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Effect of GarlicOil on Gentamicin InducedHepatorenal Toxicity in rats

اثر زيت الثوم على السموم الكبدية الكلوية المستحدثة بأعطاء دواء الجنتامايسين على الجرذان

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Abstract:

Gentamicin is an important aminoglycoside, natural or semisynthetic antibiotic commonly used against the life threating infections in human. Nephrotoxicity is the major side effect of aminoglycosides. The aim of this study is to investigate the protective/ prophylactic roles of garlic oil against gentamicin-induced abnormalities in metabolic biochemical parameters in serum and tissues of female albino rats. In this study, we used 30 Wistar albino 230-250 g female rats. The animals were randomly divided into five groups. Each experimental group consisted of six animals.Control group, they were fed with only standard rat diet and tap water for 2week. Garlic Oil: Rats were treated daily with a dose of5 ml/kg Garlic Oil(GO) via gavage, and received was start of the 5day of the experiment. Gentamicin: Rats were treated daily with a dose of 100 mg/kg gentamicin(G) asintraperitoneal(IP) at 5 day of the experiment. Gentamicin + Garlic Oil: Rats were treated with synchronisedintraperitoneal gentamicin plus garlic oil 5ml/kg every day via gavage at 5 day of the experiment. Garlic Oil (Pre) +Gentamicin: Rats were treated with 5 ml/kg/ every day via gavage garlic oil in start the first day of the experiment and then gentamicin received at the above-mentioned dose at 5 day of the experiment.a significant increase in serum levels of glucose, bilirubin, urea and creatinine as well as the activity of the AST, ALT, LDH, and ALP enzymes were observed in rats treated with gentamicin for a period of ten days. However, supplementation of gentamicin- intoxicated rats with garlic oil ameliorated the gentamicin adverse effects as evidenced by a significant increase of serum total protein content, and a decrease of serum glucose, bilirubin, urea and creatinine levels, as well as the activity of AST, ALT,LDH and ALP enzymes. Histopathological changes in liver and kidney were not observed on animals treated with Garlic oil when compared with the control group .However, animals treated with gentamicin showed fibrosis, necrosis, and fatty infiltrate in liver and showed necrosis and degeneration of glomerulus in kidney. This effect was significantly decreased in animals pretreated with garlic oil.

Keywords: Gentamicin, Hepatorenal toxicity, Garlic oil

الملخص :

الجنتامايسين هو عبارة عن مضاد حيوي شائع من عائلة الكلايكوسايد يستخدم ضد الامراض المعدية التي تهدد حياة الانسان طبيعته الكيميائية عبارة عن امينو كلايكوسيد وتعتبر هذه المجموعة الدوائية ذات اعراض سمية على الكلى و الكبد اذا اعطيت بجرعات متواصلة او عالية. الهدف من الدراسة تقييم كفائة زيت الثوم الذي يعتبر ذو خصائص مضادة للاكسدة لتقليل سمية الجنتامايسين على الكلى و الكبد و معرفة الدور الوقائي و العلاجي لهذا الزيت, اجريت الدراسة على ثلاثون جرذا وتم تقسيمها الى خمسة مجاميع كل مجموعة تضمنت ستة جرذان, اعتمدت مجموعة الضبط على الغذاء المعياري و الماء و لم يتم اعطائها اي مادة علاجية , المجماعة الثانية تم اعطائها زيت الثوم وبجرعة مقدارها 5 ملم \ كغم \ يوم عن طريق الفم , بالنسبة للمجموعة الثالثة تم حقنها بمادة الجنتامايسين و بجرعة 100 ملغ \ كغم , المجموعة الرابعة تم اعطائها زيت الثوم بعده حقنها بالجنتامايسين اما المجموعة الخامسة فقد تم اعطائها زيت الثوم ولمدة خمسة ايام قبل حقنها بمادة الجنتاميسين. اسفرت نتائج التجربة بأن اعطاء مادة الجنتامايسين للجرذان و لمدة عشرة ايام تسبب زياده معنوية في مستوى الكلوكوز في الدم ,

