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## Isolation and identification of multiple drug resistant *Neisseria gonorrhoeae* from urethritis patients.

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### Abstract:

Gonorrhea caused by *Neisseria gonorrhoeae* is one of the most common STIs and is a global health problem because of emerging antibiotic resistant strains that compromise the effectiveness of treatment. This study was carried out to determine the susceptibility and resistance of the most effective antibiotic to *Neisseria gonorrhoeae* isolated from urethritis patients. Thirty five patients with urethritis were included in this study. Bacterial isolates were identified by standard procedures resistance patterns were detected by disk diffusion test (DDT) and minimum inhibitory concentration (MIC). The minimum inhibitory concentration of all antibiotic used in this study were determined by an agar dilution method as complementary test to the previous sensitivity test to verify the rate of resistance. The results showed that *N. gonorrhoeae* were completely resistance to cephalexin, gentamicin and trimethoprim with high rate of resistance to rifampicin, doxycycline (84.21%), azithromycin (73.68%) and amikacine (68.42%) and moderate to low rate of resistance to ciprofloxacin (52.63%) and cefotaxime (42.1%). Result showed that *N. gonorrhoeae* isolates had high rate of sensitivity to levofloxacin and imipenem (94.73%) and 4 out of 19 isolate showed resistances to ceftriaxone with sensitivity rate 78.95%. The present study made to prove that there was only limited number of drugs effective against *N. gonorrhoeae*, and most probably in near future, if irrational use of antibiotic is not stopped the rate of resistance will increase.

**Keywords:** *Neisseria gonorrhoeae*, sexually transmitted diseases (STDs), disk diffusion test (DDT), minimum inhibitory concentration (MIC).

## عزل وتشخيص البكتريا البنية *Neisseria gonorrhoeae* المقاومة للمضادات الحيوية

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### الخلاصة

يعتبر مرض السيلان الناجم عن الإصابة ببكتريا النيسيرية البنية (*N.gonorrhoeae*) هو واحد من الامراض المنقولة بالاتصال الجنسي الاكثر شيوعا و يمثل مشكلة صحية عالمية بسبب ظهور سلالات مقاومة للمضادات الحيوية التي تؤثر سلبا على فعالية المضادات الحياتية. أجريت هذه الدراسة لتحديد قابلية ومقاومة المضاد الحيوي الاكثر فعالية لنيسيرية السيلان المعزولة من مرضى التهاب الاحليل . أدرج خمسة و ثلاثين من المرضى الذين يعانون من التهاب الاحليل في هذه الدراسة. وقد تم تحديد العزلات البكتيرية ثم تم الكشف عن مقاومة هذه البكتريا للمضادات الحياتية عن طريق اختبار انتشار القرص ( DDT ) والحد الأدنى من تركيز المثبط الأدنى (MIC). تم تحديد الحد الأدنى للتركيز المثبطة لجميع المضادات الحيوية المستخدمة في هذه الدراسة من خلال وسيلة نشر أجار كما تم استعمال اختبار الحد الأدنى من تركيز المثبط للتحقق من معدل المقاومة. وقد تميزت النيسيرية البنية كمقاومة إذا كان MIC اكبر من تركيز المثبط الأدنى نقطة توقف في حين سيكون عرضة اذا كان أقل من نقطة توقف. أظهرت هذه الدراسة ازدياد معدل عزلات هذه البكتريا المقاومة للمضادات التقليدية كما تم تسجيل حالات مقاومة للادوية الحديثة.

**الكلمات المفتاحية :** بكتريا النيسيرية البنية، الامراض المنقولة جنسيا، الكشف بواسطة اختبار الهلام، الحد الأدنى من تركيز المثبط.

## Introductions

Among etiological agents of treatable sexually transmitted diseases (STDs), *Neisseria gonorrhoeae* is considered to be most important because of emerging antibiotic resistant strains that compromise the effectiveness of treatment [1]. However, treatment options for gonorrhea are diminishing as *N. gonorrhoeae* have developed resistance to several antimicrobial drugs such as sulfonamides, penicillin, tetracyclines and quinolones. Antimicrobial resistance (AMR) surveillance of *N. gonorrhoeae* helps establish and maintain the efficacy of standard treatment regimens [2]. Gonorrhea caused by *N. gonorrhoeae* is one of the most common sexually transmitted infections and is a global health problem [3]. Undetected and untreated infections can lead to complications like pelvic inflammatory disease, ectopic pregnancy, tubal factor infertility, adverse pregnancy outcomes in females, testicular, prostate infections and infertility in males. Also, asymptomatic patients, unaware of their infection, may serve as a reservoir of infection to their partners [4]. A major contributing factor to the continued spread of gonococcal infections is the remarkable ability of *N. gonorrhoeae* to acquire resistance to antibiotics [5]. Over the last two decades; *N. gonorrhoeae* strains were developed high level of resistance against several antimicrobial agents in different countries

[6]. The emergence of strains resistant to extended-spectrum cephalosporins, the antibiotics used as the first line treatment for uncomplicated gonococcal infections, is a serious concern worldwide as it may pose a problem in the management of gonorrhea [7]. The emergence and spread of resistance in *N. gonorrhoeae* has occurred mainly by the acquisition of new DNA via conjugation and transformation and determinants may be located on the chromosome or on extra chromosomal elements [8]. In contrast to plasmid-mediated resistance, chromosomal resistance often occurs incrementally; Chromosomal alterations can affect permeability and simultaneously reduce susceptibility to penicillin, tetracycline and macrolides [9]. For the penicillin and quinolones there are multiple resistance mechanisms, involving porins (uptake), efflux pumps and the cellular targets of these antibiotics [10]. High-level resistance to spectinomycin and aminoglycosides probably occurs by point mutations affecting their ribosomal target sites [3].

## Materials and Methods

From April 2013 to March 2014, thirty five patients with urethritis were included in this study. All patients attended private clinical laboratories in Abu-Ghraib province and outpatient visitor to dermatology and venereology in Abu-Ghraib hospital, Baghdad. Urethral discharge from male was

collected by using cotton swab and specific small swabs were inserted into the urethral canal if there is no discharge. Female samples were taken from vagina, endocervix and urethra; specimen was inoculated immediately into Amies transport medium and transferred to the laboratory.

Samples were culture immediately on chocolate agar under 8 %CO<sub>2</sub> condition at 37°C for 24 hs. The isolated colony identified by gram stain and biochemical tests included oxidase test and sugar (glucose) fermentation test. Resistance patterns of *N. gonorrhoea* to various antibiotics were determined by disk diffusion test (DDT) and minimum inhibitory concentration (MIC). The following antimicrobial discs were tested cephalexin (30µg), cefotaxime (30µg), ceftriaxone (30µg), amikacine (30µg), gentamycin (10µg), imipenem (10µg), tetracycline (30µg), co-trimoxazole (25µg), ciprofloxacin (5µg), levofloxacin (10µg), azithromycin (30µg), rifampicin (30µg), when the incubation was complete, the diameter of the inhibition zone around the disks was measured and compared with the break points of clinical laboratory institute (CLSI) [11]. The minimum inhibitory concentration was performed by a standard agar dilution method and has been applied for determination the lowest antibiotics concentration that inhibits growth of *N. gonorrhoeae*. Stock solutions of each

antibiotic at concentrations of 10 mg/ml, 1mg/ml, and 0.1 mg/ml; then two fold dilutions from 0.5-512µg/ml for all antibiotics were prepared. Muller Hinton agar medium was prepared, sterilized by autoclaving, after cooling, 25 ml were added to each antibiotic container; the content mixed well and poured into petri dishes. The inoculum density was adjusted by using 0.5 McFarland standard tubes ( $1.5 \times 10^8$  colony-forming units/ml) and then 20 µl of each inoculum were spotted on the agar surface of Muller Hinton agar medium and incubated at 37°C for 24 h under CO<sub>2</sub> conditions [12].

## Results

This study included 35 patients with profuse, scanty, moderate watery mucopurulent or purulent urethral or vaginal discharge they were 25 (71.42%) males and 10 (28.57%) females, the age ranged from 18-30 years. Nineteen strains of *N. gonorrhoeae* were identified (out of 35 patients samples) by using chocolate agar medium, oxidase test and glucose fermentation test. By the disc-diffusion methods, all isolates (n, 19) were tested for their sensitivity to cephalexin, cefotaxime, ceftriaxone, amikacine, gentamicin, imipenem, doxycycline, trimethoprim, ciprofloxacin, levofloxacin, azithromycin and rifampicin. The result showed that *N. gonorrhoeae* isolates were completely resistant to cephalexin, gentamicin and trimethoprim, high rate of

resistance to rifampicin and doxycycline (84.21%), azithromycin(73.68%) and amikacine (68.42 %) with moderate to low rate of resistance to ciprofloxacin (52.63%) and cefotaxime (42.1%). The results also showed that the isolates had high rate of sensitivity to levofloxacin and imipenem (94.73%). Furthermore, it was found that four isolates out of nineteen showed resistances to ceftriaxone (78.95%) (Table 1).

In current study, all *N. gonorrhoeae* isolates (n,19) were resistance to more than three patterns of antibiotic which suggest that strains were multiple drug resistance (MDR), a novel four ceftriaxone resistant isolates were identified, and one strain resistant to levofloxacin and imipenem (Table 1).

