tubes were centrifuged for 5 minutes at 1000 rpm.

Fixation:

Two ml of freshly prepared fixative (3 parts methyl alcohol and one part glacial acetic acid) were added, then the cells were mixed gently and left for 30 minutes at room temperature. The solution was centrifuged for 5 minutes at 1000 rpm, then the supernatant was discarded and four ml of the fixative solution were added. This treatment was repeated three times until a clear solution was obtained.

By mean of a clean and dry Pasteur pipette small droplets from the cell suspension were dropped on a clean dry glass slides previously dipped in 60% ethanol. The slides were flamed and stained with Giemsa stain.

Staining of the chromosomes:

The fixed slides were stained by Giemsa stain according to Genest and Auger[16]. One ml of stock Giemsa 5% was diluted with nine ml of phosphate buffer (pH 6.8). The slides were stained in coplain jar for 20 minutes, rinsed by distillated water and dried in air.

Analysis of the cytogenetic data:

1- Microscopic detection:

Direct examination of the dried stained slides was carried out under low power for suitable metaphase spread, then examined under the maximum magnification using the oil immersion lens for details.

For analysis of chromosomal aberrations, fifty metaphases per animal were selected[17].

2- Photographic analysis:

Photographs intended for analysis of cytogenetic data were enlarged 3000 times. The photographic analysis included the chromosomal structure in which the chromosomes were arranged in pairs for diagnosis (Karyotyping)[18] and identified[19].

3- Chromosomal aberrations:

It was described according to Brusick[20] as follows:

a) Chromatid type aberrations

As in break and end to end association.

b) Chromosome type aberrations

As in Ring and sticky and pulverized chromosomes.

4- Mitotic Index (MI):

The mitotic index was calculated by counting 500 cells for each animal and scoring the number of cell in mitosis per 500 cells.

$$MI = \frac{\text{Number of metaphase cells x 100}}{\text{Total number of cells}}$$ (20)

II. Quantitative estimation of nucleic acids (DNA and RNA):

Samples of liver tissues were obtained from slaughtered rats for Extraction of nucleic acids[21]. DNA was determined according to the method of Dische and Schowerz[22], its optical density was determined by spectrophotometer at 600 nm. DNA content (mg/g tissue) was calculated by: Reading X 40.39. RNA was determined according to Mejbaum[23], its optical density was measured at 660 nm. RNA content (mg/g tissue) was calculated by: reading x 17.8.

III. Genetic identity and diversity analysis using Randomly Amplified Polymorphic DNA (RAPD) technique:

This technique was used to study the effect of the test drug on the molecular level and to reveal if any genetic diversity exists between groups. The technique is based on random amplification of short segments of target genomic DNA using a single oligonucleotide primer of arbitrary sequence[24].

DNA extraction:

Genomic DNA was isolated from the blood of rats in all groups using the DNA isolation Kit (Catalog of Promega Inc., USA) following manufacturers instructions then, DNA was quantified at OD = 260 nm using a UV spectrometer.

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PCR reactions:

Amplification reactions were run at a volume of 25 µl by Perkin Elemer 2400 thermocycler. The reaction mixture contained 0.4 µM of each of the four deoxynucleotides (dATP, dCTP, dGTP and dTTP); 2 mM $MgCl_2$; 0.5 U Taq-polymerase (Promega); 0.5-5 nM of primers (ISSR or RAPD/Operon Technologies) (Table 3); 2.5 µl of 10 x thermophylic buffer (50 mM KCl, 10 mM TRIS-HCl, Promega); and 20 ng template DNA[25].

PCR amplification:

RAPD amplification programs were settled on for the thermal cycler at 94°C for 2 minutes; 35 cycles of: 94°C for 30 seconds, 36°C for 45 seconds, 72°C for 1 min 30 seconds; 72°C for 20 minutes; and 4°C soak forever. The annealing temperature varied according the melting temperature (™) of each primer. The core program increased from 35 to 40 cycles, if amplification is weak, to get slight increase in the amount of PCR products.

Gel electrophoresis:

Amplified fragments, 10 ul, were separated by agarose (1.2 %, SeaKem LE, FMC) gel electrophoresis, stained with ethidium bromide (0.5 ng/µl) at 80 V in 1 x TBE buffer and photographed on a UV transilluminator (Pharmacia) by Canon S_5 digital camera with UV filter adaptor. A negative control which contained all the necessary PCR components except template DNA was included in the PCR runs.

