

## دراسة السمية الوراثية لعقاري الاندوميثاسين والكلوكوز أمين

### على بعض جينات الخصوبة لذكور الجرذان البيضاء

*A study of cytogenetic effects of Indomethacin and glucosamine drugs in some fertility genes in male Albino rats*

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### الخلاصة

درست السمية الوراثية لعقاري الاندوميثاسين والكلوكوز أمين في اربعة جينات تعد من جينات الخصوبة في ذكور الجرذان وهي ( PTGR<sub>2</sub>,GSTP<sub>1</sub>,HSP70 ,IGFBP<sub>3</sub>)، وبتركيز 7 ملغم/كغم ، 21 ملغم/كغم للعقارين على التوالي لمدة 21 يوم، وبواقع 4 مجاميع تضم كل منها 4 جرذان من ضمنها مجموعة السيطرة والتي جرعت بالماء المقطر . استخلص الحامض النووي DNA لخلايا نسيج كل من الخصى والبرابخ، شخّصت الجينات بأستعمال تقنية التسلسل المتبلر pcr والترحيل الكهربائي على جل الاكاروز. لوحظ تأثير كبير للعقارين على الجينات المعزولة من خلايا البربخ مقارنة بالخصى في جميع الجينات قيد الدراسة باستثناء الجين HSP70 اذ لم يكن للعقارين أي تأثير يذكر في كل من الخصى والبرابخ .

## Abstract

The genotoxicity of Indomethacin and glucose amine was studied in four genes of the fertility genes of male rats (PTGR2, GSTP1, HSP70, IGFBP3) and at 7 mg / kg, 21 mg / kg respectively for two drugs , drunked for 21 days and 4 groups each 4 rats, including the control group, which was drunked with distilled water. DNA was extracted for the tissue cells of both the testes and epididamises ,genes was studied by pcr technique and electrophoreses on agarose gel . A significant effect was observed on the genes isolated from the epididymal cells compared to the testes in all the genes under study except for the HSP70 gene, its not been any effect in both the testes and the epididymises.

## Introduction

Indomethacin is a non-specific anti-inflammatory drug (NSAID) that was developed specifically to abate the inflammatory responses to the indolic hormones, serotonin and tryptophan , It was introduced in 1963 for the treatment of rheumatoid arthritis, degenerative joint diseases, ankylosing spondylitis, gout, acute musculoskeletal disorders, inflammation and oedema following surgical technique and pain associated with primary dysmenorrhea [1,2]. Glucosamine and chondroitin sulfate (CS), both components to the extracellular matrix of articular cartilage, have been used for medicinal purposes for nearly 40 years<sup>[3,4]</sup> After gaining popularity in Europe and Asia for the treatment of arthritis for the last 20 years, they gained popularity in the United States after the release of several lay publications in the late 1990s.both drugs was used to treatment Osteoarthritis<sup>[5,6]</sup>. Many of the toxic effects of indomethacin in experimental animals may be due to inhibition of prostaglandin synthesis<sup>[7]</sup> but afew studies related this effected with fertility gene defected.

(PTGR<sub>2</sub>, GSTP<sub>1</sub>, HSP70 , IGFBP<sub>3</sub> ) four genes was classified as fertility genes in rat or have roles in spermatogenesis and maturation of sperms<sup>[8]</sup> . Prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase, is the key enzyme in prostaglandin biosynthesis, and acts both as a dioxygenase and as a peroxidase. There are two isozymes of PTGS: a constitutive PTGS1 and an inducible PTGS2, which differ in their regulation of expression and tissue distribution<sup>[9]</sup>. This gene encodes the inducible isozyme. It is regulate