#### **Minimum inhibitory concentration of *N. gonorrhoeae* isolates**

The MIC of all antibiotic used in this study were determined by an agar dilution method as complementary test to the previous sensitivity test to verify the rate of resistance. *N. gonorrhoeae* was characterized as resistant if breakpoint was greater than MIC defined by clinical laboratory institute (CLSI) while it will be susceptible if break point was less than the MIC (Table 2).

Results of MIC revealed that highly resistant to cephalexin (MIC 512 µg/ml) and five isolates with MIC 256 µg/ml while remain isolates with MIC 128µg/ml; for

trimethoprim and gentamicin. MIC determination showed that all the isolates were highly resistant to the antibiotic (MIC, 512µg/ml). When cefotaxime is considered, out of the eight resistant isolates two isolates were highly resistant (MIC, 512 µg/ml), one isolate with MIC 256 µg/ml and remain five isolates with MIC 128µg/ml. One isolate out of the four resistant to ceftriaxone showed an MIC32 µg/ml while the remaining three had MIC 16 µg/ml. For amikacine, nine isolates had MIC 512 µg/ml,one isolate with MIC 256 µg/ml, and three isolates with MIC 128 µg/ml. Only one isolate of *N. gonorrhoeae* exhibiting highly resistance to imipenem (MIC, 512 µg/ml). MIC of doxycycline showed that 13 out of 16 isolates resistance to this drug had MIC 512 µg/ml and remain (3 isolates) had MIC 256 µg/ml respectively. Determination of MIC of ciprofloxacin revealed that all 10 isolates resistances to ciprofloxacin had MIC 512 µg/ml. One levofloxacin resistant isolate with MIC 128 µg/ml. The MIC results of fourteen resistant isolates to azithromycin were eleven isolates had MIC 512 µg/ml; one isolate with MIC 256 µg/ml and two with MIC 128 µg/ml respectively. Out of sixteen rifampin resistant isolates fifteen isolates with MIC 512 µg/ml and only one isolate with MIC 128 µg/ml.

#### **Discussion**

Among the etiological agents of treatable STDs, *N. gonorrhoeae* stands out because of



the extent to which antibiotic resistance compromises the effectiveness of individual case management and resistance also affects control programs [13]. *N. gonorrhoeae* causes infections principally the urethra in men and the endocervix in women, although it may also infect extra genital mucosal sites. Genital infection in men usually presents with a urethral discharge, but silent infections are common in women case [14]. In the present study, antimicrobial resistance and multiple drug resistance were found in all *N. gonorrhoeae* isolates; and 100% were resistant to more than three antimicrobial agents. This finding was also observed by Tapsall *et al.* [4]. They reported that many *N. gonorrhoeae* isolates were multidrug- and extensively drug-resistant. Concurrent resistance of *N. gonorrhoeae* to a large number of antimicrobials was noted in Iraq after 1994 by Azhar A.F. impending an alarming signal [15].

The current study showed that all *N. gonorrhoeae* isolates presented similar resistance profile and they were fully resistant to cephalexin, gentamycin and trimethoprim. This result was compatible with study of Jo-anne *et al.* [16]. In a marked consistency with the results of Unemo *et al.* [17]. The resistance patterns of *N. gonorrhoeae* to several antibiotics, rifampicin (89.4%), doxycycline (84.2%), azithromycin (73.6%) and amikacine (68.4%). Fluoroquinolones showed a broad

spectrum antimicrobial activity including activity against *N. gonorrhoeae* [18]. These drugs have been demonstrated to be effective in treating gonococcal urethritis which may explain the extensive use of these drugs for treating gonococcal infections and the emerging resistance. Moreover, the use fluoroquinolones to treat other community infections might also complicated the situation [18]. Our study revealed that 10 isolates (52.6%) were highly resistant to ciprofloxacin and had MIC 512 µg/ml and this observation was in line with the findings in most previous studies [19,20]. Regarding levofloxacin, a newly introduced therapeutic agent in Iraq, 94.7 % of the isolates were sensitive to levofloxacin; the high sensitivity to levofloxacin might be attributed to the recent introduction of this agent for the treatment of gonococcal. *N. gonorrhoeae* developed resistance to multiple classes of β-lactams antimicrobial drug such as ceftriaxone, cefotaxime and imipenem and the emergence of new generation β-lactams resistant *N. gonorrhoeae* threatens effective disease control [21]. There were three sporadic reports of two ceftriaxone-resistant strains of *N. gonorrhoeae* in the past 5 years: H041 and F89. H041 was identified in only a single case involving a female sex worker in Japan in 2009 [22,23]. F89 was initially reported in France in 2010 in a man who had sex with men [22]. Subsequently, resistant strain was detected in Spain in two

homosexual men [23]. In Australia, ceftriaxone resistant *N. gonorrhoeae* was identified and called A8806 strain [24]. In this study, we identified four novel ceftriaxone resistant isolates, one of these isolate had an MIC 32 µg/ml and the remaining three isolates with MIC 16 µg/ml. The emergence of ceftriax resistant of *N. gonorrhoeae* raises concerns for controlling gonorrhoea and it was stated that it is due to the availability of ceftriaxone and their abuse by general public [25]. The result of the current study revealed high sensitivity to imipenem (94.7 %) and

this might be explained by the high cost of the agent which limits its use.

### Conclusion

Our study proved that there was only limited number of drugs effective against *N. gonorrhoeae* and most probably in near future, if irrational use of antibiotic is not stopped the rate of resistance will increase. Susceptibility testing should be carried out on all clinical isolates, and the empirical antibiotic treatment changed accordingly.

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**Table 1: Antibiotic sensitivity of different isolates of *Neisseria gonorrhoea***

Isolate N=19	Cephalexin	Cefotaxime	ceftriaxone	Amikacin	gentamicin	imipenem	Doxycycline	Trimethoprim	ciprofloxacin	levofloxacin	azithromycin	rifampicin
1	R	S	S	R	R	S	R	R	R	S	R	R
2	R	S	S	R	R	S	R	R	R	S	R	R
3	R	S	S	S	R	S	R	R	S	R	R	R
4	R	S	S	R	R	S	S	R	R	S	S	R
6	R	R	R	S	R	S	R	R	R	S	R	S
18	R	R	S	S	R	S	R	R	R	S	R	R
19	R	S	R	R	R	S	R	R	R	S	R	S
13	R	S	S	R	R	S	R	R	S	S	R	R
14	R	S	S	R	R	S	S	R	S	S	R	R
15	R	S	R	R	R	S	R	R	S	S	R	R
20	R	S	S	R	R	S	R	R	S	S	R	R
21	R	S	S	R	R	S	S	R	S	S	R	R
22	R	R	S	R	R	S	R	R	R	S	R	R
26	R	R	S	S	R	S	R	R	R	S	R	S
29	R	R	S	R	R	S	R	R	S	S	S	R
30	R	R	R	R	R	S	R	R	R	S	S	R
31	R	R	S	S	R	S	R	R	R	S	S	R
32	R	S	S	R	R	R	R	R	S	S	S	R
35	R	R	S	S	R	S	R	R	S	S	R	R

R: Resistant

S: Sensitive

Table 2: serial two fold antibiotics dilution to determine the MIC using agar dilution method.

Antimicrobial concentration Mg/ml	Volume of antibiotic Stock solution (µl)	Final concentration When adding 25 ml agar
0.1	125	0.5
0.1	250	1
1	50	2
1	100	4
1	200	8
10	40	16
10	80	32
10	160	64
10	320	128
10	640	256
10	1280	512

## Study of the function of Thyroid gland in $\beta$ - thalassemia major male patients in Kirkuk city.

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### Abstract

Thalassemia has been classified by the world health organization as a major public health problem. It occurs throughout the world and regarded as one of the major health problems in endemic regions as Middle East, Mediterranean countries, Asia and North Africa. Endocrine complications in Thalassaemia Major Patients with multitransfused Thalassaemia Major patients may develop severe endocrine complications. Due to multiple transfusions is the main cause of such complications, Iron accumulates in many tissues such as liver, heart and endocrine glands. The study aims to study the function of Thyroid gland in  $\beta$ - thalassemia Major male patients in Kirkuk city. The study was conducted  $\beta$ -thalassaemia major patients whom attended the thalassaemia center in Azadi Teaching hospital in Kirkuk Governorate from September 2015 to the end of January 2016. A total of 105 male subjects were participated in the study, (30 normal healthy subjects and 75 thalassaemic patients). Body weight height was measured. About, five ml of venous blood were obtained from all normal subjects and patients. One ml of blood sample was collected for measurement of packet cell volume (PCV) and heamoglobin (Hb). The remaining four ml of blood sample were used for serum separation. Serum used for measurement of serum thyroid stimulating hormones (TSH), T3 and T4.

**Results of study showed** a high significant decrease in body weight and height of male thalassemic patients as compare with male counterpart of control subjects of same age. Also, there was a highly significant reduction in the concentration of heamoglobin and PCV value in thalassaemic male patients as compared with control male subjects. There is no significant increase in serum Thyroid stimulating hormone (TSH) concentration in male thalassemic patients as compare with male control subjects. However, there is highly significant reduction in serum T4 and T3 concentrations in male thalassemic patients as compare with male control subjects.

