Biochemical and Cytogenetical Studies of the Non-steroidal anti-inflammatory drug (NSAID) in male mice

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Abstract: The aim of this study was to evaluate the Cytogenetic and biochemical effect of nonsteroidal anti-inflammatory drug (NSAID) vioxx (rofecoxib) on mice. **Materials and Methods:** The sample of this study was 35 adult Swiss albino male mice . Cytogenetic and biochemical analysis were performed . Cytogenetic analysis was conducted upon 15 adult mice ;which were divided into three groups (5 male mice in each). First group served as control. The two other groups treated orally with two different doses of vioxx drug (0.2 and 0.4 mg/kg.bwt) for two weeks. Biochemical analysis was conducted upon 21 adult male mice which formed three groups of adult male mice (7 male mice in each) ;control (untreated) and two treated groups; 0.2 and 0.4 mg/kg. b.wt vioxx dose groups. **Results:** revealed that there was difference between treated groups with vioxx drug by examination of chromosomal aberrations of bone marrow cells of sacrificed animals when compared to untreated groups, as well as, in chromosomes of germ cells. Biochemical studies showed that there were changes on level of total content of DNA and RNA in mouse tissues (brain, Liver, Kidney and testis) treated with vioxx drug when compared to untreated animals, as will as, urea and creatinine level (kidney function in blood) after two weeks treatment with voxx drug). Also glucose and cholesterol as similar as in cholein esterase level. In addition there was elevation in level of SGOT (ALT) and SGPT (AST) (Liver enzyme) in serum of exposed animals to vioxx drug compared to the control. On the other hand, the testosterone level hormone and haemoglobin were decreased in serum of animal was observed in treated animals.

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1. Introduction

There are many different types of non-steroidal anti-inflammatory drugs (NSIDS) vary in their strength, duration of action and the way in which they are eliminated from the body.

Non-steroidal anti-inflammatory drugs (NSAIDs) work by blocking a chemical called cyclooxygenase.

This enzyme is present in a number of places through out the body, including in inflamed tissue, the stomach lining and the kidney. Unfortunately, taking an NSAID to reduce inflammation blocked all the cyclo-oxygenase in the body often with extremely determential results.

VIOXX (rofecoxib) are common type of NSADs and first member of new drug that preferentially inhibit activity of cyclo-oxygenase-2 (COX-2) enzyme.

The recent withdrawal of vioxx and growing concern over the safety of other COX-2 inhibitors have promoted a re-evaluation of air nation's system of drug development, approval marking and monitoring. VIOXX (rofecoxib) are common types of NSAIDs and first member of new drug that preferentially inhibit activity of cyclo-oxgenase-2 (COX-2) enzyme.

VIOXX drug is called second form which doctors call Non-steroidal anti-inflammatory drugs (NSAIDs) that treat inflammation and pain of arthritis. VIOXX was approved for pain relief, Osteoarthritis, rheumatoid arthritis, short-term pain, menstrual pain, migraine headach, juvenile rheumatoid arthritis, dyspepsia and diarrhea, Sara *et al* (2000). Although generally safe "traditional" NSAIDs account for almost one fourth of all reported adverse drug event. Approximately 15 percent of NSAIDs users have gastrointestinal tract symptoms such as dyspepsia, heart bum, nausea or vomiting, Singh (1998). An estimated 16,500 NSAIDs related deaths occurred annually among patients with Osteorthritis or rheumatoid arthritis, (Wallf, 1999; Peterson, 1999 and McEboy, 2000).

2. Material and Methods:

2.1. Materials

2.1.1. Animals:

Male mice weighing (25-30 gm) obtain from a closed random-bred colony at National Research Centre were used. Males were orally administered with the different doses once daily for 15 consecutive days.

2.1.2- Chemicals:

Non steroidal anti-inflammatory drug (NSAID) VIOXX drug [(rofecoxib) tread name for the drug] is described chemically as 4-[4(methyl sulfonyl) phenyl]-3-phenyl-2(5H)-furanone.

VIOXX drug was dissolved in distilled water using vortex mixing at high speed until no traces of powder were seen in vehicle.