Fragment analysis:

Sharp PCR fragments were scored for the presence versus absence (not "ghost"). Fragments at low intensities were only scored as present when they were reproducible in repeated experiments.

Cluster analysis:

Genetic similarity was estimated on the basis of Jaccard coefficient[26]. Data from the similarity matrix were used for cluster analysis by the unweighted pair-group method with arithmetic averages (UPGMA). All the calculations were performed by using the NTSYS-pc version 2.02 software package (Numerical Taxonomy System, Exeter Software).

Statistical analysis:

Statistical analysis for MI, chromosomal aberrations and nucleic acids estimation was carried out using the Student's "t" test according to Snedecor and Cochran[27].

RESULTS

1- Mitotic Index (MI):

Analysis of the obtained results revealed that, therapeutic dose of finadyne elicited a significant ($p < 0.05$ and $p < 0.001$) decrease; 4.42 ± 0.44 and 3.2 ± 0.2 in the mean values ± SE of the mitotic index when intramuscularly injected to rats for 7 days and 30 days respectively compared with the control group; 5.92 ± 0.3 (Table 1).

2- Chromosomal aberrations:

The obtained results clearly demonstrate that, only rats given finadyne for prolonged period (30 days) displayed significant increase ($p < 0.01$); 2.6 ± 0.73 in the mean values of total chromosomal aberrations compared with the control group; 0.1 ± 0.1 and the recorded structural aberrations were ring, gap chromosomes and chromosomal deletion (Table 1 and Figure 1).

3- Quantitative estimation of nucleic acids (DNA and RNA):

The results showed that, intramuscular injection of finadyne; 2.5 mg /kg body weight daily for 30 successive days to rats evoked significant decrease; 6.01 ± 0.8 and 5.59 ± 0.5 in DNA and RNA contents respectively compared with the control while injection of the same dose of the drug for 7 days elicited non-significant changes in the contents of both nucleic acids (DNA and RNA) (Table 2).

4- Genetic identity and diversity analysis using Random Amplified Polymorphic DNA (RAPD) technique:

Random oligodeoxyribonucleotide primers were used to generate PCR-amplified fragments, termed Random Amplified Polymorphic DNA (RAPD) markers from genomic DNA of each group (Table 3).

Analysis of the obtained results revealed that seven primers (3, 4, 5, 8, 10, 12 and 13) of the 20th primers used in this study have produced different polymorphic amplified bands while the others did not (Figure 2).

By using RAPD-PCR primers it was shown that the numbers of produced polymorphic bands were found to be variable among groups using different primers.

The total numbers of amplified polymorphic fragments from all primers for each group are summarized in Table (4).

From this table, we can observed that DNA extracted from blood of rats injected with finadyne for 30 days showed the lowest numbers of polymorphic with unique bands compared with the control.

The dendrogram based on the correlation matrix between different groups according to their amplification pattern after using random primers displayed high similarity between amplified polymorphic bands of DNA obtained from the control and those of DNA obtained from rats treated with finadyne for 7 days. There was significant genetic diversity between DNA obtained from rats treated with finadyne for 30 days compared with those obtained from the control (Figure 3).

Table (1): Effect of intramuscular administration of the finadyne at a dose rate; 2.5 mg /kg body weight once daily for different periods; B and C on the mitotic index and chromosomal aberrations in rats

Group	Number of examined metaphase cells	Type of aberration			Total damage (n = 10)			Mitotic Index (M.I)		
		Deletion	Ring	Gap	No	%	Mean ± SE	Number of cells counted	Number of metaphase cells	Mean ± SE
A	500	0	1	0	1	0.2	0.1 ± 0.1	5000	296	5.92 ± 0.3
B	500	1	2	0	3	0.6	0.36 ± 0.11	5000	221	4.42 ± 0.44*
C	500	11	10	5	26	5.2	2.6 ± 0.73**	5000	160	3.2 ± 0.2***

A: control rats.
B: rats intramuscularly injected with finadyne 2.5 mg /kg body weight once daily for 7 successive days
C: rats intramuscularly injected with finadyne 2.5 mg /kg body weight once daily for 30 days.
*p < 0.05 **p < 0.01 ***p < 0.001