**Key words:** PCV, Hb, Weight, height, thyroid gland, T3, T4, male, Thalassemia, Kirkuk, Iraq.

## دراسة وظيفة الغدة الدرقية لدى الذكور من مرضى التلاسيميا الكبرى نوع بي في كركوك

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### الخلاصة

تم تصنيف مرض فقر الدم التلاسيميا من قبل منظمة الصحة العالمية كمشكلة صحية رئيسية عامة. ويوجد مرض التلاسيميا في جميع أنحاء العالم، ويعتبر واحد من المشكلات الصحية الرئيسية في المناطق الموبوءة كالشرق الأوسط وشمال أفريقيا ودول البحر الأبيض المتوسط وآسيا.

ان مضاعفات الغدد الصم لدى مرضى التلاسيميا المصاحب مع تعدد في نقل الدم في التلاسيميا الكبرى مضاعفات شديدة و بسبب النقل المتعدد كونه السبب الرئيسي لمثل هذه المضاعفات، يتراكم الحديد الزائد في العديد من الأنسجة مثل الكبد والقلب والغدد الصماء الغدد. تهدف الدراسة الحالية دراسة وظيفة الغدة الدرقية لدى مرضى التلاسيميا الكبرى في مدينة كركوك. **المرضى والطرق:** أجريت دراسة مقطعية على مرضى التلاسيميا الكبرى-β الذين حضروا الى مركز التلاسيميا في مستشفى ازادي التعليمي في محافظة كركوك من أيلول 2015 إلى نهاية كانون الثاني 2016. وشارك ما مجموعه 105 طفل من الذكور في هذه الدراسة، (30 طفل سليم و 75 مريض). وقد تم قياس وزن و طول الجسم. تم الحصول على خمسة مل من الدم الوريدي من جميع المشاركين. وقد تم جمع واحد مل من عينة من الدم لقياس حجم الخلايا المتراسة و خضاب الدم (الهيموغلوبين). وقد تم جمع أربعة مل المتبقية من عينة الدم في أنبوب عادي، لفصل مصل الدم. استخدم المصل لقياس هرمون منشط الغدة الدرقية ((TSH، T3 و T4). **النتائج:** كان هناك انخفاض معنوي في وزن و طول الجسم لدى مرضى التلاسيميا الذكور مقارنة مع نظيرهم من الذكور في مجموعة الضابطة من نفس الفئة العمرية. أيضا، كان هناك انخفاض كبير جدا في تركيز خضاب الدم وقيمة حجم الخلايا المتراسة في مرضى التلاسيميا الذكور مقارنة مع نظيرهم من الذكور في مجموعة الضابطة من نفس الفئة العمرية. كذلك هناك زيادة غير معنوية في تركيز هرمون محفز الغدة الدرقية في مصل الدم (TSH) في مرضى التلاسيميا الذكور مقارنة مع نظيرهم من الذكور في مجموعة الضابطة. ومع ذلك، هناك انخفاض معنوي في تركيز هرمون الثايروكسين (T4) في مصل الدم وتركيز ثلاثي ايودييد الثايرونين ( T3 ) في مرضى التلاسيميا الذكور مقارنة مع الاطفال في المجموعة الضابطة.

### الكلمات المفتاحية:-

حجم الخلايا المتراسة، خضاب الدم ، وزن الجسم، هورمونات الغدة الدرقية، الأطفال الذكور، كركوك ، العراق.

## Introduction

Thalassemias are a group of hereditary anemias which occur as a result of genetic disorders that affect the synthesis of normal hemoglobin (Hb), in which a reduced rate of synthesis of one or more of the globin chains leads to defective Hb production, and damage to the red cells [1,2]. Thalassemia occurs throughout the world and regarded as one of the major health problems in endemic regions as the, Middle East, Mediterranean countries, Asia and North Africa [3,4]. The two main types of thalassemia are called alpha and beta thalassemia, Individuals with alpha thalassemia do not produce enough alpha globin. Those with beta thalassemia do not produce enough beta globin. There are a number of different forms of alpha and beta thalassemias, with symptoms ranging from mild to severe [3,4].

Beta thalassemia in turn is classified into two categories: beta plus where the beta –chain production is reduced and beta zero, where there is no  $\beta$ -chain production found [5,6]. Beta-thalassemia probably is the most common single gene disorder causing a major genetic health problem in the

world. There are at least two hundred and forty million carriers for hemoglobinopathies throughout the world [3]. Thalassemia is a congenital hemolytic anemia caused by partial or complete deficiency of globulin protein chain synthesis resulting in microlytic anemia of varying degrees [6]. Due to multiple blood transfusions is the main cause of such complications. Iron accumulates in many tissues such as liver, heart and endocrine glands [7-9]. Thyroid gland hormones are responsible for raising the level of activity in the systems essential for exercise performance [10-12].

The **aim** of study is determining the effect of iron overload in Thalassemic patients on thyroid gland function.

## Patients and Methods

The study was conducted  $\beta$ -thalassaemia major patients whom attended the thalassaemia center in Azadi Teaching hospital in Kirkuk Governorate from September 2015 to the end of January 2016. One hundred and five male subjects were participated in the study. Seventy five  $\beta$ - thalassemia major male patients aged 8 to 16 years



and thirty male subjects apparently healthy, with no family history of hereditary blood diseases attendants to out-patient pediatric clinic, who were assessed by a pediatrician, all control healthy subjects aged 8 to 16 years. Body weight was measured and body height was measured in centimeter (cm).

Five ml of venous blood were obtained from all patients in this study by antecubital venipuncture, between 8.00 am and 10.00 am and distributed in the following manner; 1 ml of blood sample was collected into ethylene diaminetetracetic acid (EDTA) tube, with gentle shaking for proper mixing with anticoagulant, to be use for packet cell volume (PCV) and heamoglobin (Hb) measurements.

The remaining 4 ml of blood sample was collected into a plain tube, then incubate for 30 min and then centrifuged for serum separation so that the samples for males were subdivided and labeled for measurement serum Thyroid stimulating hormone (TSH), T3 and T4 for females were measured [13,14].

All data were presented as a mean and standard deviation (SD), unpaired student T test was used to compare between the mean of variables.

Probability value less than  $P \leq 0.05$  and 0.01 levels were considered to be a significant deference.

## Results

There was a significant decrease ( $p < 0.01$ ) in body weight of thalassaemic male patients ( $37.42 \pm 7.34$  kg) as compared with control male subjects ( $58.62 \pm 9.32$  kg) as shown in table (1). Also, there was significant reduction ( $p < 0.01$ ) in body height of thalassaemic male patients ( $132.64 \pm 7.4$  cm) as compared with control male subjects ( $151.24 \pm 9.5$  cm) as shown in table (1).

### Heamoglobin and PCV in male subjects:

There was a highly significant reduction ( $p < 0.01$ ) in the concentration of heamoglobin in thalassaemic patients ( $8.23 \pm 0.276$  gm/dl) as compared with control male subjects ( $13.965 \pm 0.97$  gm/dl) as shown in table 2. Also, there was a highly significant reduction ( $p < 0.01$ ) in the PCV value of thalassaemic male patients ( $29.98 \pm 2.76$  L/L) as compared with control subjects ( $40.32 \pm 2.37$  L/L) (table 2). There was a highly significant increase ( $p < 0.01$ ) in the serum ferritin concentration of thalassaemic male patients ( $3558.43 \pm$

298.4 ng/ml) as compared with control subjects ( $59.87 \pm 8.13$  ng/ml) (table 2).

### Thyroid hormones

No significant increase in serum TSH concentration in male thalassaemic patients ( $4.412 \pm 0.21$   $\mu$ U/ml) as compare with male control subjects ( $3.22 \pm 1.23$ ). There is highly significant reduction in serum T4 concentration ( $P \leq 0.01$ ) in male thalassaemic patients ( $0.683 \pm 0.213$   $\mu$ g/dl) as compare with male control subjects ( $6.542 \pm 0.62$   $\mu$ g/dl). Moreover, there is significant reduction in serum T3 concentration ( $P \leq 0.05$ ) of male thalassaemic patients ( $1.067 \pm 0.273$  ng/ml) as compare with male control subjects ( $1.994 \pm 0.39$  ng/ml).

### Discussion

In present study, significant reduction in body weight and length of male body of thalassaemic patients as compared with control subjects. Possible reasons are persistent anaemia due to inadequate transfusion and complications of iron overload in addition to other factors [5,6]. Similar finding was reported by previous study [7]. In present study, there was a highly significant increase in the serum ferritin concentration of

thalassaemic male patients as compared with control subjects. Similar results was reported in previous studies [11,12]. Key contributing factors to stunted growth in patients with thalassaemia major (TM) may include chronic anaemia, transfusional iron overload, hypersplenism, and chelation toxicity [4,12]. **Thyroid dysfunction** in  $\beta$ -thalassaemic patients has been reported in various prevalence, ranging from a low prevalence of 0-12% [13,14]. The abnormal thyroid function found in the present patients was the slight elevation of TSH, which was consistent with the diagnosis of compensated hypothyroidism, the most common thyroid dysfunction in all previous reports [16]. Impaired thyroid function is frequent among present thalassaemia major patients and this necessitates regular follow up and early commencement of chelation therapy to prevent such complication [17].

In present study, there is significant reduction in serum T3 and T4 concentrations in thalassaemic patients as compare with control subjects. Previous study was done in Irbil –Iraq, it was found that the mean levels of thyroid hormones; T3 and T4 were significantly lower ( $P < 0.001$ ) among

thalassaemia patients, while the mean TSH level was higher compared to the control group [18,19].

