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Effects of Glutathione and the aqueous extract of *Nigella sativa* seeds on liver, kidney and testis tissues damaged by hydrogen peroxide in male mice

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Abstract:

Objective :This study was designed to investigate the effect of glutathione and the aqueous extract of *Nigella sativa* seeds extraction liver, kidney and testis tissues of male mice have been treated with H_2O_2.

Methods: The mice were divided into four groups (each group contain eight male mice), the first group (control) injected with 0.1ml (normal saline) /daily for 30 days, the second group injected with 0.1 ml H_2O_2 (0.5%) / daily for 20 day, the third group injected with 0.1 ml H_2O_2 (5%) /daily for 20 days and treated by Glutathione 100 mg/kg/ daily/ for 10days, the fourth group injected with 0.1 ml H_2O_2 (0.5%) daily for 20 days and treated with 100 mg/kg from the extract of *Nigella Sativa* seeds for 10days.

Results: The results showed a significant decrease ($p\leq0.05$) in liver enzymes (AST, ALT and ALP) in all groups compared with control group and a significant increase in the third and fourth group compared with the second group. As the results showed a significant increase ($p\leq0.05$) in urea and Creatinine concentration in the second group compared with control group. The result of histological study showed inflammation , hypertrophy of nuclei hepatocytes, degenerated of hepatocytes increase Kupffer cell and bloody congestion in liver of mice and there are hemorrhage, fibrosis, apoptosis and hyperplasia in kidney of the second group. Furthermore, the results revealed a significant decrease ($p\leq0.05$) in Spermatogonia in all groups compared with control group and a significant decrease in primary spermatocytes and spermatids in the second group compared with other groups. As the results showed a significant decrease($p\leq0.05$) in the sperm count in all groups compare with the control group and a significant increase in the third and fourth group compared with the second group. While there were a significant increase in rate of sperm abnormalities in the second group compared with the other groups.

Conclusion: The Results indicated that there were negative effects of hydrogen peroxide in male mice and the treatment of mice with glutathione and aqueous extract of *Nigella sativa* seeds reduced those effects at different rates.

Keywords: Hydrogen peroxide , Glutathione , *Nigella Sativa* , liver, kidney, testis.

تأثيرات الكلوتاثيون والمستخلص المائي لبذور الحبة السوداء على تلف انسجة كبد وكلى وخصى ذكور الفئران المختبرية المعاملة ببيروكسيد الهيدروجين

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الخلاصة:

الهدف: صممت الدراسة الحالية لبحث تأثير الكلوتاثيون والمستخلص المائي لبذور الحبة السوداء على تلف انسجة كبد وكلى وخصى ذكور الفئران المختبرية المعاملة ببيروكسيد الهيدروجين.

طرائق العمل: قسمت ذكور الفئران المختبرية الى اربعة مجاميع (كل مجموعة مكونة من ثمان حيوانات)المجموعة الاولى (السيطرة) حقنت بـ 0.1 مل من المحلول الفسيولوجي يوميا ولمدة 30 يوم ، المجموعة الثانية حقنت بـ 0.1 مل من بيروكسيد الهيدروجين (0.5%) يوميا ولمدة 20 يوم ، المجموعة الثالثة حقنت بـ 0.1 مل من بيروكسيد الهيدروجين (0.5%) يوميا ولمدة 20 يوم ثم عوملت بـ 100 ملغم / كغم من الكلوتاثيون لمدة 10 ايام المجموعة الرابعة حقنت بـ 0.1 مل من بيروكسيد الهيدروجين (0.5%) يوميا لمدة 20 يوم ثم عوملت بـ 100 ملغم / كغم من المستخلص المائي للحبة السوداء ولمدة 10 ايام.

النتائج : اظهرت النتائج وجود انخفاض معنوي في مستوى انزيمات الكبد (ALP, ALT , AST) في كل المجاميع مقارنة مع مجموعة السيطرة كما سجلت ارتفاعا معنويا في المجموعتين الثالثة والرابعة مقارنة مع المجموعة الثانية. كما اظهرت النتائج ارتفاعا معنويا في تركيز اليوريا والكرياتينين في المجموعة الثانية مقارنة مع مجموعة السيطرةفيمابينت نتائج الدراسة النسجية لكبد الفئران وجود ارتشاح التهابي مع تضخم في انوية الخلايا الكبدية وانحلال تلك الخلايا مع زيادة في اعداد خلايا كوبفر واحتقان دموي كما سجل وجود نزف وتليف وموت خلوي مبرمج وفرط تنسج في كلى فئران المجموعة الثانية.