Administration was effected in a volume of 1ml/animal daily for two week. Two different doses of one time were used in this study (0.2 and 0.4 mg/kg b.wt) which similar to human therapeutic dose.

2.2. Methods:

2.2.1. Experimental design:

A total number 36 of adult albino mice were used for this study .Cytogenetic and biochemical analysis were performed. Animals were divided into three groups for each test. Each group contained five animals in Cytogenetic assay and seven animals in biochemical assay, their details were as following:

Group I: animals were orally administered with distilled water daily.
Group II: animals were orally administered with 0.2 mg/kg.bw daily of VIOXX solution for two weeks.
Group III: animals were orally administered with 0.4 mg/kg.bw of VIOXX solution daily for two weeks

2.2.2. Cytogenetic Examination

Fifteen male mice were sacrificed 24 hour after last treatment and chromosomes smears of bone marrow and testes were prepared according to Yosida and Amano (1965) and Evans *et al*. (1964). To obtain the frequencies of chromosomal aberration in bone marrow cells and spermatocytes of male mice; 50 metaphases from each animal were examined for a total 250 cells per each dose and control.

2.2.3. Biochemical examination:

1- Determination of total content of Nucleic acid (DNA and RNA) in brain, liver, kidney and testes tissues of male mice according to Peares (1985).
2- Determination of total content of protein according to methods of Peters (1968) give blue colour read at wave length 545nm using spectrophotometer.
3- Determination of cholesterol in serum according to Method of Richmond (1973) and determination of glucose in serum according to Tinder (1969).
4- Determination of heamoglobin in serum according to Drobkin (1982).
5- Determination activity of liver enzymes glutamic-oxaloacetic transaminase GOT (AST) and glutamic-pyruvic transaminase GPT (ALT) according to the method or Reitman and Frankel (1957) at wave length (490-520nm).
6- Determination of concentration of urea and creatinine (indictor of kidney function) in serum was carried out according to Henry (1974) ; Coulamb and Favreau (1963) using spectrophotometer at wave length 492nm (490-570).
7- Determination of cholinesterase activity in serum and brain was carried out according to Jakobs *et al* (1990) using spectrophotometer at wave length 405 (400-440nm).
8- Determination testosterone level was estimated in serum by ELISA (Micro-well method) according to Parker (1981).

2.2.4. Statistical analysis:

Data are expressed as mean $\pm$ SE according to using standard T-test.

3. Results

3.1. Cytogenetic effect of VIOXX drug:

In present study, in comparison to untreated group, VIOXX drug (NSAIDs) induce sings of clinical toxicity in somatic cells of male mice were observed in the chromosomal aberration which recorded different changes after treatment with two doses dependent.

The chromosomal aberration were in the form of gap, break, deletion, centromeric attenuation and fragments. In our study we show the value of gap, break and deletion centromeric attenuation and fragments. In our study we show the value of gap, beak and deletion give Non-significant change in two doses when compared to control. In other hand, Centromeric attenuation and fragments give significant chang value when compared to control. Table (1). The total number chromosomal aberration were increased significantly in treated animals dose dependent. The numerical aberration observed non significant value in low dose but there was significant value with high dose when compared to control Table (1).

Moreover, in germ cells of male mice observed from testis which treated with two doses of VIOXX drug for two weeks the chromosomal aberration (Spermatocyte) were increased Table (2). Spermatocyte were in the form of chain, autosomal and x-y univalent. There was significant increase only in x-y univalent with two dose when compared to untreated animals. But in chain and outosomal univalent there was significant increase only with high dose when compared to control. Also, there was no significant changes in numerical aberration of Spermatocyte cells when compared to control Table (2).

Table (1): Effect of VIOXX drug on the frequency of somatic chromosomal aberration in male mice compared to control.