d by specific stimulatory events, suggesting that it is responsible for the prostanoid biosynthesis involved in inflammation and mitogenesis.. A single-nucleotide polymorphism at position 313 in The resulting isoleucine<sup>[10]</sup> . Glutathione S-transferases gene polymorphisms could impair the capability of defense against oxidative stress and result in the development of some cancers One of the determinant factors of susceptibility of spermatozoa to oxidative damage <sup>[11]</sup> . Heat shock protein 70 (HSP70)is produced by the HSP70 gene, The HSP70 gene is encoded by a single exon. The open reading frame the gene is 1926 bp and its protein includes 641 amino acids and includes a family of HSPs which range in size from 68 to 73 KDa<sup>[12]</sup>. HSP70 plays a protective role in reaction to hyperthermia as well as other stress conditions by providing a balance between synthesis and degradation of cellular proteins .Elliott et. al. (2009) found that HSP70, as sperm-binding oviduct AL proteins, increase longevity and viability of sperm in bull and boar,It was reported that semen quality may be influenced by levels of HSP70 protein in boars <sup>[13]</sup>. Govin et al.(2006)found

association between HSP70 function and spermatid DNA-packaging proteins during spermatogenesis<sup>[14]</sup>. Insulin-like growth factor binding protein- 3 (IGFBP-3) is a multifunctional molecule with many mechanisms of action, their receptors, and their binding proteins play key roles in regulating cell proliferation and apoptosis<sup>[15]</sup>. Among the several roles of IGFBP3 are its function as the major carrying protein for IGF1 and IGF2 in the circulation, and its action as a modulator of IGF bioactivity and as a direct growth inhibitor in the extravascular tissue compartment, where it is expressed in a highly regulated manner <sup>[16]</sup>. The effects of the GST genes (GSTM1, GSTT1 and GSTP1) on male factor infertility have not been well elucidated. A case–control study was performed to investigate the association between functionally important polymorphisms in GST genes and idiopathic male infertility<sup>[17,18]</sup>. The present study aims to study the effect of indomethacin and Glucoseamine on the four genes under study using pcr technique to determine the risk of these drugs on male fertility.

## Materials and methods

### Chemicals

Indomethacin tablets in (25 mg/tablet) was obtained from sigma chemical company.

Glucose amin gelatinous [capsules](#)(500 mg/capsule) was obtained from sigma

chemical company.

### Experimental animals

Sixteen adult male [albino](#) rats , age from (12-16) weeks and their weights (200–250 gm) were brought at the National Center for Control and Pharmaceutical Research in Baghdad... The animals were acclimatized for two weeks prior to the experiment. They were kept in plastic cages under adequate temperature and [ventilation](#). All rats were provided with free access to water and food with a 12 h light/dark cycle.

### Experimental design

The rats were classified into four groups:

- Group I (control group): Comprised of 4 rats. They are received saline

orally by gavage in a daily for 4 weeks.

- Group II: (Indomethacin group): Included 4 rats fed on basal diet and given Indomethacin dissolved in saline orally by gavage in a daily dose (7 mg/kg) for 4 weeks.

Group III: (Glucose Amin group): Included 4 rats fed on basal diet and given Glucose Amin dissolved in saline orally by gavage in a daily dose (21 mg/kg) for 4 weeks.

Group IV: (Indomethacin and Glucose Amin group): Included 4 rats fed on basal diet and given both drugs dissolved in saline orally by gavage in a daily for 4 weeks.

At the end of the fourth weeks, animals were anesthetized and decapitated and the the testes with epididymis was harvested by careful rapid dissection and was immediately extracted DNA by DNA Extraction Kit was equipped by Gene aid Total DNA Mini Kit.

The quality of the DNA was also reported by passing the samples on the agarose gel by electrophoresis <sup>[19]</sup>.

Genetic analysis was carried with sample PCR .