اضافة الى ذلك اشارت النتائج الى وجود انخفاض معنوي في اعداد سليفاتالحيامن في كل المجاميع مقارنة مع مجموعة السيطرة وانخفاضا معنويا في اعداد خلايا الحيامن الاولية وطلائع الحيامن في المجموعة الثانية مقارنة مع بقية المجاميع وقد اشارت النتائج الى وجود انخفاضا معنويا في اعداد الحيامن في كل المجاميع مقارنة مع

مجموعة السيطرة ووجود ارتفاعا معنويا في المجموعتين الثالثة والرابعة مقارنة مع المجموعة الثانية بينما سجل ارتفاعا معنويا في معدل تشوهات الحيامن في المجموعة الثانية مقارنة مع بقية المجاميع

الاستنتاج : اشارت النتائج الى وجود تأثيرات سلبية لبيروكسيد الهيدروجين على ذكور الفئران المختبرية وان معالجتها بالكلوتاثيون ومستخلص بذور الحبة السوداء قلل من تلك التأثيرات بنسب مختلفة.

1- Introduction:

Glutathione one of the most important antioxidant which widespread in human, animals, fungi, some prokaryotic and plant it composed of three amino acids (Glycins,Cystein and glutamin) as tripeptides it characterized as be soluble in water.(Danyelle, 2003). Concentration of GSH (0.5 – 10 mM) intracellular while extracellular less than that(Maher, 2005),and this value dependent on balance of consumption, production and transporting of glutathione (Ocsi *et al*., 2004). There are several function in the body of GSH such as DNA synthesis, production of new cells and protection of the body against tumor, evasion of apoptosis, resistance of radiation and multidrug.(Angell *et al*., 2011). There are an imbalance in GSH concentration is observed in many diseases such as cancer, cystic fibrosis and neurodegenerative (Danyelle, 2003).

The Hydrogen peroxide produced from water and ozone when atoms of hydrogen and oxygen are bind with unstable bonds to form a strong oxidizer caused breaking the bond between oxygen –oxygen to release free radicals (OH).It acts as disinfect of pathogens by destroyed out the inner cell structures of them (Tote *et al*., 2009).Also it considered important to life and whole health therefore all organisms are produced H_2O_2(Campbell,2015).Antioxidant is molecule act to defense against free radical by give electron to oxidant molecules. Hydrogen peroxide (H_2O_2)act as free radical substance induce oxidative stress which caused liver damage (Goraca *et al*,2015). The one of major classes of antioxidant enzymes is Glutathione reductase (GR). Reduced

glutathione (GSH) is a major intracellular redox buffer glutathione functions as a direct free-radical scavenger, as a co-substrate for glutathione peroxidase activity, and as a cofactor for many enzymes (Meister and Anderson, 1983).It is a key enzyme of the antioxidative system that protects cells against free radicals. This enzyme reduced glutathione (GSSG) to GSH by the NADPH-dependent mechanism. Decreased GSH/GSSG ratio contributes to oxidative stress due to its important role (Warsy and el-Hazmi,1999 & Kamerbeek. *et al*.,2007). This enzyme is more stable than the other cytosolic enzymes and it can protect its activity at high temperatures. Inhibition of GR and disturbance in the cellular oxidant-antioxidant balance lead to intracellular GSSG accumulates, and the loss of thiol redox balance may cause loss of cellular homeostasis and numerous diseases (Tandogan, and Ulusu, 2006 & Packer and Cadenas, 2007).

Nigella sativa is one of the medical plants with pervasive in various locations of the world, the seeds of this plant used as stimulant, bitter, anthelmintic and diuretic (Padmaa, 2010). *N. sativa* high rich with antioxidants which inhibit the free radicals that caused several chronic diseases, it contain many essential fatty acids, nickel, thimoquinine as antioxidant, phenols and amino acids (Al-Johar and Shinwari, 2008).

2- Materials and Methods:

2-1:Study Design

The mice were divided into four groups (each group contain eight male mice)

1-The first group (control) :Intraperitoneally (i.p). injected with 0.1ml normal saline/daily for 30days.

2- The second group :i.p. injected with 0.1ml H_2O_2(0.5%)/daily for 20days.

3- The third group : i.p. injected with 0.1 ml H_2O_2 (5%)/daily for 20days and treated with Glutathione 100mg/kg/daily for 10days.

4- The fourth group : i.p. injected with 0.1 ml H_2O_2(0.5%) daily for 20 days and treated with 100mg/kg from extract of *Nigella Sativa* seeds for 10 days.

2-2: Extraction of the *Nigella Sativa* :

According of Mashhadian and Rakhshandeh,(2005) method for extraction (aqueous extract) of the *Nigella sativa* seed.

2-3: Determination of serum Aspartate Transaminase enzyme (AST), Alanine Transaminase enzyme (ALT) & alkaline phosphatase (ALP):

Using of reagents were supplied by Biolabo (France). Enzymatic method described by (Reitmanand Frankel, 1957).And method of Kind & King (1954) to measured ALP.