Table (2): DNA and RNA contents (mg/gm tissue) in rats intramuscularly injected with finadyne in a dose of 2.5 mg/kg body weight once daily for different periods B and C

Group	DNA	RNA
A	8.51 ± 0.12	8 ± 0.22
B	7.99 ± 0.39	8.02 ± 0.45
C	6.01 ± 0.8*	5.59 ± 0.5**

A: control rats
B: rats intramuscularly injected with finadyne, 2.5 mg /kg body weight once daily for 7 successive days.
C: rats intramuscularly injected with finadyne; 2.5 mg /kg body weight once daily for 30 days.
*P < 0.05 **P < 0.01

Table (3): Random primers used in this study and their sequences and the percentages of the G/C content of them

Primer	Sequence	GC%
1	5-GTA GAC CCG T-3	60
2	5-GGA CCC TTA C-3	60
3	5- GTC GCC GTC A-3	70
4	5- GGT CCC TGA C-3	70
5	5- TGG ACC GGT G-3	70
6	5-AGG GGT CTT G-3	70
7	5-TTC CCC CGC T-3	70
8	5-TTC CCC CCA G-3	70
9	5- ACT TCG CCA C-3	60
10	5- CAA TCG CCG T-3	60
11	5- AGG GAA CGA G-3	60
12	5-TGC GCC CTT C-3	70
13	5-TTC GCA CGG G-3	70
14	5-GTG AGG CGT C-3	70
15	5-CAA ACG TCG G-3	60
16	5-CTG CTG GGA C-3	70
17	5- GTG ACG TAG G-3	60
18	5- CCA CAG CAG T-3	60
19	5-TGA GCG GAC A-3	60
20	5- GTG AGG CGT C-3	70

Table (4): Total lanes polymorphism at all treated DNA using all the tested primers

Total lanes polymorphism	Groups		
	C	B	A
Monomorphic bands	0	0	0
Polymorphic (without unique)	0	2	2
Unique bands	1	5	11
Polymorphic (with unique)	1	7	13
Total numbers of bands	1	7	13

A:DNA from control.
B:DNA from rats intramuscularly injected with finadyne; 2.5mg/kg body weight once daily for 7 successive days.

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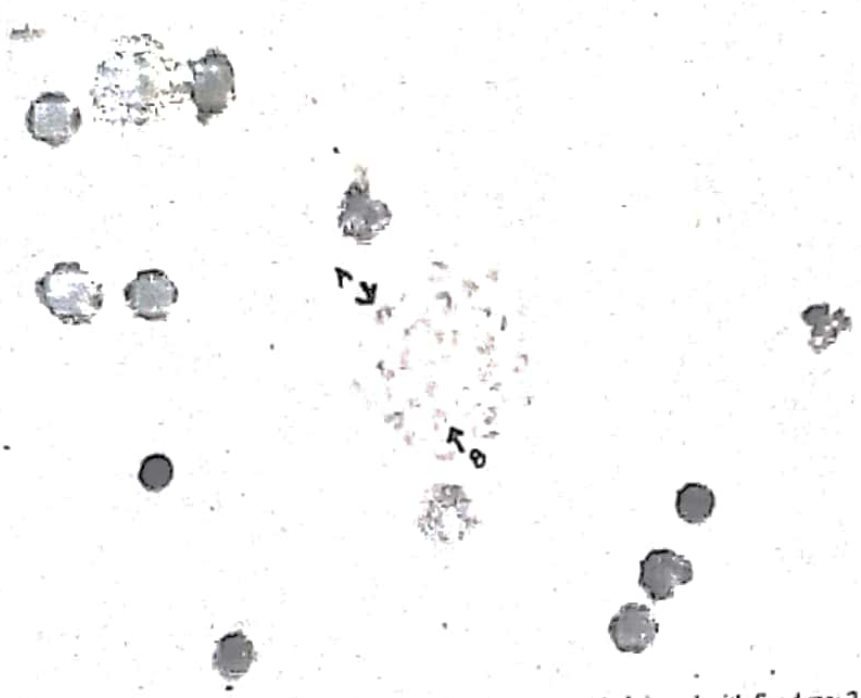

Figure (1): Metaphase spread obtained from femur bone marrow culture of rats intramuscularly injected with finadyne; 2.5 mg/kg body weight once daily for 30 days displaying gap chromosome (A) and chromosomal deletion (B)