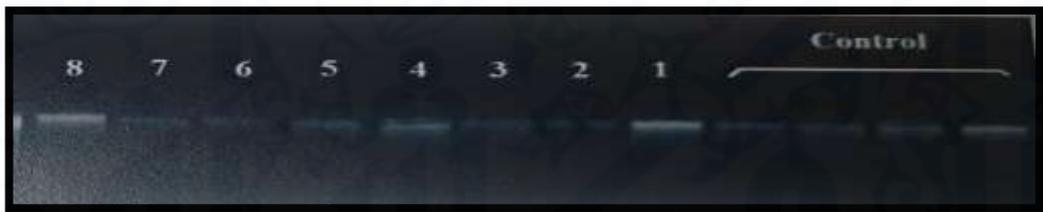
Four Primers equipped with Bioneer company (showed in table no.1) were used to amplify products. Applied Biosystem Veriti™ Thermal Cycler was used for deoxyribonucleic acid (DNA) amplification .The polymerase chain reaction (PCR) cycling condition consisted of an initial denaturation for 2min at 95C°, followed by 1 cycle of 95 C° for 5 min, 95C° 20 sec ,and 60 C° for 1min, and a final extension at 72 C° for 2 min .polymerase chain reaction products were electrophoresed on a 2% agarose gel and visualized under ultraviolet( figur1) .

| Primers  |   | Sequences                   | Size   |
|----------|---|-----------------------------|--------|
| IGFBP -3 | F | 3'CTGCAAGGCAACACCATTCA 5'   | 845 Pb |
| IGFBP -3 | R | 3'ATGGGGGTGCCAATCATCTG 5'   |        |
| HSP70    | F | 3'TTCTCCCAGTGCTTGTTGGA 5'   | 1005pb |
| HSP70    | R | 3'GGTAGGAAGGCATTAGGACGA 5'  |        |
| GSTP1    | F | 3' TCTGGCCGTTGTTCTGTAA 5'   | 1176pb |
| GSTP1    | R | 3'TGACCTGAGGTTGCTTTGAGG 5'  |        |
| PTGR2    | F | 3' AGTGTGTGGTTACGGCTTTCA 5' | 1027pb |
| PTGR2    | R | 3'GCATGCCAAATGGCAGAAAAA 5'  |        |

Table 1: showed the four primers used with their sequences and size of bands.

## Results

DNA samples isolated from both the testis and the epididymis showed high purity when measured with a Nano drop( 1.8 - 2) with concentrated 25 to 125 nm /  $\mu$ l in Fig.1



**Fig1:the electrophoreses of hole DNA extracted from testis and epididymis for four groups of animals with control.**

The results of the polymerase chain reaction(pcr) of multiplying the IGFB3 gene in the 854 Pb in the DNA samples from the testis and epididymis for the experimental animals under study with the three treatment (indomethacin, glucose amine and indomethacin with glucose amine) were reported to be electrophoresed on agarose gel 2% after staining by ethidium bromide stain , The occurrence and dispersion of the band in each of the testes and epididymis in three treatments compared to the standard sample as shown in Figures 22 and 3 respectively.

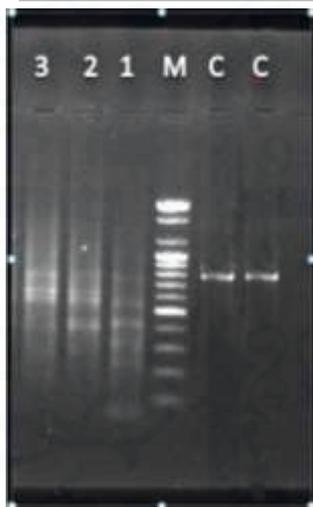


Fig.2:show the IGFP3 gene in IGFP3 gene in Bands size 845pb for DNA extracted DNA extracted from testes(1:indomethacin (1:indomethacin2:glucose amine 3:indomethacin 3:indomethacin with glucose amine and C:control) and C:control)

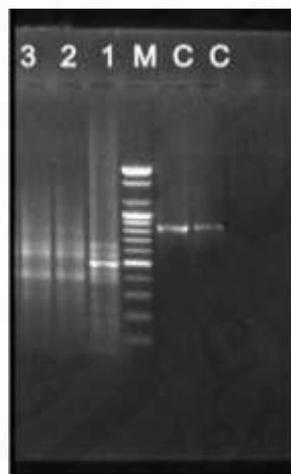


Fig.3:show the Bands size 845pb for from epididymis 2:glucose amine with glucose amine

fig.4 and fig.5 in respectively was showed the HSP70-2 gene pcr with band in size 1005 pb ,NO effect for the all treatment you can see both testes or epididymis samples .