Treatment	Structural chromosomal aberration Mean ± S.E.						Numerical aberration		
	Gap	Break	Del	C.A.	Frag	Total aberration	Poly polidy	Hypo polidy	Hyper polidy
Control	1.2 ± 0.545	0.8 ± 0.5447	1.6 ± 0.447	2 ± 0	0.4 ± 0.447	6 ± 1.581	0.0 ± 0.0	0.4 ± 0.4	0.4 ± 0.4
Low dose	1.6$^{N.S.}$ ± 0.447	1.6 $^{N.S.}$ ± 0.45	2.4 $^{N.S.}$ ± 0.447	14.4***+ 1.643	2.4* ± 0.447	22** ± 2.8	0.4$^{N.S.}$ ± 0.44	2$^{N.S.}$ ± 0.707	2.8 ± 1.140
High dose	1.6$^{N.S.}$ ± 0.45	2 $^{N.S.}$± 0.0	3.6 $^{N.S.}$ ± 0.837	28***± 1.581	3.6** ± 0.836	39.2*** ± 5.650	0.4 ± 0.497	2.4 ± 0.832	4** ± 0.707

C.A. Centromeric attenuation N.S.: Non significant * Significant 0.05
** Very high significant 0.01 *** Very high significant 0.001

Table (2): Induced Chromosomal aberration in germ cells of male mice compared to control with VIOXX drug

Treatment	Structural aberration			Numerical aberration		
	Chain	Autosomal	x-y univalve	Poly polidy	Heype polidy	Hayper polidy
Control	0.0 ± 0.0	0.8 ± 0.49	1.6 ± 0.447	0.0 ± 0.0	0.00 ± 0.00	0.0 ± 0.0
Low dose	2.0$^{N.S.}$ ± 10.07	3.2$^{N.S.}$ ± 0.894	4.0* ± 0.707	0.4 ± 0.01	0.8 ± 0.545	0.8 ± 0.55
High dose	3.2* ± 0.894	4.4* ± 0.837	5.6** ± 0.837	0.4$^{N.S.}$ ± 0.4	0.2 $^{N.S.}$ ± 0.55	1.2 $^{N.S.}$ ± 0.54

3.2. Biochemical effect of VIOXX drug:

In our study we has been investigate the changes in different parameters of biochemistry in male mice when treated with two doses of VIOXX drug for two week compared to untreated animals.

3.2.1. Changes in DNA and RNA contents:

The DNA and RNA content in male mice tissues reduced by VIOXX drug administration in dose dependent the maximum reduction was recorded with high dose in all tissues (brain, liver, kidney and testes) Table (3).

Table (3):Changes total content of nucleic acid in different tissues of male mice after treatment with VIOXX drug Compared with control level

Treatment	Brain		Liver		Kidney		Testes	
	DNA (mg/g)	RNA (mg/g)	DNA (mg/g)	RNA (mg/g)	DNA (mg/g)	RNA (mg/g)	DNA (mg/g)	RNA (mg/g)
Control	0.365± 0.006	0.293 ± 0.005	0.390 ± 0.006	0.331 ± 0.006	0.358 ± 0.01	0.283 ± 0.006	0.376 ± 0.007	0.319 ± 0.007
Low dose	0.317* ± 0.82	0.238** ± 0.011	0.336** ± 0.009	0.250** ± 0.013	0.332 ± 0.007	0.232** ± 0.008	0.329** ± 0.011	0.242** ± 0.011
High dose	0.252*** ± 0.008	0.167** ± 0.005	0.264*** ± 0.009	0.204** ± 0.006	0.249 ± 0.008	0.160** ± 0.007	0.269*** ± 0.008	0.187** ± 0.005

* Significant 0.05; ** high significant 0.01; *** Very high significant 0.001

3.2.2. Changes in total protein contents:

Data in Table (4) showed that the administration of VIOXX drug into male mice reduced total protein contents in all tissues of male mice the reduction was dose dependent since the high dose gave highest reduction when compared with untreated group.