2-4: Determination of serum urea concentration:

Using of reagents were supplied by Biomerieux (France). Enzymatic method described by (Patton and CrouchR.1977).

2-5: Determination of serum Creatinine concentration:

Using of reagents were supplied by Biolabo company (France).this method described by (Tietz, N.W.1999).

2-6: Histological study:

The organs (Liver, kidney, Testis) were fixed by 10% formaldehyde solution, dehydration in ethanol, embedded in paraffin wax, sectioned on 5µ and stained with hematoxyline and eosin according to (Luna ,1968) method.

2-7: Statistical Analysis Statistical analysis were performed by SPSS version 17.0. The results were expressed as mean ± standard deviations (mean ± SD). One way ANOVA was used to compare parameters in different studied groups. P-value ($P \leq 0.05$) was considered statistically significant.

3: Results:

3-1.Effect of glutathione and *Nigella sativa* on liver enzymes level in male mice treated with hydrogen peroxide.

The results in table 1 showed a significant decrease ($P\leq 0.05$) in concentration of liver enzyme in all the groups compared with the control group but there was a significant increase ($P\leq 0.05$) in the third and fourth groups compared with the second group, while a significant decrease ($P\leq 0.05$) in ALT and AST of the fourth group compared with the third group.

Table1: Effect of glutathione and *Nigella sativa* on the liver enzymes level in male mice treated with hydrogen peroxide (Mean ± stander error).

liver enzymes / group	ALT(IU/L)	AST(IU/L)	ALP(U/L)
Frist group (control)	25.66±0.66 a	24.83± 0.65a	129.66± 1.0 a
second group	4.66±0.33 b	5.00±0.36 b	38.00± 0.96 b
third group	10.38± 1.13 c	12.50± 0.76 c	60.66± 1.72 c

Fourth group	7.00± 0.57 d	8.33± 0.76 d	57.66± 1.8 c
LSD	1.80	1.60	3.54

The different letters refer to the a significant differences at (P≤ 0.05).

3-2:Effect of glutathione and *Nigella sativa* on the kidney function in male mice treated with hydrogen peroxide.

The results of the current study showed significant increase (P≤0.05) in urea and Creatinine concentration in the second group compared to the control group, but there was a non-significant difference (P≤0.05) in the third and the fourth groups compared to the control group, and a significant decrease in the third and the fourth groups compared with the second group.

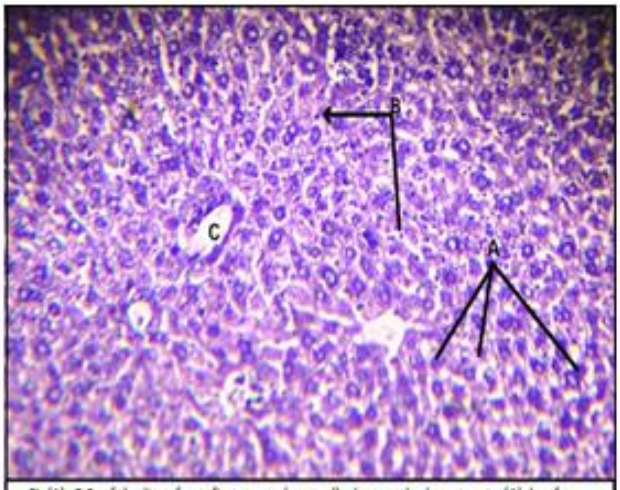
Table2: The effect of glutathione and *Nigella sativa* on kidney function in male mice treated with hydrogen peroxide. (mean ± stander error).

kidney function / group	Creatinine (mg/dl)	Urea(mg/dl)
First group (control)	0.56±0.02a	53.33 ± 1.22 a
Second group	0.66± 0.03 b	70.00 ± 3.14 b
Third group	0.56 ± 0.03 a	45.66 ± 1.56 a
Fourth group	0.55 ± 0.02 a	57.33 ± 0.55 a
LSD	0.07	4.58

The different letters refer to the a significant differences at (P≤ 0.05).

Histological Study:

3-3: The effect of glutathione and *Nigella sativa* on liver damage in male mice treated with hydrogen peroxide: Histological change examination of liver sections in the control group showed a normal composition(Hepatocytes, kupffer cell and central vein) (Fig.1). In the second group, (hydrogen peroxide), there were changes (degenerated hepatocyte, some vacuolated, hypertrophy of nucleus and infiltration of inflammatory cells) (Fig.2). While, in the third group (with H_2O_2 and glutathione) and the fourth group(with H_2O_2 and extract of *Nigella sativa* seeds) showed absence of all these negative histological changes(Fig.3 and 4).