Other factors in addition to Iron overload, like hypoxia due to persistent anemia and perfusion defect, also contribute to the derangement. Hypothalamic pituitary axis, thyroid, para-thyroid, adrenal, pancreas, gonads, all showed hypoactivity [15,16].

Previous study found that the mean T4 of cases was significantly lower than that of controls. The mean TSH level was significantly higher ( $p < 0.01$ ) in cases as compared to controls [20]. Iron overload causes deposition of iron in the thyroid gland, with consequent fibrosis of the glandular parenchyma, and progressive thyroid dysfunction going through different degrees of severity up to overt hypothyroidism [21].

Thyroid dysfunction is known to occur frequently in thalassaemia major, but its prevalence and severity varies in different cohorts, and long-term natural history is poorly understood [20,22].

A wide spectrum of pathogenic mechanisms is involved. Tissue chronic hypoxia and iron overload have a direct

toxic effect on the thyroid gland. High concentrations of labile plasma iron and labile cell iron which are considered responsible in the formation of free radicals and the production of reactive oxygen species (ROS) may lead to cell and organ damage [23,24]. From the present study and previous reinforce the importance of the regular follow up of patients with  $\beta$ -thalassaemia major and thalassaemia intermedia for early detection and management of associated complications. In this way, the future prevalence of endocrine abnormalities can be lessened [19,20].

Subclinical hypothyroidism may be associated with male and female gonad dysfunction and interferes with their reproductive ability. The awareness of the thyroid status in any infertile couple is crucial, because of its significant, frequent and often reversible or preventable effect on infertility [25,26]. Most complications are caused by increased iron sedimentation in tissues like heart, endocrine glands and these results in heart failure, arrhythmia, hypothyroidism and diabetes mellitus. Most of these complications occur slowly and appear in the second decade of a patient's life [27,28].

In the present study, It was found that highly significant reduction in the concentration of hemoglobin and packed cell volume (PCV) in thalassaemic male patients as compared with control male subjects. PCV values found in previous study that in Mosul city was the similar to the PCV result of the present study [29]. Also, it was found that Thalassaemic patients had a low PCV and low hemoglobin concentration as compared with their counterpart of same age and gender [30,32].

Present study **conclude** the Thalassaemic male patients had a lower T3 and T4 as compare with normal healthy male control subjects of same age. However, there is a non significant increase in serum TSH concentration as compare with male control subjects. Present study **recommends** assessment of pituitary hormone especially growth hormone by carry out hormonal test for both genders as a routine follow up of thalassaemic patients.

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**Table 1** The mean & standard deviation (SD) of age, body weight, and height of patients and controls.

Parameters	Control (30)	Patients (75)	P value
Age (years)	14.91 $\pm$ 2.11	13.84 $\pm$ 1.98	NS
Body weight (Kg)	58.62 $\pm$ 9.32	37.42 $\pm$ 7.34	0.001
Height (Cm)	151.24 $\pm$ 9.5	132.64 $\pm$ 7.4	0.001

**Table 2** The mean and standard deviation of hemoglobin, PCV and serum ferritin of male thalassaemic patients and control male subjects:

Parameters	Thalassaemic males (n=75)	Control males (n=30)	P. value
Hb (g/dl)	8.23 ± 0.276	13.965 ± 0.97	0.01
PCV (L/L)	29.98 ± 2.76	40.32 ± 2.37	0.01
Ferritin (ng/ml)	59.87 ± 8.13	3558.43 ± 298.4	0.01

**Table 3** Show the mean & standard deviation (SD) of serum TSH, T3 & T4 hormones concentrations in patients and control subjects.

Parameters	Control	Patients	P value
TSH (µU/ml)	3.22 ± 1.23	4.412 ± 0.21	NS
T4 (µg/dl)	6.542 ± 0.62	0.683 ± 0.213	0.01
T3 (ng/ml)	1.994 ± 0.39	1.067 ± 0.273	0.05



## FBG as a Medical Thermometer

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### **Abstract**

An FBG thermometer was designed for medical applications in human body. In this work, a short period Fiber Bragg Grating (FBG) temperature sensor system were demonstrated and investigated according to the measurement of the Bragg wavelength shift. This FBG designed by using OptiGrating 4.2 software and various temperature where applied according to human body temperature ranging from hypothermia (35 °C) to hyperthermia (42 °C) and taking into consideration the normal human body temperature as (37 °C) which is taken as a reference temperature in the simulation design. There was shifting in Bragg wavelength for each temperature degree. The applied temperature of FBG was so small so the strain effect is neglected.

**Keywords:** *Fiber Bragg grating; temperature sensor; medical thermometer.*

## كمقياس حرارة طبي FBG

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### الخلاصة:

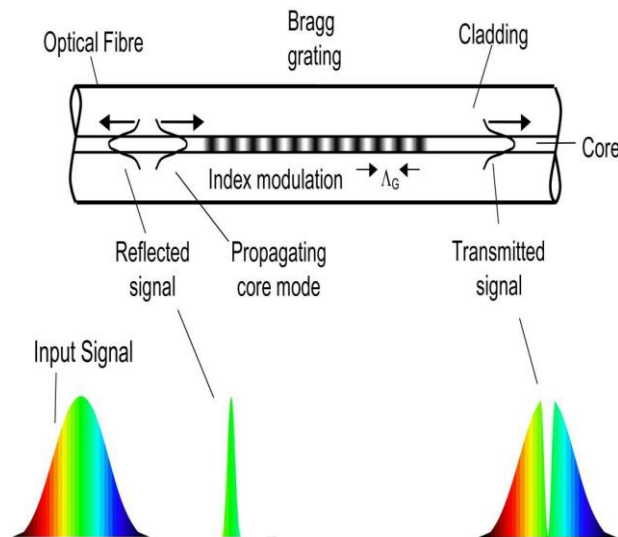
في هذا البحث تم تصميم محرار يستخدم للأغراض الطبية باستخدام ليف محرز براغ. حيث تم بناء مجس حراري باستخدام محرز براغ للاليف البصرية ذي الدورة القصيرة بالاعتماد على الزحزحة في الطول الموجي لبراغ باستخدام برنامج النمذجة (OptiGrating 4.2) بتسليط درجات حرارة مختلفة وضمن حدود حرارة جسم الانسان وهي 35 درجة مئوية كحد ادنى و42 درجة مئوية كحد اقصى للحرارة التي يصلها الجسم وتم اخذ درجة الحرارة 37 درجة مئوية كدرجة حرارة مصدر للقياس، لوحظ ان هناك زحزحه لمقدار الطول الموجي لمحزر براغ بتغير درجة الحرارة مع اهمال تأثير الاستطالة بسبب انخفاض درجات الحرارة المسلطة.

## Introduction

Human body temperature comprises temperature of the core and the shell. The core temperature refers to the temperature of the abdominal, thoracic and cranial cavities, and the shell temperature refers to the temperature of the skin, subcutaneous tissue and muscles. The brain is responsible for control the core temperature, while the skin, subcutaneous tissue and muscles affected on the shell temperature. [1] The beginning of optical fiber application in medicine was done using the fiber as illumination environment of the fiber optic endoscopy [2]. Fiber optical sensor is used to monitor variable chemical and physical parameters related to medical field. These sensors are commonly grouped in two classes: in the first class the sensing element represented by the optical fiber itself this called, intrinsic sensors, in the second class which called the extrinsic sensors the optical fiber act as the medium for conveying the light whose characteristics (e.g., intensity, frequency, phase) are modulated by the measurand, in this class the basic component of fiber optical sensor is spreading away from the sensing element, in order to develop small size sensor hybrid solutions. [3], [4].

Sensing There are many inveterate advantages that make them attractive as sensing technologies for wide range of industrial and medical sensing applications. They are typically small in size, passive, immune to electromagnetic interference,

resistant to cruel environments and have a capability to perform distributed sensing [5]. Although developed initially for the telecommunications industry in the late 1990's, fiber Bragg gratings (FBGs) are increasingly being used in sensing applications and are enjoying widespread acceptance and use. The FBG is an optical filtering device that reflects light of a specific wavelength and is present within the optical fiber core waveguide. The wavelength of light that is reflected depends on the spacing of a periodic variation or modulation of the refractive index that is present within the fiber core. This grating structure acts as a band-rejection optical filter passing all wavelengths of light that are not in resonance with it and reflecting wavelengths that satisfy the Bragg condition of the core index modulation. A diagram of an FBG is shown in Figure (1) [6]. In the last decade, fiber Bragg gratings (FBGs) have shown a great potential for applications in the field of biomechanics and rehabilitation engineering due to their prominent advantages such as their small size, biocompatibility, chemical inertness, immunity to electromagnetic interference (EMI), High sensitivity, light weight and multiplexing capability. These characteristics make FBGs suitable for human body uses that adapt to the sensor material so that they can be used for in vivo measurement and can be left for long-term monitoring [7]



**Figure1.** Schematic diagram of an FBG having an index modulation of spacing inside a single-mode optical fiber [5]

**FBG TEMPERATURE SENSOR**

There are many material properties have temperature reliance. In order to utilize temperature effects on measurement is required. Examples of such temperature reliance are density, electrical conductivity, refractive index, rigidity and diffusion. Temperature measurement also plays an important role in health monitoring of electric circuits. There are different types of fiber optic temperature sensors can be used. The most common fiber optic temperature sensors are:

- Fiber Bragg gratings, where the temperature dependence of distributed optical reflection is used.
- Extrinsic interferometric optical structures, which show a temperature dependent behavior.
- Raman scattering distributed temperature sensors, which use the temperature dependence of inelastic scattering on optical phonons [8].