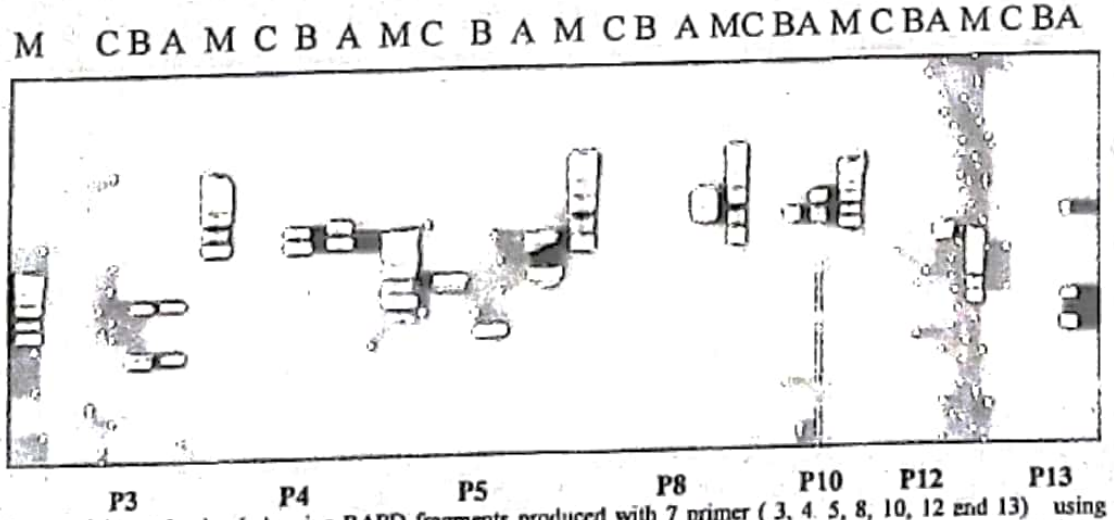

Figure (2): Ethidium bromide stained gel showing RAPD fragments produced with 7 primer (3, 4 5, 8, 10, 12 and 13) using genomic DNA extracted from blood of rats belongs to different groups; control group (A), treated with finadyne for 7 days (B), treated with finadyne for 30 days (C), notice the variation in the number of polymorphic bands which reflect the genetic diversity of DNA of treated rats compared with the control. M: Marker, P: Primer.