Fig(1): C.S. of the liver from first group (control), showes the hepatocyte (A), kupfer cell (B), central vein (C), (H & E) 100X

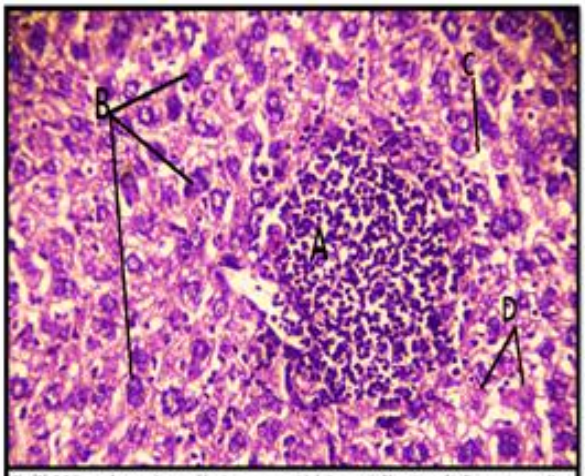
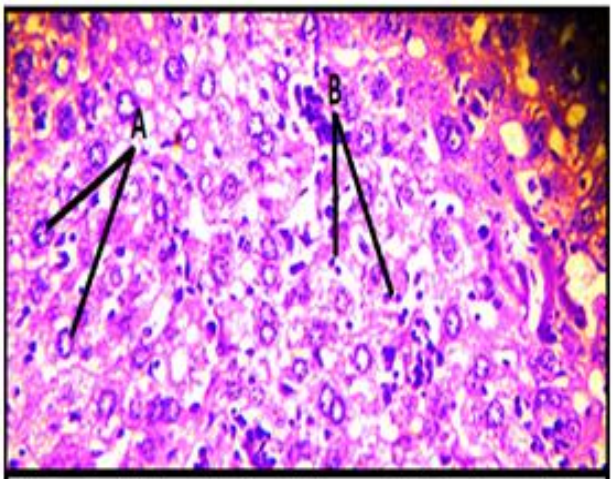
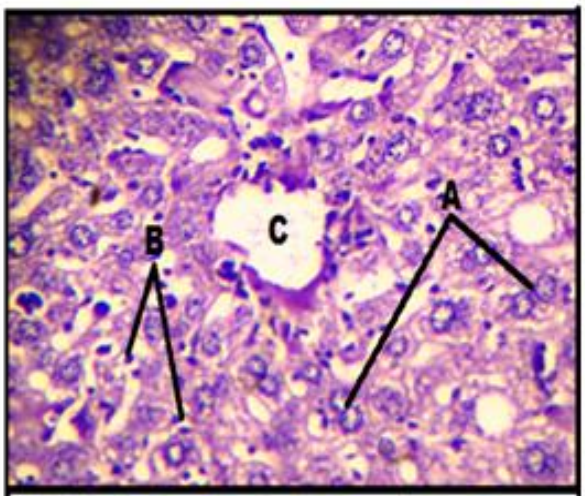


Fig.(2):C.S.liver of second group(hydrogen peroxide) showes infiltration of inflammatory cells (A), hypertophy of nucleus(B), sinuses(C), degenerated of hepatocytes(D), (H & E)100X.



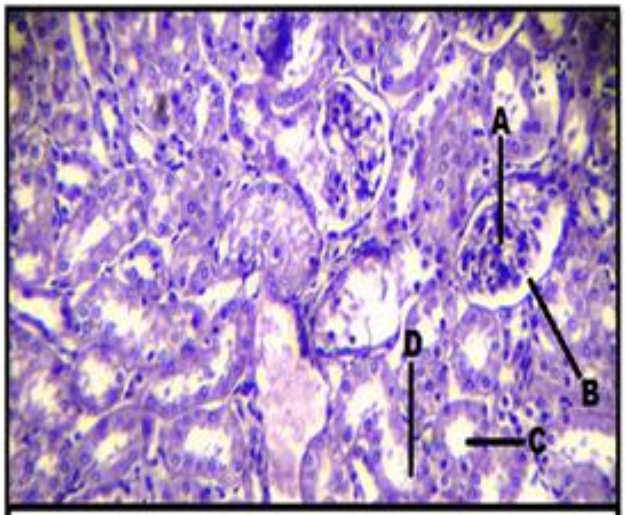
Fig(3): C.s. liver of third group (glutathion and hydrogen peroxide), showes hypatocyte (A), kupffercelle (B) and non degenerative of hypatocytes and nucleus and absence of inflammatory cells, (H&E), 100X.



Fig(4): C.S. liver of fourth group (Nigelle sativa and hydrogen peroxide) showes the hepatocytes (A)kupffercells (B), central vein (C), non degeneration of hypatocytes, (H&E) 100X.

3-4:The effect of glutathione and *Nigellasativa* on kidney damage in male mice treated with hydrogen peroxide.