In this paper, we will focus on FBG temperature sensor when broadband light passes through the FBG, the narrowband spectral component at the Bragg wavelength is reflected by the FBG. The basic principle of FBG’s is to measure the shift of reflected Bragg wavelength ( $\lambda_B$ ), which is related to the effective refraction index ( $n_{eff}$ ) and the periodicity ( $\Lambda$ ) of the index variation of the grating area in fiber core. The Bragg wavelength of FBG is described as: [9].

$$\lambda = 2n_{eff} \Lambda \dots\dots (1)$$

Any disturbance that can change effective index ( $n_{eff}$ ) and periodicity ( $\Lambda$ ) will result in a shift in Bragg wavelength.

The temperature sensing of Bragg grating occurs principally through the temperature effect on the index of refraction and to a lesser extent through the expansion coefficient. It is remarkable that temperature sensitivity can be enhanced by suitable bonding to other materials. The maximum operating temperature may be around (500 °C); however this may depend on the fabrication condition of the Bragg grating [10].

The wavelength sensitivity of Bragg grating is governed by the elastic-optic and thermo-optic properties. From equation (1), a theoretical analysis shows that if there is a short period grating with period  $\Lambda$  under influence change  $\Delta T$  :

$$\frac{d\lambda_B}{dT} = 2[\Lambda \frac{d\lambda_B}{dn_{eff}} \frac{dn_{eff}}{dT} + n_{eff} \frac{d\lambda_B}{d\Lambda} \frac{d\Lambda}{dT}] \dots\dots (2)$$

From the above equation, it can be seen that the contribution to the thermal induced shift is a function of change in refractive index with temperature  $\frac{dn_{eff}}{dT}$  while the waveguide effect is dependent on the variation in grating period with temperature. From equation 2, we get:

$$\Delta\lambda_B = 2\left[\frac{1}{n_{eff}} \frac{d\lambda_B}{dn_{eff}} \frac{dn_{eff}}{dT} + \Lambda \frac{d\lambda_B}{d\Lambda} \frac{1}{a} \frac{da}{dT}\right] \dots (3)$$

Where;  $a$  core radius, and  $\frac{d\Lambda}{dT} = \frac{da}{dT}$

The shift in Bragg wave grating center wavelength due to temperature can be given by:

$$\Delta\lambda_B = \lambda_B (\alpha_\Lambda + \alpha_n) \Delta T \dots (4)$$

Where,  $\alpha_\Lambda = \left(\frac{1}{\Lambda}\right) \left(\frac{\partial\Lambda}{\partial T}\right)$ .

The thermal expansion coefficient for the fiber (approximately  $0.55 \times 10^{-6} \text{ 1/}^\circ\text{C}$  for Silica).

$\alpha_n = \left(\frac{1}{n_{eff}}\right) \left(\frac{\partial n_{eff}}{\partial T}\right)$  Represents the thermal – optic coefficient, which is approximately equal to ( $8.6 \times 10^{-6} \text{ 1/}^\circ\text{C}$ ) for the Germanium – doped, Silica – core fiber [11], [12].

### 1. Material and Methods

A complex grating is approached by a series of uniform segments, and analyzed by connecting the segments with the well-known Transfer Matrix Method. This will provide the required information about to test and optimize grating designs to the designer.

The optical FBG used in this work is designed by OptiGrating 4.2 software Short period FBG designed with grating length ( $l = 50000 \mu\text{m}$ ), and periodicity ( $\Lambda = 0.5338 \mu\text{m}$ ), refractive index of the core ( $n_{core} = 1.46$ ) and for the cladding ( $n_{cladding} = 1.45$ ) and difference of refractive index ( $\Delta n_{eff} = 0.0030$ ) as shown in figure (2) then we obtain the grating spectrum reflection and transmission at different applied temperature. The inverse scattering solver is only used on the reflection at zero temperature to create a reference spectrum.

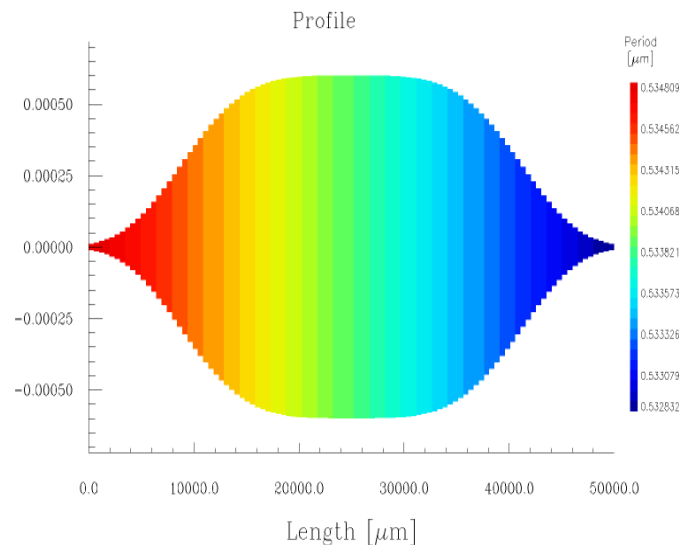
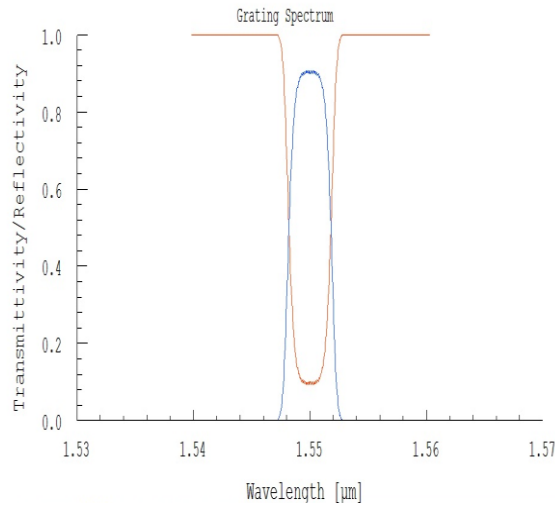


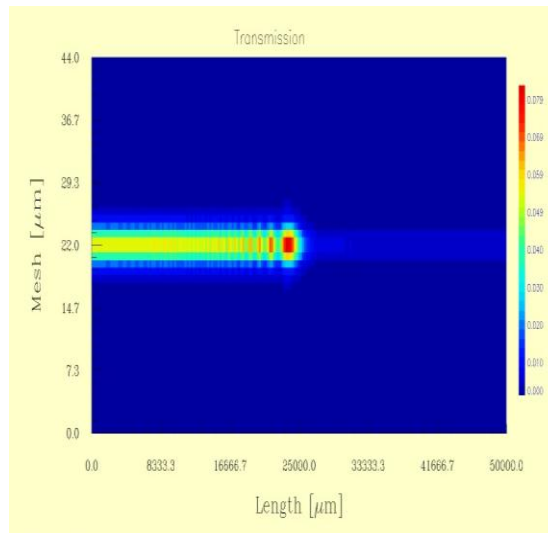
Figure 2: Profile of changing of refractive index along grating length.

**2. Result and Discussion:**

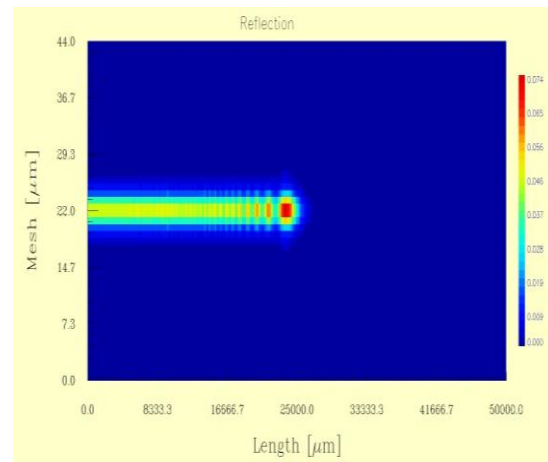
According to eq. 1 the Bragg wavelength of FBG is 1.55  $\mu\text{m}$ . Figures 3 and 4a, b showed the spectrum of FBG, transmission of Bragg wavelength at 37 °C, reflection of Bragg wavelength at 37°C respectively.