There was no effect on the GSTP1 gene for DNA samples of the testis tissue, while primer did not recognize the gene in the samples of the nucleic acid of the epididymis as the gene was affected by the two drugs under study show fig.6 and 7.Same results we can see in PTGR2 gene as fig.8,9

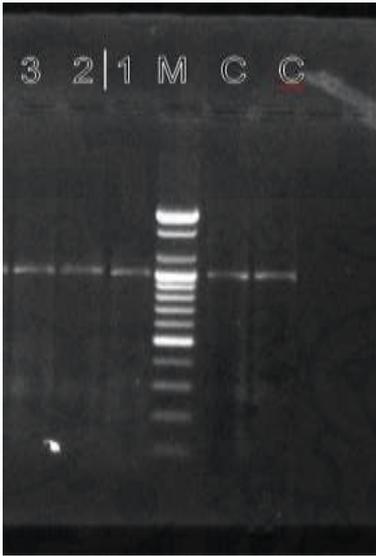


Fig.4:show the HSP70-2 gene in  
gene in  
Bands size 1005pb for DNA extracted  
DNA extracted  
from testes(1:indomethacin  
(1:indomethacin  
2:glucose amine 3:indomethacin  
3:indomethacin  
with glucose amine and C:control)  
C:control)

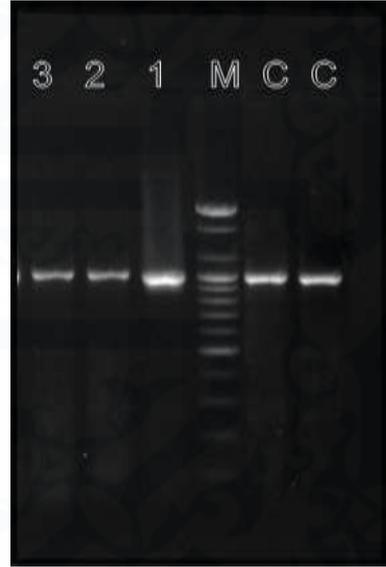


Fig.5:show the HSP70-2  
Bands size 1005pb for  
from epididymis  
2:glucose amine  
with glucose amine and

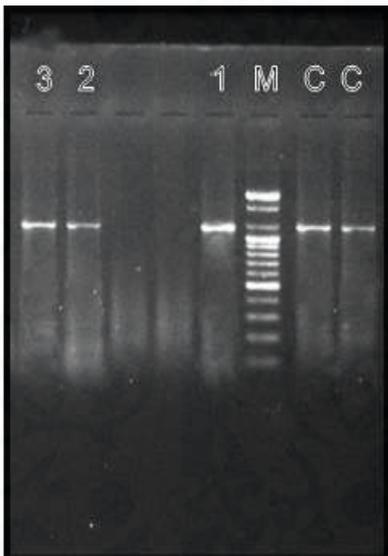


Fig.6:show the GSTP1 gene in gene in DNA extracted

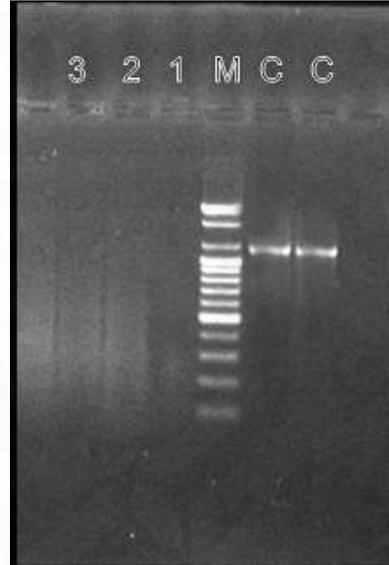


Fig.7 :show the GSTP1 Bands size 1176 pb for DNA extracted from testes(1:indomethacin 2:glucose amine 3:indomethacin with glucose amine and C:control)