Histological examination of kidney sections of the control group clarified renal glomerulus, Bowman's capsule, proximal convoluted tubule and distal convoluted tubule (Fig.5), while in the second group it showed: hemorrhage , fibrosis , apoptosis and hyperplasia in kidney(Fig.6).There are non-fibrosis tissue, , absence the hemorrhage and clarity the Proximal convoluted tubules and distal convoluted tubules in the third group (with H_2O_2 and glutathione) and the fourth group(with H_2O_2 and extract of *Nigella sativa* seeds). (Fig.7&8).



Fig(5): C.S. kidney of first group (control) showes Renal glomerulus (A), Bowman's capsule (B), proximal tubulus (D), distal tubulus (C), (H & E) 100X.

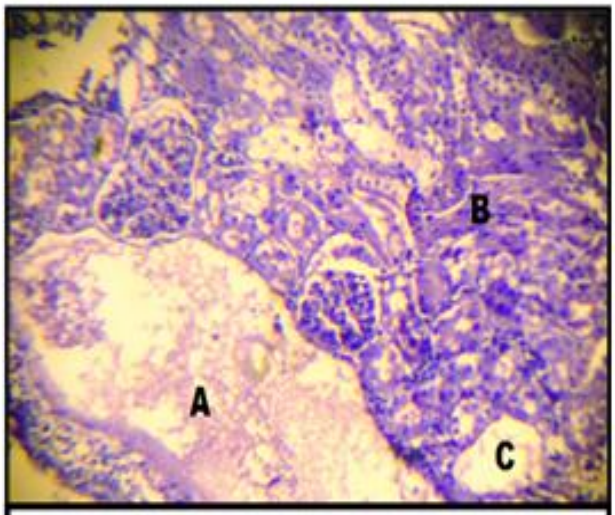
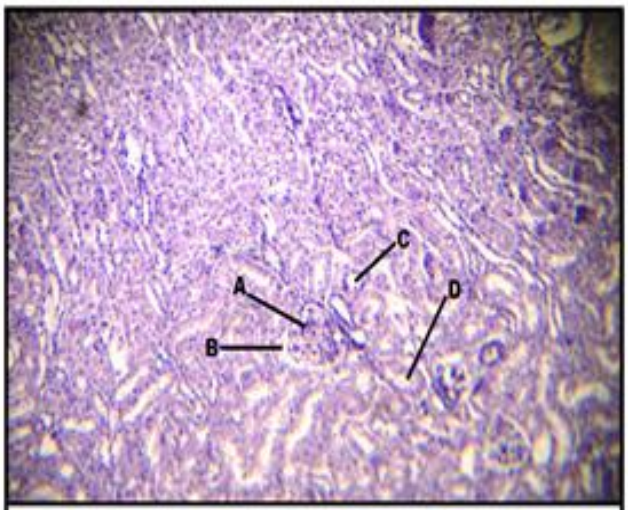
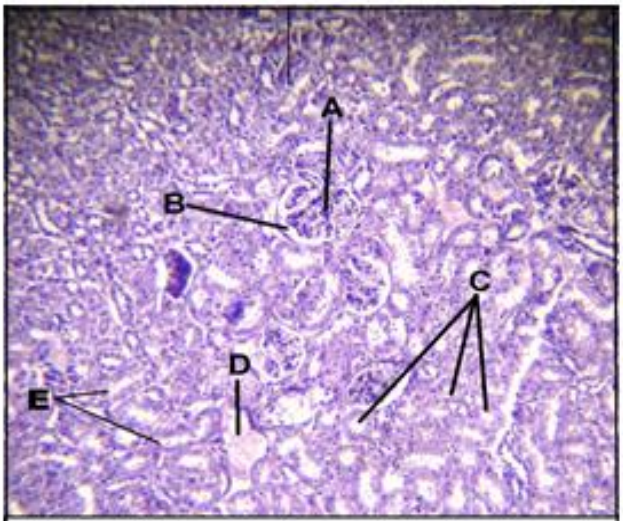


Fig.(6): C.S. kidney of second group (hydrogen peroxide), showes hemorrhag(A), fibrosis (B), apoptosis (C), (H & E) 100X.



Fig(7): C.S. kidney of third group (glutathione and hydrogen peroxide), shows glomerulus (A), Bowman's capsule (B), proximal tubulus (C), distal tubulus (D), (H&E) 100X



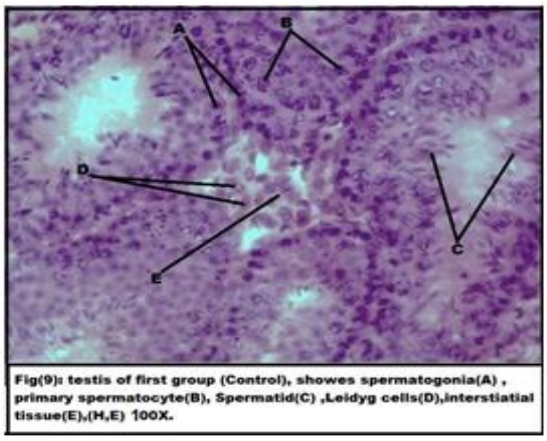
Fig(8): C.S. kidney of fourth group (*Nigella sativa* and hydrogen peroxide) ,showes the glumerulus (A), Bowmans capsule (B), proximal tubulus (C), distal tubulus (E), bleeding (D), (H&E) 100X