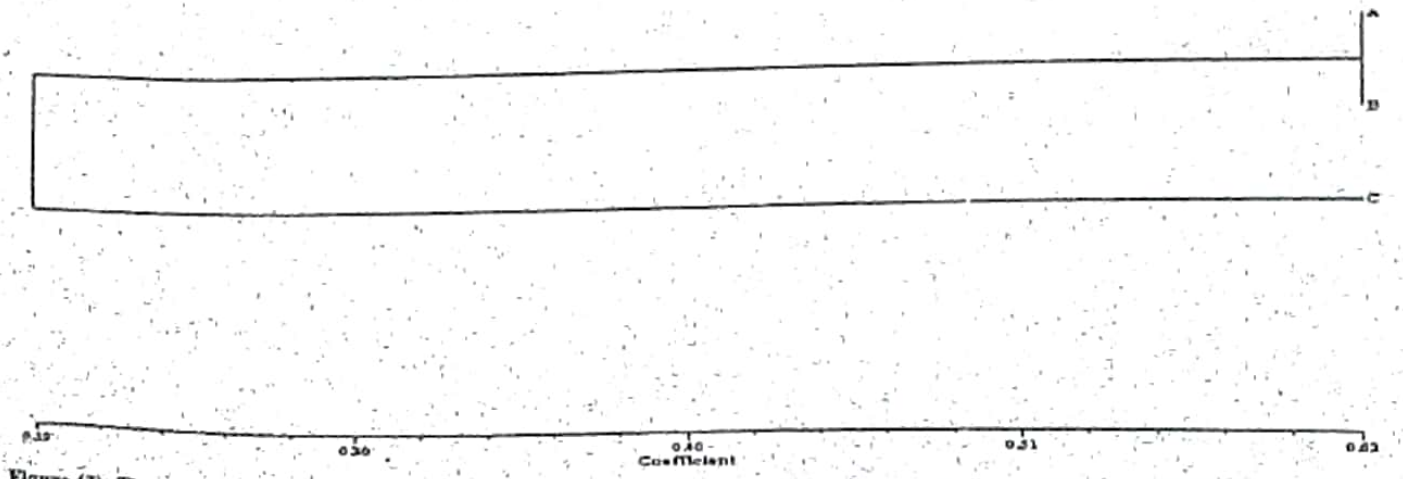

Figure (3): The dendrogram based on the correlation matrix between different groups according to their amplification pattern after using random primers showing high genetic similarity between A "DNA of control" and B "DNA obtained from blood of rats treated with finadyne for 7 days" while there was significant genetic diversity between C "DNA obtained from blood of rats treated with finadyne for 30 days" and both A and B

DISCUSSION

It has been well established through various experimental and clinical studies that, there is a strong relationship between cyclooxygenase inhibitors and DNA damage and cell cycle arrest[10] and this relationship becomes strongly prominent with prolonged administration of cyclooxygenase inhibitors[11].

As a matter of fact, the obtained results fit in neatly with the above mentioned concept . We recorded significant decrease in the mitotic index in rats intramuscularly injected with finadyne for 7 days and highly significant inhibition in the mitotic index was observed after prolonged administration of the drug (for 30 days).

Several studies supported our results and stated that, non-steroidal anti-inflammatory drug as Sulindac and its metabolites, such as sulindac sulfide, inhibited the proliferation in cultured colon cancer cells and induced DNA strand breaks and cell death by apoptosis (cell cycle arrest) with reduction of both the number and size of the cells[28].

Numerous investigations have shown that NSAIDs exerted direct toxicity to the renal inner medullary collecting duct cells (by evaluating the effects on cell number, cell cycle, and cell death) and toxicity is considerably greater in rapidly proliferating cells than in more slowly growing. confluent cells. The authors attributed this effect to the direct inhibition of the ribonucleotide reductase, which reduces cell growth by stopping DNA replication. Then, the relative number of cells in the S phase decreases[29].

Possible mechanisms that explained the antimitotic activity of cyclo-oxygenase inhibitors include the ability for damaging the DNA during the S-phase of the cell cycle by blocking the formation of the mitotic spindle in M-phase or by interfering with DNA integrity and thereby exhibits strong anti-mitotic activity both *in vivo* and *in vitro*[30].

In the same line, Claudinéia et al.[28] stated that the cytotoxic effect of NSAID in *A. nidulans* diploid cells might be explained by cell cycle delay or by a cell death mechanism.

Another school of thought reported that, cyclooxygenase enzyme and its major final product, PGE_2, protect cells against apoptosis. In fact, a possible mechanism for enhanced apoptosis by cyclooxygenase inhibitors may not likely to be related to a reduction in prostaglandins but rather to elevation of the prostaglandin precursor Arachidonic Acid (AA), which stimulates ceramide production, a strong mediator of apoptosis[8].

In the current work, it has been pointed out that, finadyne exerted significant increase in the mean values of the total structural chromosomal aberrations of various types and significant suppression in the DNA and RNA contents when administered to rats for 30 days.

Admittedly, the formentioned findings could be tightly knotted together with the previous reports.

Bishop et al.[11] stated that increases in Sister Chromatid Exchanges (SCE) and Micronuclei (MN) frequencies have been reported in lymphocytes of patients maintained on cyclooxygenase inhibitor salicylazosulfapyridine (SASP) therapy for up to 21 months and increases in MN were observed in the peripheral blood erythrocytes of male and female mice administered 675, 1350 or 2700 mg/kg SASP by gavage for 90 days. Weak but significant dose-related increases in MN were also observed in the bone marrow cells of male mice administered 500, 1000 and 2000 mg/kg SASP for 3 days.

Cast in the same mold, the genotoxic potentiality of nimesulide, cyclooxygenase inhibitor, was evaluated in vivo in murine bone marrow cells. The human equivalent prophylactic dose of nimesulide (5 mg/kg body wt/day) was given to animals orally, once daily for seven consecutive days. Metaphase chromosome analysis revealed the significant increase in the incidence of chromosomal aberrations with preference to structural over the numerical ones. It therefore suggested the clastogenic effect of the nimesulide[31].