**Figure (3): Spectrum of Grating.**

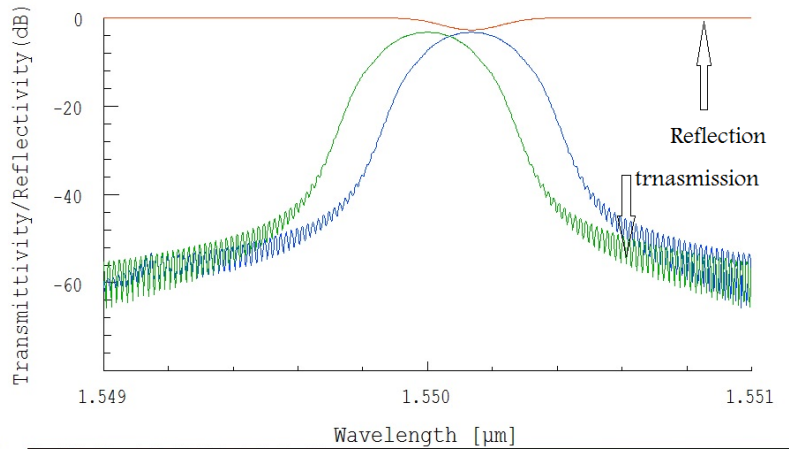


**Figure (4a): transmission of Bragg wavelength at 37 °C (reference temperature)**

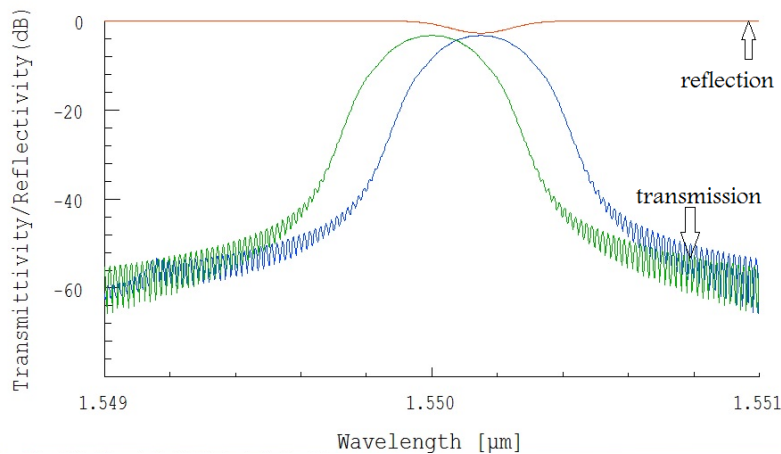


**Figure (4b): reflection of Bragg wavelength at 37°C (reference temperature)**

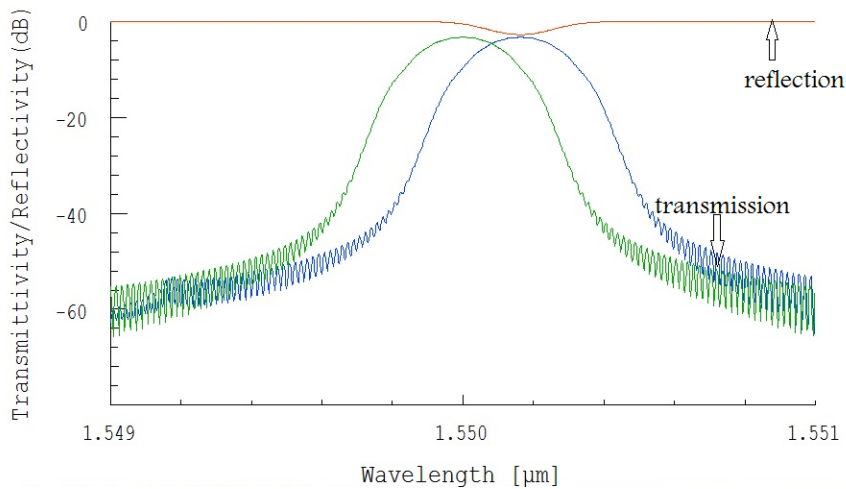
By taking natural temperature of human body as a reference temperature which is 37 °C and apply different values of temperature from hypothermia (35 °C) to hyperthermia (42 °C) with interval of (1 °C). The peak wavelength of FBG sensor was recorded at different temperature as shown in figures (5, 6, 7, 8, 9, 10, 11 and 12). The relation between the applied temperature and shifting Bragg wavelength is linear as it clears in figure (13).



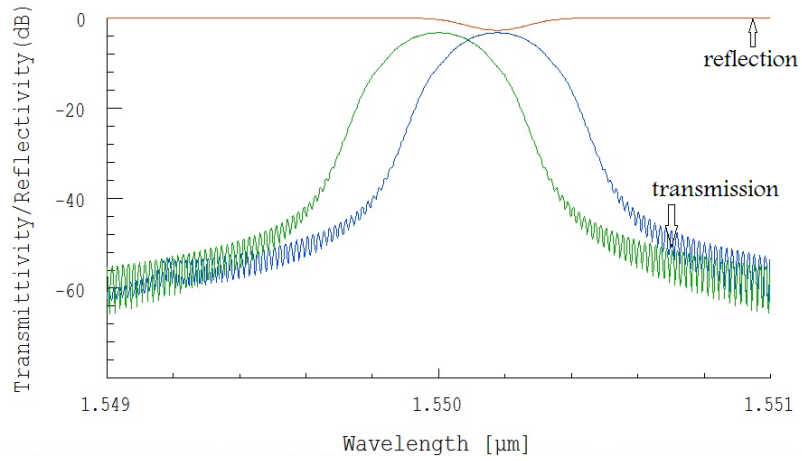
**Figure (5): transmission and reflection spectra for shifted Bragg wavelength at 35 °C**



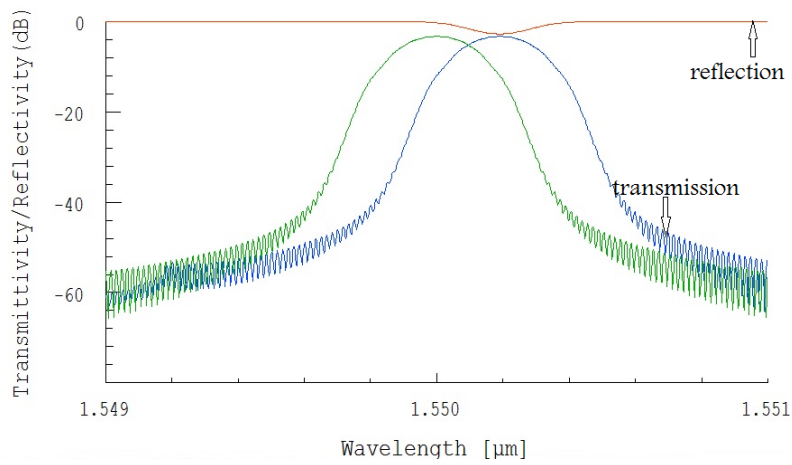
**Figure (6): transmission and reflection spectra for shifted Bragg wavelength at 36 °C**



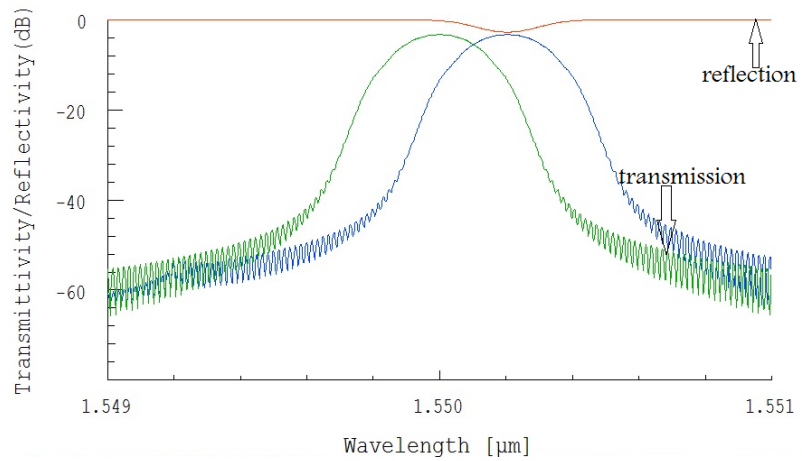
**Figure (7): transmission and reflection spectra for shifted Bragg wavelength at 37 °C**



**Figure (8):** transmission and reflection spectra for shifted Bragg wavelength at 38°C



**Figure (9):** transmission and reflection spectra for shifted Bragg wavelength at 39°C



**Figure (10):** transmission and reflection spectra for shifted Bragg wavelength at 40°C



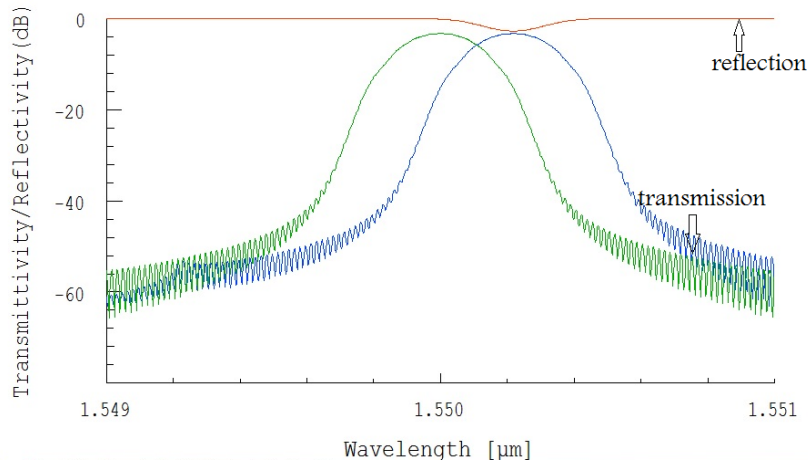


Figure (11): transmission and reflection spectra for shifted Bragg wavelength at 41°C