البليروبين, اليوريا و الكرياتنين بالاضافة الى مستوى فعالية انزيمات وظائف الكبد بينما لوحظ اعطاء زيت الثوم للجرذان المحقونة بدواء الجنتامايسين ادت الى انخفاض معنوي في القياسات البايوكيميائية اعلاه. كما تم التوصل بأن هنالك تغيرات نسيجية مرضية في كل من النسيج الكبدي و الكلوي في الجرذان المحقونة بمادة الجنتامايسين في حين قلت التغيرات النسيجية المرضية بعد اعطتئها زيت الثوم.
مفتاح البحث: الجنتامايسين ,السموم الكبدية الكلوية,زيت الثوم

Introduction:

Gentamicin is an important aminoglycoside,natural or semisynthetic antibiotic commonly used against Gram negative bacterial infections.Nephrotoxicity is the major side effect of aminoglycosides,which may occur in 13-30 %of treated patients[1-3].Gentamicin is execrated through kidneys without degradation or metabolic changes,with 5 to 10 dose is concentrated in proximal tubules vastly exceeding the concurrent serum concentration[4].The accumulation of gentamicin in kidney cortex can cause oxidative stress and lipid peroxidation[5]. Several studies have shown that reactive oxygen metabolites including reactive oxygen species likesuperoxide,hydroxyl radical anion and hydrogen peroxide are important mediators of tissue injury[6-9].Gentamicin causes the activation of platelet activation factor ensuing local vasoconstriction and thus reduces the renal blood flow and glomerular filtration rate. Gentamicin induced renal injuries are mostly localized to the proximal tubules because of association of gentamicin with polyanionic inositol phospholipids and megalin, a receptor, for uptake of gentamicin. Gentamicin resists the degradation of phospholipids that compromised the lysosomal membrane integrity and eventually the leakage of enzymes. Gentamicin behaves as iron chelator and iron-gentamicin complexes are thought to be involved in free radical formation[10-13].

Garlic is a member of the lily family,contains more than 200 chemical compounds. Some of its more important ones includevolatile oil with sulphar containing compound(allicin, allin and ajone), and enzymes (allinase,peroxidase and myrosinase)[14,15]. Ancient Egyption records mentioned that use of garlic as a remedy for avariety of disease[14].

Garlichas antioxidant propertiesthat been shown to inhibit lipid peroxidation (LPO)[16], and dose-dependent induction of endogenous antioxidantsin rat kidney and liver[17].And also have antimutagenesis,xanthine oxidase inhibitor,anticarcinogenesis,antiinflammatory,antiviral,antifungal,antiatherogenicand antithromboitic effects[18-22].

The aim of this study is to investigatethe protective/prophylacticroles of garlic oil against gentamicin-induced abnormalities inmetabolic biochemical parameters in serum and tissues of female albino rats.

Material and Methods:

Chemicals:

Gentamicin (80 mg/2ml) was obtained from the Essential Drug Company (Baghdad,Iraq),and given byintraperitoneally injectionat a dose of 100mg/kg body weight as previouslydescribed[10]. Garlic oil was purchased from local market(Kerbala, Iraq). Garlic oil was given by gavages at a dose of 5ml/kg as described[23].

Animals :

In this study, we used 30 female Wistar albino (230-250 g) rats which were housed in wire bottom cages, free standarddiet, tap water and with a 12 h light/ dark cycle for 2 weeks (the experimental period).The animals were randomly divided into five groups, each experimental group consisted of six animals.

Control group, they were fed with only standard diet and tap water for 2weeks.

Garlic Oil group,rats were treated with garlic oil with a dose of 5 ml/kg dailyvia gavage started from the 5th day to last day of the experimental.

Gentamicin group,rats were treated with 100 mg/kg gentamicin daily byintraperitonealinjectionstarted from the 5th day to last day of the experimental.

Gentamicin-Garlic Oil group, rats were treated with gentamicin with a ratio of 100 mg/kg by intraperitoneal injection at the same time garlic oil with ratio of 5ml/kg was given via gavage daily started from the 5th day to last day of the experimental.

Garlic Oil-Gentamicin group, in this group, rats were treated with garlic oil with a ratio of 5 ml/kg daily via gavage startedfrom the first dayto the last day of the experiment while gentamicinwas givenwith a ratio of 100 mg/kg by intraperitoneal injection from the 5th day to last day of the experimental.