Table (4): Effect of VIOXX drug on total protein content in different tissue for male mice compared to control

Treatment	Protein g/g tissues			
	Brain	Liver	Kidney	Testis
Control	6.658 ± 0.117	9.121±0.192	6.751±0.088	7.85±0.157
Low dose	5.841** ±0.171	8.383* ±0.243	5.357*** ±0.127	6.94** ±0.107
High dose	5.074*** ±0.12	7.184*** ±0.112	4.687*** ±0.131	6.077*** ±0.139

* Significant 0.05** High significant 0.01
*** Very high significant 0.001

3.2.3. Changes in serum level of glucose, cholesterol and hemoglobin

The level of serum when determined the values of glucose, cholesterol and heamoglobine in male mice there was reduced by VIOXX drug in treatment of hemoglobin but there was increase significantly in cholesterol and in glucose there was the elevation give non significant when compared to control group (Table 5).

Table (5): Determination serum level of glucose and cholesterol with serum level of hemoglobin in male mice with VIOXX drug compared to control

Treatment	Glucose mg/dl	Cholesterol mg/dl	Hemoglobine g/dl
Control	70.33 ± 0.680	155.729 ± 1.537	7.168 ± 0.318
Low dose	71.771 N.S. ± 0.753	168.939** ± 1.123	6.797** ± 0.277
High dose	72.6354 N.S. ± 1.158	179.943** ± 1.287	5.215*** ± 0.751

N.S. non significant ** high significant 0.01
*** Very high significant 0.001

3.2.4. Determination of kidney function(Creatinine and Urea):

The effect of two doses VIOXX drug on creatinine and urea level in serum of treated mice was studied Table (6). The result showed that the VIOXX drug were increased dependent dose. Hence the increased in serum level of male mice was high significant

Table (6): Determination Serum level of kidney function (Urea and Creatinine) in male mice after treatment with VIOXX drug compared to control

Treatment	Creatinine mg/dl	Urea g/dl
Control	0.507 ± 0.014	30.753 ± 0.512
Low dose	0.886*** ± 0.028	48.716*** ± 0.316
High dose	1.205*** ± 0.057	54.447*** ± 2.070

*** Very high significant 0.001

3.2.5. Determination the change of liver enzymes (GOT and GPT)

The changes in serum level of GOT and GPT (liver enzyme) of male mice was significantly increased by VIOXX drug. The study show increased in enzyme activity in Table (7) by dose when compared to control animals.

Table (7): Serum level of liver transaminase enzymes (GOT and GPT) in male mice after treatment with VIOXX drug compared to control.

Treatment	GOT U/ml	GPT U/ml
Control	51.00 ± 2.437	68.285± 4.32
Low dose	60.0* ± 3.144	84.143* ± 5.031
High dose	73** ± 5.073	91.571* ± 7.281

* Significant 0.05 ** high significant

Table (8): Effect of VIOXX drug on serum level of testosterone and activity of cholinesterase enzyme in male mice compared to control.

Treatment	Cholein esterase U/ml	Testestrone ng/ml
Control	5.245 ± 0.151	13.869 ± 0.227
Low dose	6.797** ± 0.227	12.541v ± 0.245
High dose	7.162* ± 0.312	11.643*** ± 0.163

* Significant 0.05** High significant 0.01
*** Very high significant 0.001

3.2.6 Determination activities of cholinesterase enzyme and testosterone hormone:

The effect of VIOXX drug on cholinesterase activity (brain enzyme) and testestrone level (hormone) in serum of treated male mice was studied (Table 8). Male mice showed increase in the blood enzyme activity and decrease the hormone level in serum.

4. Discussion

Eventually all NSAIDs should be sulfated to such long-term tests. What is safe? Nothing really is safe, but the relative dangers have been evaluated or even recognize. American who use VIOXX to relieve pain is VIOXX safe or dangerous? Both are true depending on the circumstances.

The present investigation after treatment of commonly NSAIDs VIOXX were used. VIOXX works by reducing substances that cause inflammation pain and fever in body. The manufactures of VIOXX has announced a voluntary withdrawal of drug from the U.S. and would wide market. This withdrawal is due to safety concerns of an increased risk of cardiovascular events in patients taking mis-represents the safety profile for VIOXX (FDA 2001); Mukherjee *et al*., (2001).

In present study oral administration of VIOX drug (NSAIDs) induced a statistically significant increase in frequencies of chromosomal aberration in all tested cell types. This means that the VIOXX has a possible genetic activity (Ostensen *et al*., 2006).