In keeping with this line, it was found that paracetamol concentration of 200 microg/ml expresses certain genotoxic effects evaluated by micronucleus cytokinesis-block assay, chromosome aberration analysis, and nuclear division index. in human peripheral blood lymphocytes[12].

In addition, Paracetamol 400 mg/kg as intraperitoneal administration in mice leads to expression in chromosome aberrations such as gaps, chromatid breaks, acentric fragments and polyploid metaphases[13].

Many investigations recorded the direct effect of the cyclooxygenase inhibitors on DNA, inducing the occurrence of DNA breaks and cell cycle alterations[28].

It seems essential, from this prospective, to emphasize that DNA damage leads to sister chromatid exchange and chromosomal aberrations due to inhibition of nucleotide excision repair[29].

The achieved results not only documented the significant decrease in the DNA quantity but also on the level of DNA structure using RAPD technique, the results demonstrated marked changes in the structure of DNA using different primers of different genetic sequences and we recorded that, prolonged administration of finadyne (for 30 days) showed more detectable changes in the DNA structure.

In this context, it is akin to mention that, there was limited evidence that Flunixin-meglumine was an *in-vitro* mutagen[32].

Available data point to three proposed mechanisms of possible cyclooxygenase inhibitors-induced genotoxicity and mutagenicity:

1- Inhibition of ribonucleotide reductase;
2- Increase in cytosolic and intranuclear Ca^{2+} levels; and
3- DNA damage after glutathione depletion[33].

CONCLUSION

It could be concluded that, prolonged administration of flunixin meglumin is not recommended due to the detectable hazard of cytogenetic influences.

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تقييم التأثير الوراثى الخلوى لشبط اثريم السيكلواوكسيجينيز, فلونكسين ميجلومين (فينادين) فى الفئران

نجلاء زكريا حلمى عليوه

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اجريت هذه الدراسة لتقييم التأثير الوراثى الخلوى للفلونكسين ميجلومين (الفينادين) فى الفئران و ذلك من خلال دراسة تأثيره على معدل الانقسام الميتوزى ، نسبة التشوهات الكروموسومية ، محتوى الأحماض النووية (الحامض النووى الدي أوكسى ريبوزى- الحامض النووى الريبوزى) وكذلك دراسة قدرتة على احداث تغيرات وراثية على مستوى جزىء الحامض النووى الدى اوكسى ريبوزى باستخدام تقنيات الحامض النووى الدى أوكسى ريبوزى المكبر و المتعدد الأوجه العشوائى. تم استخدام ٣٠من الفئران البيضاء والذين قسموا الى ٣ مجموعات متساويه هى : المجموعه الأولى الضابطة و المجموعه الثانية التى تم حقنها عضليا بالفينادين بجرعة ٢,٥ مجم/كجم من وزن الجسم يوميا لمدة ٧ أيام متتالية اما المجموعه الثالثة فقد تم حقنها عضليا بنفس الجرعه السابق ذكرها من الفينادين لمدة ٣٠ يوما.

و لقد أسفرت النتائج عن عن و جود نقص معنوى فى معدل الانقسام الميتوزى فى الفئران التى حقنت بالفينادين لمدة ٧ أيام أو ٣٠ يوم وكذلك ظهور زيادة واضحه فى نسبة التشوهات الكروموسومية الكلية فى الفئران التى اخذت الفينادين لمدة ٣٠ يوما فقط كما لوحظ حدوث نقص معنوى فى محتوى الأحماض النووية(الحامض النووى الدي أوكسى ريبوزى- الحامض النووى الريبوزى) فى نفس المجموعه فقط (التى اخذت الفينادين لمدة ٣٠ يوما) .

و بتحليل التغيرات الوراثية التى احدثت فى جزئ الحامض النووى الدى أوكسى ريبوزى المكبر و المتعدد الأوجه العشوائى وجد أن الحامض النووى الدى أوكسى ريبوزى الماخوذ من دم الفئران التى حقنت بالفينادين لمدة ٣٠ يوما اظهر تغييرا واضحا فى ترتيب قواعدة الوراثية مقارنة بالمجموعه الضابطة .

و من كل ما حصلنا عليه من نتائج فان الدراسة توصى بعدم استخدام الفلونكسين ميجلومين (الفينادين) لفترات طويله تتعدى الفترة العلاجية المقرره له و ذلك لما اظهره من تأثير سيئ من الناحية الوراثية الخلوية و الجزيئية .