The end of the experiment rats weresacrified 24h after the last garlic oil and gentamicin received, and blood samples were collected in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at 860g for 20 min, and then frozen at -20°C for biochemical analysis. One gram of liver or kidney tissue samples were homogenized(Heidolph® homogenizer) with 4 ml 0.9%NaCl at 4000 rpm for 1.5 min and then extract by centrifuge at 2000 rpm for 20 min,then stored at -20°C for subsequent measurements.Liver and kidney were excised,tissue samples were rinsed with % 0.9 cold NaCl solutions and stored at -80 °C,and embedded on paraffin for histopathologic research.

Biochemical analysis:

The serum glucose was estimated by glucose kit (Randox®)[24].Serum alanine aminotrasferase(ALT) and aspartate aminotransferase (AST) activities were estimated according to Reitman and Frankel methods[25].Creatinine levels was determined using Diamond Diagnostic kit®according to Owens *et al.* method,[26] also urea was estimated by the use Diamond daignostic kit ®as previously reported[27].Total protein and bilirubin were determined using Diamond diagnostic kit® as described[28].Alkaline phosphatase was determined according to Belfield and Goldbergmethod[29].Lactate dehydrogenase were determined using commercially available diagnostic kit(Biomerieux®) as described[28].The cholesterol and triglycerides were estimated by the use Biolabo®daignostic kit as described [30,31].

Preparation of kidney and liver tissues for Histopathology:

The specimens were fixed in formalin 10 % solution for 72 hours. After fixation, the tissues were washed under running tap water for 24 h and dehydrated with 50, 60, 70, 80, 90, 96 and 100% concentrated ethanol. The specimens were then laid in a 1:1 ratio of immersion oil and absolute alcohol for 1h, followed by immersion oil overnight, for transparency. After the application of xylol, the specimens were made into paraffin blocks using a 1:1 xylol and paraffin mixture for 1h and paraffin for 6 hours in an incubator. 10 micron thick sections were rehydrated and dyed with Masson's trichrome (Bio-Optica Masson tricromica cat no 04-010802, Milano S.p.a, via San Faustino, 58,20134 Milano, ITALIA) technique.

Statistical analysis:

The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means ± SE and $P < 0.05$ was considered to be statistically significant.

Results:

As shown in table 1, 2 and 3 a number of biochemical parameters were determined in the serum collected from each group .While rats fedon standard diet supplemented with garlic oil did not show any significant changes in the majority of the parameters examined, a significant increase in serum levels of glucose, bilirubin, urea and creatinine as well as the activity of the AST, ALT,LDH, and ALP enzymes were observed in rats treated with gentamicinfor a period of ten days. The total protein content significantly decreased in serum. However, supplementation of gentamicin-intoxicated rats with garlic oil ameliorated the gentamicin adverse effects as evidenced by a

significant increase of serum total protein content, and a decrease of serum glucose, bilirubin, urea and creatinine levels, as well as the activity of AST, ALT,LDH and ALP enzymes .At the same time there was a significant increase in total protein and a significant decrease of serum glucose,bilirubin,urea and creatinine levels,as well as the activity of AST,ALT,LDH and ALP enzymes when supplementation of garlic oil with gentamicin intoxicated rats.

As shown in table 4,5 the same parameters were estimated in liver and kidney tissues. In the gentamicin-treated rats, a significant inhibition in total protein contents, and the activity of the enzymes ALT and AST as well as the levels of renal urea, creatinine.The activity of hepatic ALP was significantly increased in the gentamicin-treated rats. However, administration of garlic oil to the gentamicin-intoxicated rats significantly restored these parameters in the liver and kidney organs.

Histopathological changes in liver and kidney were not observed on animals treated with Garlic oil when compared with the control group (Figure 1 and 2 for liver) and (figure 5 and 6 for kidney), however animals treated with gentamicin(100mg/kg i.p.)showed fibrosis, necrosis, and fatty infiltrate in liver(Figure4)and showed necrosis and degeneration of glomerulus in kidney (Figure 7). This effect was significantly decreased in animals pretreated with garlic oil (Figure 3 for liver and Figure 8 for kidney).

Table (1): Shows the serum liver function tests in different rat groups.