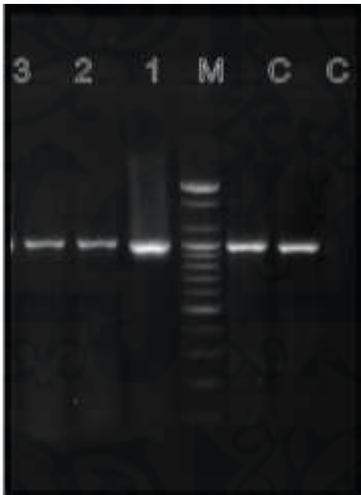


Fig.8:show the PTGR2 gene in gene in



Fig.9 :show the PTGR2

|   |  |
|---|--|
| Bands size 1027 pb for DNA extracted<br>DNA extracted<br>from testes(1:indomethacin<br>(1:indomethacin<br>2:glucose amine 3:indomethacin<br>3:indomethacin<br>with glucose amine and C:control)<br>C:control) | Bands size 1027 pb for<br>from epididymis<br>2:glucose amine<br>with glucose amine and |
|---|--|

## Discussion

Several previous studies have indicated that there is no hereditary toxic effect of indomethacin and glucose amine but indomethacin inducers of apoptosis and inhibitors of cell proliferation<sup>[20,21]</sup>, but used both drugs as cofactor for treatment of number of cancers [22] . while the present study showed genetic variability of the two drugs on four genes under study. The result of electrophoreses of four genes (IGFP3, HSP70-2, GSTP1 and PTGR2) clear effects of both drug on this gene specially on epididymis DNA samples comparative with testes DNA samples , only HSP70 gene no effected was showed in both samples . This may refer to the effect of both drugs on the shape and effectiveness of sperm as previous studies indicate The increased HSP 70 expression has positively correlated to DNA damage detected in mice sperm<sup>[13]</sup>. This is what the current study has shown for the other three genes, which have shown that the DNA of the epididymis has been

destroyed. The reason for the non-susceptibility of the genes to the testicular specimens may not be due to the high protection of the individual by increasing the specificity of the material being carried from the cell into the cells. Effect of the two drugs on the composition of the sperm to the extent of their ability to fertilize. The previous study have indicated to the role of IGFBP-3 and BAX interaction activates germ cell apoptosis via the mitochondria-dependent pathway and this regulating germ cell homeostasis that may have significance for male fertility testicular disease<sup>[19]</sup>. Indomethacin and glucose amine also showed a clear effect on the GSTP1 gene ,which proved its role in maintaining the integrity of the sperms and its germ cells as an antioxidant gene<sup>[21,22,23]</sup>. The gene PTGR2 was also affected by the both drugs and showed several breakdowns in DNA samples of the epididymis of this gene plays an important role responsible for producing an enzyme PTGR2 is an enzyme involved in the metabolism of prostaglandins. The encoded protein catalyzes the NADPH-dependent conversion of 15-keto-prostaglandin E2 to 15-keto-13,14-dihydro-prostaglandin E2. This protein may also be involved in regulating activation of the peroxisome

proliferator-activated receptor. Alternative splicing results in multiple transcript variants<sup>[24,25]</sup>.

## Conclusion

We conclude from this study that the two drugs have a hereditary effect on these genes and the long-term use may cause fertility problems in males in further.

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