3-5:The effect of glutathione and *Nigella sativa* on testis damage in male mice treated with hydrogen peroxide. The results of the present study showed a significant decrease ($P \leq 0.05$) in Spermatogonia count of all groups compared with the control group, and in primary spermatocytes and spermatid count of the second group compared with all groups.(Table 3), As a results showed blood congestion, decomposition of interstitial tissue and decrease of the leydig cells(Fig 9-12).

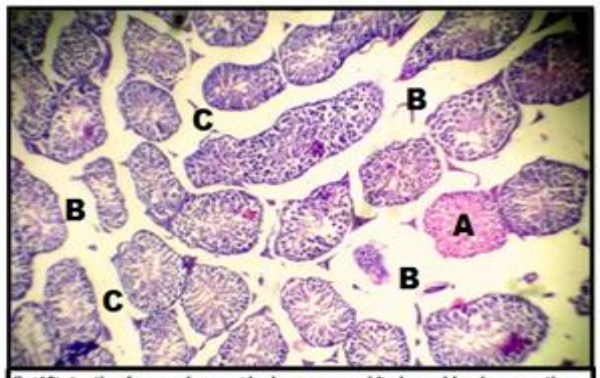
Table3: Effect of glutathione and *Nigella sativa* on testis damage in male mice treated with hydrogen peroxide (mean ± stander error).

Parameters / Groups	Spermatogonia	Primary spermatocyte	Spermatid
First group(Control)	50.161 ±1.99 a	42.502 ± 1.76 a	40.831 ± 1.70 a
Second group	28.33 ± 3.48 b	37.16 ± 1.24 b	23.66 ±2.91 b
Third group	37.831 ± 2.70 b	40.66 ± 2.34 a	36.16 ± 4.79 a
Fourth group	31.50 ± 2.36 b	36.82 ± 5.10 a	33.31 ± 6.44 a
LSD	9.36	10.39	15.20

The different letters refer to the a significant differences at (P≤ 0.05).



Fig(9): testis of first group (Control), showes spermatogonia(A) , primary spermatocyte(B), Spermatid(C) ,Leidyg cells(D),interstiatial tissue(E),(H,E) 100X.



fig(10): testis of second group(hydrogen peroxid),shows blood congestion (A) , decomposition of interstitial tissue (B),decreased number of leidyg cells (C), (H,E), 100X.

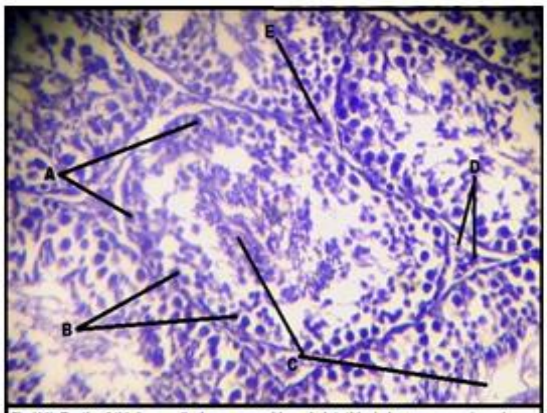


Fig (11): Testis of third group (hydrogen peroxide and glutathion),showes spermatogonia (A),primaryspermatocytes(B),spermatid(C),leidyg cells (D),interstiatial tissue(E),(H, E),100X

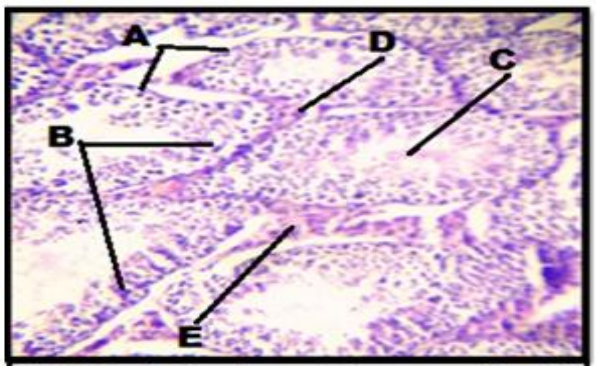


Fig (12): Testis of fourth group (hydrogen peroxide and *N. sativa*), showes spermatogonia (A), primary spermatocytes (B), spermatid (C), leidyg cells (D), interstiatial tissue (E), (H, E), 100X.