	ALT U/L	AST U/L	ALP U/L	Bilirubin mg/dl	LDH U/L
Control	21.00 ± 1.86^{a}	85.00 ± 2.89^{a}	20.17 ± 1.97^{a}	0.280 ± 0.007^{a}	50.17 ± 2.47^{a}
Gnt	39.83 ± 2.89^{b}	135.33 ± 1.54^{c}	30.17 ± 0.95^{b}	0.583 ± 0.023^{b}	71.17 ± 3.09^{a}
Gar	19.67 ± 1.26^{a}	84.50 ± 1.38^{a}	19.33 ± 1.15^{a}	0.273 ± 0.007^{a}	49.50 ± 1.59^{c}
Gnt-Gar	24.00 ± 1.15^{a}	113.50 ± 2.28^{b}	20.83 ± 0.83^{a}	0.330 ± 0.016^{a}	59.67 ± 1.58^{b}
Gar-Gnt	22.00 ± 1.86^{a}	109.83 ± 2.21^{b}	20.33 ± 0.71^{a}	0.320 ± 0.014^{a}	58.50 ± 1.69^{ab}

Gen Gentamicin; Gar garlic oil; ALT alanine aminotransferase; AST aspartate aminotransferase; ALP alkaline phosphatase; LDH lactate dehydrogenase; Values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

Table (2): Shows the serum biochemical parameters in different rat groups.

	Glucose	Cholesterol	Triglyceride
Control	101.17 ± 3.11^{a}	7.00 ± 0.86^{a}	13.33 ± 0.95^{a}
Gentamicin	251.00 ± 2.84^{c}	18.67 ± 1.09^{b}	22.50 ± 1.52^{b}
Garlic oil	99.50 ± 2.20^{a}	6.17 ± 0.65^{a}	11.83 ± 1.01^{a}
Gentamicin -Garlic oil	150.50 ± 2.45^{b}	9.50 ± 0.99^{a}	15.17 ± 1.14^{a}
Garlic oil -Gentamicin	148.67 ± 3.32^{b}	8.83 ± 0.95^{a}	14.00 ± 1.15^{a}

Values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b, c) differ significantly, P<0.05

Table (3): Shows the serum kidney function tests in different rat groups.

	Creatinine	Urea	Protein
Control	1.44 ± 0.10^{a}	35.17 ± 1.35^{a}	35.50 ± 2.83^{a}
Gentamicin	2.25 ± 0.38^{b}	52.33 ± 1.93^{b}	61.33 ± 2.14^{b}
Garlic oil	1.42 ± 0.10^{a}	34.67 ± 1.45^{a}	35.17 ± 1.40^{a}
Gentamicin -Garlic oil	1.29 ± 0.05^{a}	37.50 ± 2.90^{a}	37.83 ± 0.65^{a}
Garlic oil -Gentamicin	1.27 ± 0.05^{a}	36.17 ± 2.79^{a}	36.83 ± 0.48^{a}

Values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b) differ significantly, P<0.05

Table (4): Shows the hepatic tissue function tests in different rat groups.

	AST U/mg	ALT U/mg	ALP U/mg
Control	11.70 ± 0.38^{b}	2.80 ± 0.39^{b}	171.62 ± 2.56^{a}
Gentamicin	7.60 ± 0.50^{a}	1.10 ± 0.04^{a}	272.65 ± 5.24^{b}
Garlic oil	12.20 ± 0.32^{b}	3.00 ± 0.39^{b}	169.27 ± 2.58^{a}
Gentamicin -Garlic oil	11.98 ± 0.59^{b}	2.70 ± 0.42^{b}	234.68 ± 26.08^{ab}
Garlic oil -Gentamicin	12.05 ± 0.54^{b}	2.80 ± 0.37^{b}	234.80 ± 36.50^{ab}

ALT alanine aminotransferase; AST aspartate aminotransferase; ALP alkaline phosphatase;

Values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b) differ significantly, P<0.05

Table (5): Shows the renal tissue function tests in different rat groups.

	Urea	Creatinine
Control	33.73 ± 3.72^{b}	1.04 ± 0.01^{b}
Gentamicin	12.42 ± 0.07^{a}	0.63 ± 0.09^{a}
Garlic oil	34.50 ± 3.46^{b}	1.06 ± 0.01^{b}
Gentamicin + garlic oil	26.00 ± 0.68^{b}	1.16 ± 0.04^{b}
Garlic oil + gentamicin	27.93 ± 0.69^{b}	1.15 ± 0.01^{b}

Values are expressed as mean ± SME (n=6) P values where calculated by student t-tests, mean with different superscripts (a, b) differ significantly, P<0.05

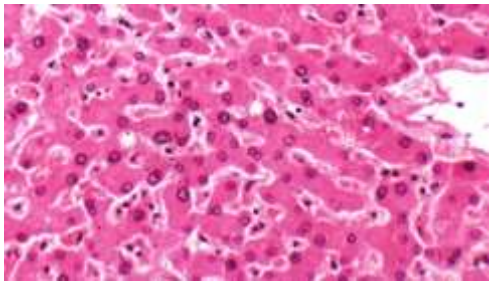

Figure (1) Liver section from a control rat showing normal morphology.