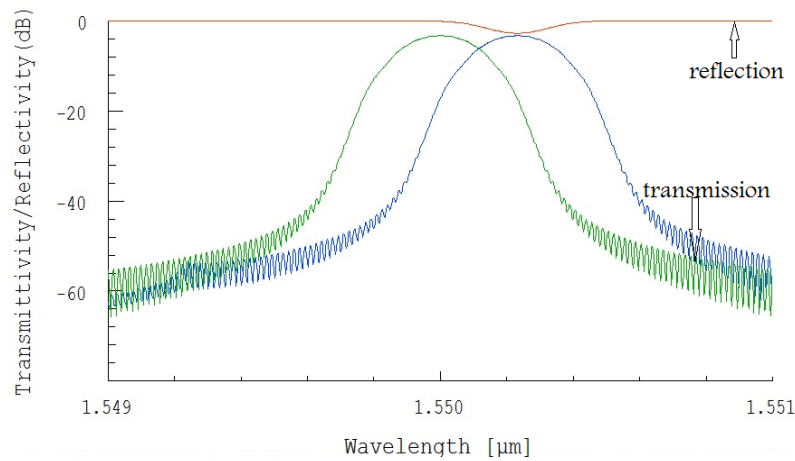


Figure (12): transmission and reflection spectra for shifted Bragg wavelength at 42°C

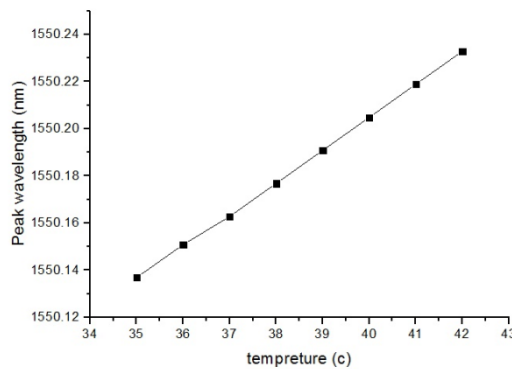


Figure (13): The Relation between shifting in Bragg wavelength with applied temperature

### Conclusion

An important application of FBG technology was sensing. The sensitivity of the Bragg wavelength to temperature arises from the change in the refractive index of the optical fiber. The used FBG in this work is very sensitive to the variation of the temperature degrees; the sensitivity was (0.014 nm/°C). The relation between the applied temperature and shifting Bragg wavelength is linear. There was a little shifting in wavelength of FBG which indicate it is sensitive to

temperature arise so this simulation result will help to improve this work to practical area and synthesized an implantable thermometer used for emergent cases which include sudden temperature shift.

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## Histopathological changes induced after oral administration of acetamiprid in kidneys of male albino mice

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### Abstract

Repeated oral administration of (10 and 20 mg/ml) of Acetamiprid (ACP) - a neonicotinoid insecticide that is effective against both soil and plant insects (LD50=200mg/kg), for 14 days in male albino mice aged (6-7weeks) induced significant

changes in the histoarchitecture of the kidneys included marked congestion, tubular cell degeneration and sloughing of epithelial cells. haemorrhage and severe necrosis observed depend on the dose . The oral toxicity study of (ACP) revealed that this neonicotinoid insecticide is of highly risk in albino mice

**Key words:** Pesticide, Neonicotinoid, Acetamiprid, Histopathology.

## التغيرات النسجية المرضية في كلى ذكور الفئران البيضاء والمستحثة بفعل الجرع الفموية للاسيتامبريد

مها عبد النبي غثوان

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### الخلاصة:

عرضت مجموعه من ذكور الفئران البيض بعمر (6-7) أسابيع، ولمدة 14 يوم، لجرع متكررة بلغت 10-20 ملغم /مل من مبيد الاسيتامبريد (ACP)، وهو مبيد حشري من النيونيكوتينويدات الفعالة ضد كل من حشرات التربة والنباتات، وقد اظهرت النتائج وجود تغيرات مرضية نسجية شملت البنية النسجية للكلية، واحتقان ملحوظ فضلاً عن تنكس خلايا النبيبات الكلوية وانسلاخ الخلايا الظهارية مع نزف وتخر نسيجي حاد، مع زيادة الجرعة المعطاة. وافادت الدراسة بخطورة المبيد على حيوانات التجربة.

## Introduction

Insecticides are chemical substances used to control insects by killing them or preventing them from engaging in undesirable or destructive behaviors [1]. Insecticides could affect the physiological make-up of the target pests by causing changes in growth, development and reproduction parameters, or by causing changes in the nutritional contents of the host plants, which may result in enhanced developmental time, decreased survival, fecundity and reproduction or other changes in the behavior of the target pest. Insecticidal effects on biological parameters of insects potentially have an ecological impact [2].

Neonicotinoids are the latest major class of insecticides with a novel mode of action. These insecticides are very important in agriculture because they are efficient against a broad spectrum of insect pests [3]. Most neonicotinoids are water-soluble and break down slowly in the environment, so they can be taken up by the plant and provide protection from insects as the plant grows. Neonicotinoids are currently used on corn, canola, cotton, sorghum, sugar beets and soybeans. They are also used on the vast majority of fruit and vegetable crops, including apples, cherries, peaches, oranges, berries, leafy greens, tomatoes, and potatoes. The use of neonicotinoids has been linked in a range of studies to adverse ecological effects, including honey-bee colony collapse disorder (CCD) and loss of birds due to a reduction in insect populations [4,5,6,7].

Acetamiprid is a neonicotinoid insecticide, which is a class of neuro-active insecticides modeled after nicotine. Nicotine was identified and used as an insecticide and rat poison as early as the 1600's. Its effectiveness as an insecticide spurred a search for insecticidal compounds that have selectively less effect on mammals, which led to the discovery of neonicotinoids. Neonicotinoids, like nicotine, bind to nicotinic acetylcholine receptors of a target cell [8,9]. These compounds are extensively applied to control pest insects in different agricultural crops; however they can also affect non target organisms (humans or

biota). Still a limited number of studies are referring to neonicotinoids in terms of potential hazard for additive/cumulative effects on human health and to toxic effects of their transformation products on aquatic non target organisms [10-12].

## Materials and Methods

Eighteen animals (aged 6-7 weeks) of albino male mice were used and distributed into three groups, each with 6 mice. First group was normal controls, which were administered orally with 0.1 ml of distilled water. Second group (A GROUP) included mice orally administered with acetamiprid (10 mg/ml), for two weeks. Third group (B GROUP) was orally administered with acetamiprid (20 mg/ml) for two weeks.

## Histopathology

Half of the mice were sacrificed (under anesthesia) on day 7, and the rest on day 14, and were examined by conducting postmortem examination for the presence of gross pathological changes and then tissue samples (kidney) were dissected out and cleaned with physiological saline solution (0.89%). The tissues were immediately put in 10% neutral formalin solution for subsequent processing and histopathological studies. The formalin fixed tissues were thoroughly washed in running tap water, dehydrated in ascending grades of alcohol and acetone, cleared in xylene, and embedded in paraffin wax at 58 °C. Five microns thickness sections from paraffin embedded tissues were stained with haematoxyline and eosin (H&E) stain [13].

## Results

There was a significant change in the histoarchitecture of the kidneys, especially in the second half of the experiment for both concentrations (10, 20 mg/ml) of acetamiprid. Photomicrographs of a section of the kidneys after 7 days of insecticide exposure (Fig 1 and 4) showed mild tubular cell hydropic degeneration with cellular swelling. Some sections of kidneys of administration of acetmiprid (10 mg/ml) for

day 7 showed shrunken glomerulus and nucleated tubules filled with protein cast (fig 3).

The nephritic changes continued as the experiment progressed, with marked congestion, and necrotic tubular epithelium (pyknotic nuclei and acidophilic cytoplasm), and collecting duct degeneration and sloughing of epithelial cells becoming evident on day 14 of treatment, for the two concentrations, (Fig. 2, 5). At day 7 of 20mg/ml of pesticidal administration, there may be a small but significant increase of focal to multifocal foci of tubule basophilia, nuclear crowding, peritubular basement membrane thickening, and variable infiltration by mononuclear inflammatory cells, and hyaline casts are prominent (Fig 6).

### Discussion

All new pesticides are tested to establish the type of toxicity and the dose necessary to produce a measurable toxic reaction. In order to compare the results of toxicity tests done in different labs, there are strict testing procedures. Toxicity testing is extensive (involving many phases) and therefore expensive. Humans, obviously, cannot be used as test subjects, so toxicity testing is done with animals and plants. Since different species of animals respond differently to chemicals, a new chemical is generally tested in mice, rats, rabbits, and dogs. The results of these toxicity tests are used to predict the safety of the new chemical to humans [14]. Histopathological biomarkers can be indicators of the effects on organisms of various pollutants and are a reflection of the overall health of the entire population in the ecosystem. The alterations in cells and tissues in vertebrates are recurrently used biomarkers in many studies. Histopathological biomarkers embody tissue lesions arising as a result of a previous or current exposure of the organism to one or more toxins. Well-documented lesions based on experimental data in liver, ovary, skeleton system and skin have been used as biomarkers to date [15]. Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants

have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. histopathological lesions may arise from pollutants or diseases, provoking necrotic and degenerative alterations to which the organism responds with an inflammatory, defensive reaction [16, 17]. An increased number of macrophagic aggregates can be found in the liver, kidney and spleen in fish exposed to chemical pollutants, bacteria, fungi or parasites [18]. Degeneration of the epithelial cells of the renal **proximal convoluted tubule** (PCT) has been found in the toxicity of asbestos [19]. Severe congestion of the blood vessels, desquamation or necrosis of the epithelial cells of the tubules and proliferation of the endothelial cells of the glomeruli were seen in the kidney of goats due to cypermethrin intoxication [20]. Whereas, mild degenerative changes such as cellular swelling and necrosis were noticed in rats receiving cypermethrin [21, 22] mentioned that moderate degree of degenerative and necrotic changes in proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) was found to be noted in the rats of 25 mg/kg of acetamiprid administration, while the rats of 100 mg/kg group there were congestion and hemorrhages in kidney. Moderate degenerative and necrotic changes were noted in the rats of 100 mg/kg group of (ACP). Rats of 200 mg/kg group revealed degenerative and necrotic changes in PCT and DCT of kidney. In some area of kidney tubular cells had undergone complete lyses leaving reticular framework and that was near to this study observation, Coagulative necrosis and degeneration of tubular epithelium were reported by [23,24] in NDEA induced oxidative stress in albino rats.