FDA News (2004) reported that genotoxicity of NSAIDs may appear in DNA damage or involving multiple interactions with non. DNA damage or involving multiple interactions with non. DNA. This recorded of chromosomal aberration obtained by VIOXX treatment, might be attributed to inhibition of DNA agreement with present study.

Also VIOXX cause in germ cells depression Cleary in increased of x-y univalent and decreased in testosterone hormone in blood which lead to infertility, Martini *et al*., (2003); Nagalakshi and Tong-man (1999) showed that NSAIDs causing gene mutation or chromosomal aberration due to genetic alteration in germ cells lead to reproductive failure or genetic disorder in subsequent generation NSAIDs in all animal species studied including abnormalities induced in animals mice, rat, rabbit and monkey by specific of gestation showed brain malformation defective limbs and facial abnormalities (Kennely *et al*., 2001; Monike *et al*., 2006).

Genetic variants may play an important part in the development of disease. The apparent substantial heritability of late-onset AD (Alzheimer disease) is inadequately explained by genetic

variation within the wall-replicated genes Nancy (2010).

Monique and Breteler (2010) study identified and strengthened associations of additional loci (the position of a gene on a chromosome with AD and confirm these in an independent sample.

The biochemical study data in the present work showed that oral administration NSAIDs in male mice caused a dose dependent reduction of kidney (urea and creatinine which may risk in kidney and increase liver enzyme (GOT and GPT) which indicated risk function of liver. Stanfield *et al*., (2003) recorded that there was reduction in prostaglandin formation precipitate over renal decompensation patients at greatest risk of this reaction are those with impaired renal function, heart failure, liver dysfunction those taking diuretics and ACE inhibitors and elderly. Peruzzi *et al*., (2001), Stika (2002) noticed borderline elevation one or more liver tests may occur in up to 15% of patients taking NSAIDs and not able elevations of ALT and AST (GOT and GPT) (approximately three or more times the upper limit of normal).

Anemia is some times observed in patients receiving VIOXX.Patients on long-term treatment with VIOXX should check their hemoglobin or hematocrit if they exhibited any signs or symptoms of anemia or blood loss. VIOXX does not generally affect platelet counts, Prothrombin time (PT) or partial thromboplastin time (PTT) and does not inhibit platelet aggregation at induced dosages. Penta-saccharides, which are molecules that induce a conformational change in antithrombin molecule so that can bind and inactive activated coagulation factor X, are logical alternatives for low-molecular-mass heparin (Turpie *et al*., 2003).

Although COX-2 inhibitors are safe in the majority of patients under certain condition they may induce prothrombotiz effects, few patients with predisposed thrombosis may be at risk for cardiovascular and ocular thrombotic events (Meyer *et al*., 2005).

A statistical studies of drugs VIOXX sometimes reveal on unexpected ,beneficial side effects even dangers after taking VIOXX, as selective COX-2 inhibition that is an anti-inflammatory drug. This results found that an anti-inflammatory drug developed Alzheimer's disease (Zandi 2006).

Scientists at Georgetown university found that high cholesterol levels increase the rate at which beta amyloid from its "parent" protein and accumulated into the plaques found in Alzheimer's disease. They also shown cholesterol increase ,the production of another protein called APOE, which contributes to nerve cell toxic over-produced. Beta amyloidal is a protein that all accumulated in the brain in Alzheimer's disease. The group has also shown that raising cholesterol level beta amyloid production.

Johnsen *et al*., (2004) shown that small tumors of sympathetic nervous system (neuroblastoma) have abnormal levels of COX-2 expressed.

A few large studies have investigated the finding also the combination hormone replacement did not protect against a milder form of memory mild cognitive in parent. Mild cognitive impairment is characterized by memory lapses that can accompany aging and eventually lead to Alzheimers. Additional study on possible preventive measures during normal aging is now under way (Kristina 2003).

This present work of the NSADIs VIOXX controversy including specific information regarding the misleading and harmful role played by animal tests during all aspects or pre-approval testing.

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