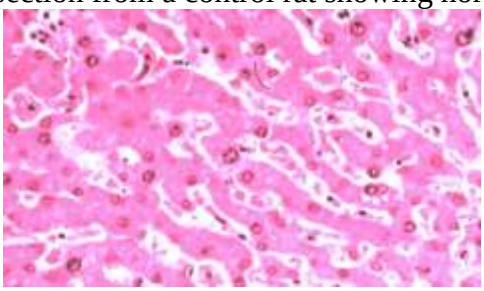

Figure (2) Liver section from rat treated with garlic oil showing normal morphology when compared with control rat.

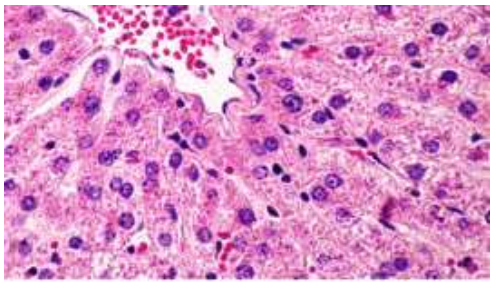

Figure (3) Liver section from rat treated with garlic oil prior to and during treatment with gentamicin, the section shown reduction of fibrosis, necrosis, and fatty infiltrate when compared with animals treated with gentamicin alone .

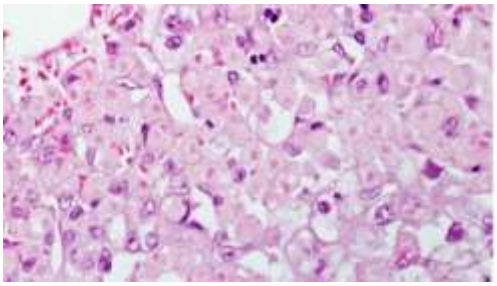

Figure (4) Liver section from rat treated with gentamicin showing fibrosis, necrosis, and fatty infiltrate.

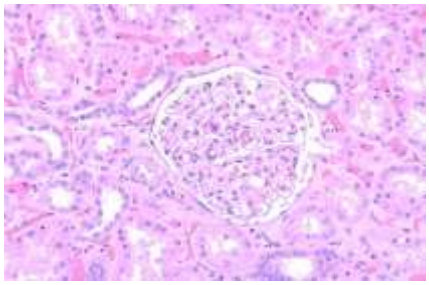

Figure (5) Kidney section from control group showing the normal structure of glomerulus.

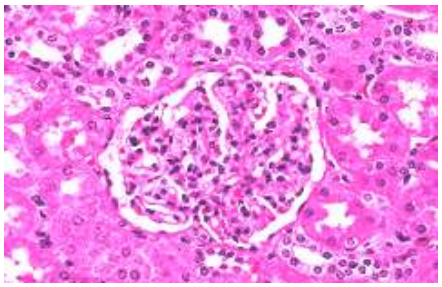

Figure (6) Kidney section from rat treated with garlic oil showing normal morphology when compared with control rat.

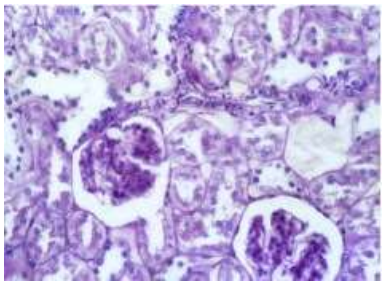

Figure (7) Kidney section from rat treated with gentamicin showing necrosis and degeneration of glomerulus.