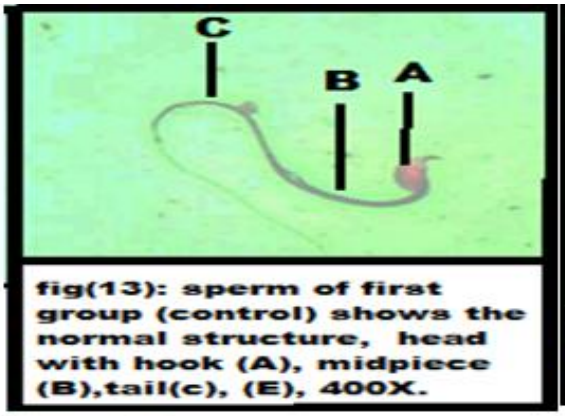
3-6:.The effect of glutathione and *Nigella Sativa* on count and Abnormalities rate of Sperms in male mice treated with hydrogen peroxide. The results of the current study showed a significant decrease (P≤ 0.05) in the sperm count of all groups

compared with the control group and a significant increase (P≤0.05) in the third and the fourth groups compared with the second group. On the other hand, the results appeared a significant increase (P≤0.05) in the rate of the abnormalities sperms in the second group compared with the all groups (Table 4) , (Fig. 13-16).

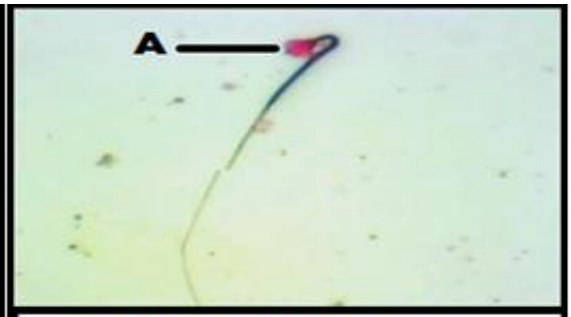
Table4: Effect of glutathione and *Nigella Sativa* on count and Abnormalities rate of the sperms in male mice treated with hydrogen peroxide (mean ± stander error).

Parameters / Group	sperm count ×104			Sperm abnormalities (%)		
First group	825.66	±11.61	a	12.07	± 0.57	a
Second group	446.66	± 27.02	b	21.16	± 1.07	b
Third group	674.83	± 29.19	c	15.66	± 1.05	a
Fourth group	605.83	± 17.84	d	15.83	± 2.52	a
LSD	80.65			3.65		

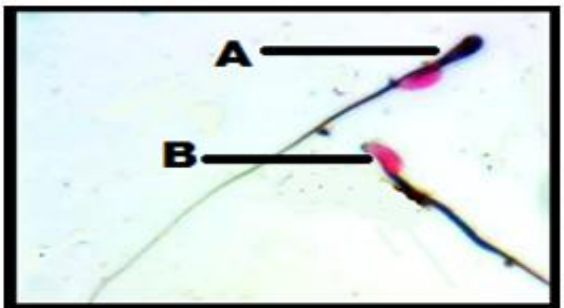
different of letters refer to the a significant differences.



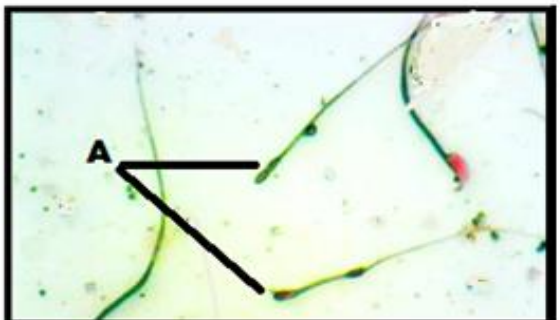
fig(13): sperm of first group (control) shows the normal structure, head with hook (A), midpiece (B),tail(c), (E), 400X.



FIG(14): Sperm of second group (hydrogen peroxide), shows head distorted (A), (E),400X.



fig(16): sperm of second group (hydrogen peroxide) shows inclined piece(A),loss of hook(B), (E), 400X



fig(15):sperm of second group (hydrogen peroxide) shows reduced of head,(E),(400x).

4:Discussion:

4-1:The effect of glutathione and *Nigella sativa* on liver enzymes in male mice treated with hydrogen peroxide

The results showed a significant decrease in the level of liver enzymes (AST, ALT & ALP), this enzymes that reflect liver function due to the high concentrations present in liver cells. AST found in high concentrations in the liver, heart, muscle structure, red blood cells and kidneys, while high concentrations of ALT in the liver and low concentration in the pancreas and skeletal muscle (Bennett & Plum,1996&Cori*et al*.,2009). The important fact is that the liver is consider the essential site of enzymes secretion so that any decline or a rise level enzymes indicates adamage in body tissues, particularly in the liver.