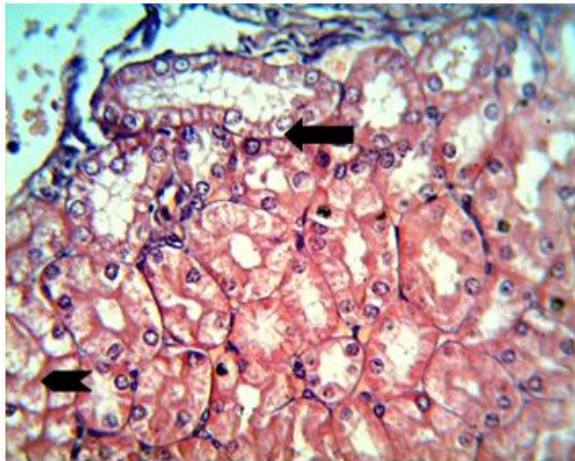
In the present study possibly progressive dehydration in (A&B groups) causing decrease in glomerular filtration rate and lesser blood supply through efferent artery to PCT and DCT resulted in low nutrient supply leading necrosis and lysis of the cell. similar to the results of [25]. As an analysis parameter the (ACP) has induced histopathological effects on mice all dose

levels when exposed for a period of 14 days , It seems that the ACP at the dose dependent levels tested. in the present study for a period

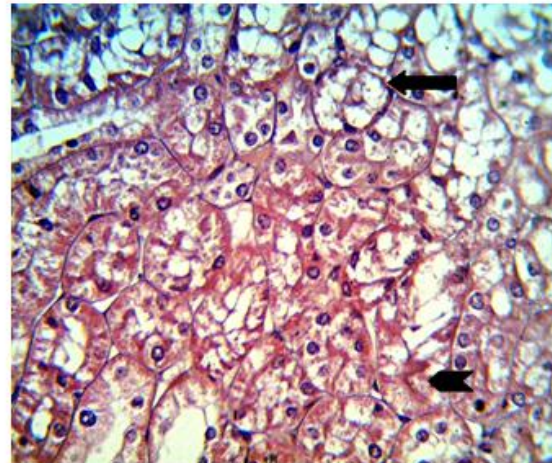
of 14 days The above findings suggested that the kidney as the excretory organ suffered the maximum damage. as mentioned by [26].

**Conclusion:**

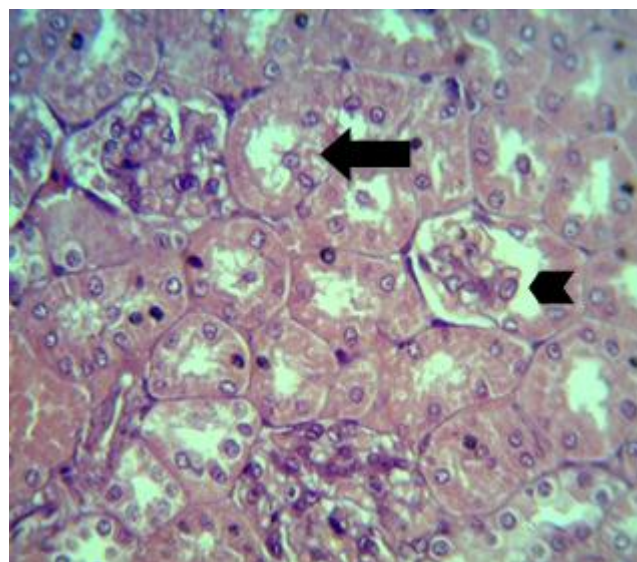
It is now clear that more studies are required to understand the toxicity of ACP on animal health hazards and establish guidelines for acceptable residues in the environment.



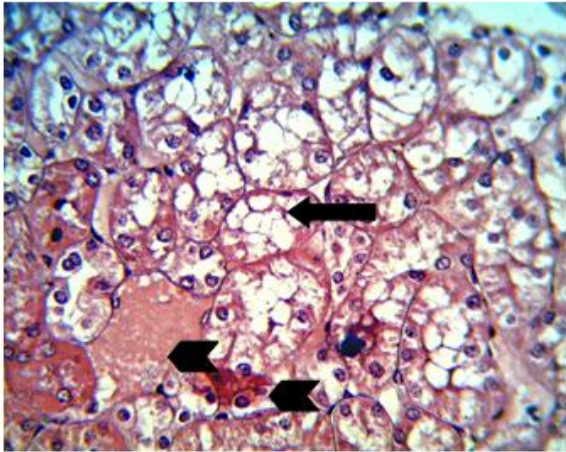
(Fig. 1): Photomicrographs of a section of a kidney of the albino mouse after the administration of acetmiprid (10 mg/ml) day7, showing: mild tubular epithelial cells degeneration (arrow) with cellular swelling (arrow head). H&E. x 400.



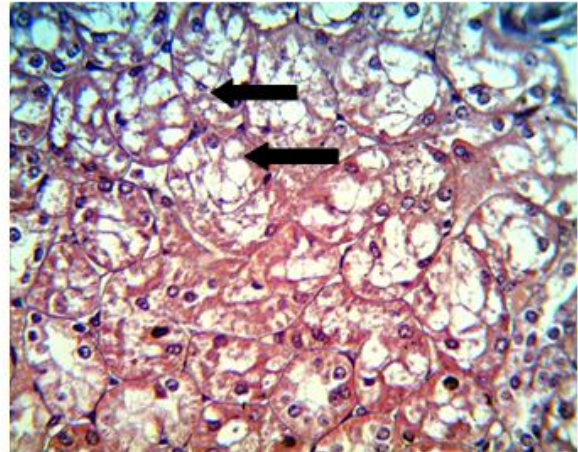
(Fig. 2): Photomicrographs of a section of a kidney of the albino mouse after the administration of acetmiprid (10 mg/ml) day14, showing: sloughed off tubular epithelial cells lying in the lumen of convoluted tubules (arrow) with mild tubular necrosis (arrow head). H&E. x 400.



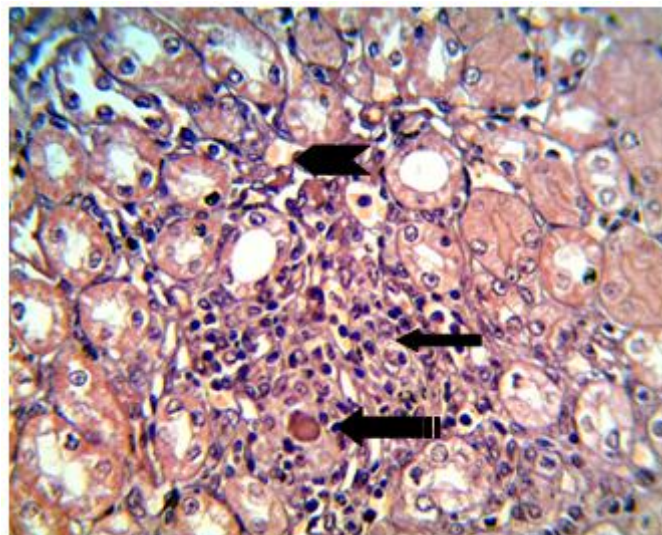
(Fig. 3): Photomicrographs of a section of a kidney of the albino mouse after the administration of acetmiprid (10 mg/ml) day7, showing: shrunken glomerulus (arrow) and nucleated tubules filled with protein cast (arrow head). H&E. x 400.



(Fig.4): Photomicrographs of a section of a kidney of the albino mouse after the administration of acetmiprid (20 mg/ml) day7, showing: sever degeneration of tubular epithelial cells(arrow) with heamorage(arrow head). H&E. x 400.



(Fig. 5): Photomicrographs of a section of a kidney of the albino mouse after the administration of acetmiprid (20 mg/ml) day14, showing: severe acute tubular necrosis(arrows). H&E. x 400.



(Fig. 6) Photomicrographs of a section of a kidney of the albino mouse after the administration of acetmiprid (20 mg/ml) day7, showing: small but significant increase of focal to multifocal foci of tubule basophilia(striped arrow), nuclear crowding, peritubular basement membrane thickening, and variable infiltration by mononuclear inflammatory cells(arrow), and hyaline casts are prominent.(arrow head). H&E. x 400.



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