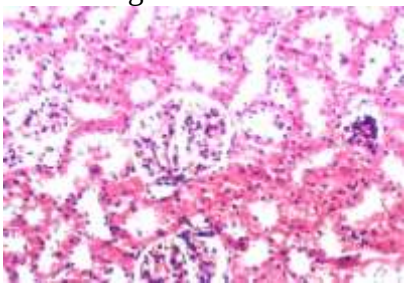

Figure (8) Kidney section from rat treated with gentamicin and garlic oil showing a reduction of necrosis and degeneration of glomerulus.

Discussion:

Gentamicin treatment causes significant increase in the serum level of liver and kidney function markers such as creatinine, urea, total cholesterol, triglycerides, bilirubin and glucose as compared to control indicating hepatorenal dysfunction.These injuries may be due to the production of freeradicals and involvement of oxidative stress to hepatorenaltoxicity caused by gentamicin treatment. Gentamicinmay influence the various metabolic pathways of liver thereby enhancing the level of total cholesterol, triglycerides,glucose,bilirubin, urea and creatinine inserum.

This study evaluated kidney function by measuring serum creatinine and urea values. Gentamicin treatment is found to elevate creatinine and urea levels in serum, but decreased in the kidney, suggesting an impairment of kidney functions. These effects could also be attributed to the aminoglycoside induced nephrotoxicity is characterized by a decrease in the glomerular filtration rate and direct tubular injury .These observations are generally in agreement with other studies[32,33].Creatinine and urea are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase of these parameters, as observed in this study, confirms an indication of functional damage to the kidney [34]. Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases the serum creatinine level[35].However,adminstration of garlic oil along with gentamicin caused significant decrease in urea and creatinine suggested the protective effects of garlic oil.This results agree with hassan et al[36],who reported the administration of garlic oil significantly reduced the concentration of urea and creatinine in sodium nitrite induced rats.

The present study showed that the injection of 100mg/kg gentamicin once daily for ten days to rats lead to a marked elevation in the levels of serum AST, ALT ,LDH and ALP which is indicative of hepatocellular damage. This elevation may be attributed to the release of these enzymes from the cytoplasm into theblood circulation after rupture of the plasma membrane and cellular damage. Serum AST, ALT ,LDHand ALPare biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damage [37].Increased level of LDH in serum indicated the toxic effects of gentamicin in rat.This results obtianed in this study are agree with other reports[10].

Garlic has been found to have an important dietary and medicinal role for centuries. Most of its prophylactic and therapeutic effects are ascribed to specific oil and water soluble organosulfur compounds. Thiosulfinates and other secondary metabolites of garlic, including steroids, terpenoids, flavonoids and other phenols, may be responsible for reported therapeutic effects of garlic. Reuter *et al*[38]have reviewed the therapeutic effects ofgarlic on cardiovascular system as well as its antibiotic, anticancer, antioxidant,immunomodulatory,anti-inflammatory, hypoglycemic and hormone-like effects. Garlic also increases anti-inflammatory monocyte IL-10production and decreases proinflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8, T cell interferon gamma, IL-2.[38-40]Administration of garlic oilsignificantly reduced the activity of liver enzymes in gentamicin induced rats . Due to its ability to reduce free radical-induced oxidative damage in the liver, [41]garlic extract has been shown to decrease liver enzymes in serum and prevent liver damage of rats with liver fibrosis [42].

There was a significant decrease in the concentration of serum total protein in gentamicin group as compared to control group, might be depressed as a result of defective protein synthesis. However,adminstration of garlic oil along with gentamicin caused significant increase in total protein suggested the protective effects of garlic oil.

There wasasignificant increase in levels of serum ALT,AST,LDH,ALP,urea,creatinine,glucose,cholesterol,triglycerides and bilirubin in gentamicin group as compared to control group.However,administration of garlic oil for 5 days before treatment with gentamicin caused significant decrease in levels of serum of ALT,AST,LDH,ALP,urea,creatinine,glucose,cholesterol,triglycerides and bilirubin as compared to gentamicin groups suggested the prophylactic roles of garlic oil.

Conclusion

From this study we can conclude there are a protective and prophylactic effects of garlic oil against gentamicin induce damage, that return the abnormal value of the serum biochemical parameters of ALT,AST,ALP, bilirubin, lactate dehydrogenase, glucose, cholesterol, triglyceride, creatinine , urea and protein as well as hepatic tissue AST,ALT and ALP and renal tissue urea and creatininehistopathological changes in renal and hepatic tissue to normal.

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