The decrease of these enzymes may be due to theoxidative stress which causes an increase in free radicals production and a decrease in antioxidant, that causes the breakdown of plasma membrane of hepatocytes by oxidation of fats and protein of those membranes, in addition the effect of free radicals on DNA and RNA of those cells, which causes their decrease ability to secrete those enzymes.Where as it explained by Palanisamy *et al*.(2012);Ferrari (2000); Giboney (2005);Median & Moreno (2005).They showed that the free radical causes oxidation of fats, damage of proteins and segmentation of DNA and RNA, while explained by Lee *et al*.(2006) and Ray *et al*.(2001) that oxidative stress that occur by hydrogen peroxide causes structure and functional changes of liver cells as a result of decomposition of the plasma membrane of the hepatic cells.

4-2:The effect of glutathione and *Nigella sativa* on urea and creatinine concentration in male of mice treated with hydrogen peroxide.

The results showed a significant increase in urea and creatinine concentration ($P \leq 0.05$) in the second group which treated with hydrogen peroxide, compared to the control group.

Creatinine and urea metabolic products of protein in the body which excretion out by kidney, so that the reason of high level of creatinine and urea perhaps due to the inability of the kidney to output of urine leads histological changes in them due to hydrogen peroxide such as hemorrhage , fibrosis , apoptosis and hyperplasia in kidney, the results of the current study may reflected negatively on kidney function, re-absorbed in the renal tubules and the level of creatinine and urea in urine. This increase may explain the role of hydrogen peroxide impact on the renal ability to

maintain creatinine and the absorption process. Where explained by Chen *et al*.(2005) & Noiri *et al*.(2001); Noiri *et al*.(2001), that treatment of animal by hydrogen peroxide causes changes may be caused the high level of creatinine and urea in the blood serum and within cells lining the tubules kidney .

The oxidative stress produced by hydrogen peroxide causing the oxidation of fats, proteins and bilateral lipid components of the plasma membrane of the tubules renal (Dani *et al*.,2008; Sharma *et al*.,2006). which affects the permeability membranes and thus increase the flow of creatinine and urea into the bloodstream, causing a rise in blood serum.

The high level of creatinine and urea may be due to inflammatory diseases as a result of exposure to hydrogen peroxide (Tak *et al*.,2001),where treatment of animal with hydrogen peroxide causes the activation of a wide range of receptors inflammatory such as cytokines, which cause a variety of damage at the kidney with a decrease in their ability to function and thus increase urea and creatinine concentration .

4-3:Effect of glutathione and *Nigella sativa* on liver and kidney damage in male mice treated with hydrogen peroxide.

The results of the present study showed that there were changes in tissues of the liver and kidney of male laboratory mice (degenerated hepatocyte, some vacuolated, hypertrophy of nucleus and infiltration of inflammatory cells) in liver, and (hemorrhage , fibrosis , apoptosis and hyperplasia in kidney) in kidney, the reasons for these changes may be because the effects of oxidative stress resulting from the treatment of mice with hydrogen peroxide on cells by oxidation of proteins, fats nucleic acids and the gene expression, while Yang *et al*.(2005) explained that the free radicals cause oxidation of macromolecules and damage of liver cells and induce the apoptosis, also it has an agreement with (Hiroto *et al*.,2009). As Abheri *et al*.(2010) and Fernando *et al*.(2005) pointed out that the free radicals caused kidney tissue damage especially the renal tubules and loss ability to function.

4-4:Effect of glutathione and *Nigella sativa* on spermatogenesis in male mice treated with hydrogen peroxide.

The results showed there area significant decrease in the count of spermatogonia, primary spermatocyte and spermatids, also there was blood congestion, decomposition of interstitial tissue and decrease of the leydig cells count in the second group which treated with hydrogen peroxide.

The spermatogenesis required a testicular tissue integrity and its hormone side(testosterone, LH, FSH), so any testicular dysfunction causes a defect in this processes.

The decrease in the count of spermatogonia, primary spermatocyte and spermatids may be causes of the effect of oxidative stress and free radicals stress in the testicular tissue which is due to the effect on the plasma membrane, macromolecules and DNA which is negatively reflected on the production a new cells during division (mitosis and meiosis), As Wulf (2002) & Halliwell, and Gutteridge (1995) showed that the high concentration of free radicals (ROS) are harmful for all parts of living body and physiological functions, it causes a damage in macromolecules (lipid, protein, carbohydrate, DNA and RNA), and become unable to perform their functions, while Ashok *et al.*(2005) showed the oxidation stress causes damage the plasma membrane of the sperm cells, caused destruction and decrease their count.

The Results indicated that there were negative effects of hydrogen peroxide in liver, kidney and testis functions and tissues of male mice and the treatment of mice with glutathione and aqueous extract of *Nigella sativa* seeds reduced those effects at different rates.

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