Effect of gentamicin and thioacetamide toxicity on serum proteins

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ABSTRACT

Forty-four male Wister rats were divided into four groups. group one (CN) (control group for gentamicin) was used as control(n=9). group two (Neph) (treated group for gentamicin) was injected subcutaneously with 100 mg/kg body weight /day/3 days(n=15). group three(CC) (control group for thioacetamide)was used as control (n=10).group four (cirrhosis)(treated group for thioacetamide)was injected 200mg/5ml saline /kg body weight of thioacetamide I/P for three times per week for 7 weeks(n=10).Results showed that there was significant decrease in total protein, albumin and A/G ratio in(Neph) (treated group for gentamicin) when compared to its control group (CN), furthermore there was significant decrease in concentration of total proteins, albumin, α1gloublin, α2 globulin and β globulin in treated group for thioacetamide. these results showed that different changes of serum protein in rats

Keywords: Serum protein, gentamicin, thioacetamide, rat.

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1. INTRODUCTION

The blood serum is composed of hundreds of different proteins and concentrations of total proteins and several specific proteins are of clinical value *(Joliff, 1992)*. Majority of plasmatic proteins is synthesized in hepatocytes, with albumin representing their largest quantitative part *(Elmaouhoub et al. 2007)*. Serum protein bands or peaks visualized by electrophoresis include albumin and α1, α2, β, and γ globulins. Collectively, the proteins in these bands or fractions serve various functions, including maintaining colloid osmotic pressure and acting as Enzymes, hormones, and antibodies *(kaneko, 1997)*. Albumin is the primary and most homogenous fraction, comprising 35% to 50% of total serum protein in animals. A key metabolic function of albumin is its role as a general binding and transport protein. Albumin is a negative acute-phase protein (that is, its quantity decreases during the acute-phase response), whereas α, β, and γ globulins are positive acute-phase proteins and increase in quantity during the acute-phase response *(Vaden et al., 2009)*. Globulin fraction of serum proteins determined by electrophoresis involves α1, α2, β1, β2 and γ-globulins. Alpha1-globulins include α1-glycoprotein, α1-antitrypsin (AAT), α1-fetoprotein (AFP); α2-globulins include ceruloplasmin and haptoglobin, β-globulins include transferrin, fibrinogen and complement components. Gamma-globulins include individual immunoglobulin classes *(Baumann and gaudie, 1994)* the globulins comprise the positive acute-phase proteins involved in the response to injury, inflammation, and stress. Significant changes in the absolute value of the globulin fractions occur with growth to maturity *(Ganheim et al., 2007)*. Separation of serum protein fraction is very important for the diagnosis of different disease like paraproteinemias, haemoglobinopathies, immune deficiency, various protein abnormalities, determination of the underlying nature of hyperproteinemia or hyperglobulinemia and genetic abnormalities. It is also helpful along with other investigations in acute, chronic liver disease, kidney disease and collagen disease. In multiple myloma it is also helpful in monitoring the treatment *(Whicher et al., 1987, Lomborg et al., 2008)*. The concentration of serum proteins can be used as a measurement of liver function, especially when they are compared with proteins that are not produced in the liver, such as the immunoglobulins *(Zaki, 2005)*.

Therefore, this experiment was designed to perform a comparative study on serum proteins

changes in experimentally induced hepatitis, hepatic fibrosis, renal disturbance and bacterial infection. In addition, other serum biochemical changes including liver and kidney function tests, lipid profile, blood picture and histopathological changes were also evaluated.

2. MATERIAL AND METHODS

2.1. Animals

Forty-four male Wister rats weighing about 100-120 gm were obtained from the Animal House, Faculty of Veterinary Medicine, Benha University, Egypt. All animals were caged and maintained on a standard powder diet, with free access to tap water and were acclimatized for 1 week before starting the experiments. Body weights of the rats in all the groups were measured using an electronic balance. Baseline of body weights was taken before treatment in all groups. Animals were weighed weekly to adjust the dosage of chemicals.

2.2. Gentamicin:

Gentamicin was obtained by Alexandria Company for pharmaceuticals and chemical industries, Alexandria, Egypt and injected subcutaneously in strength equivalent 100 mg/kg body weight daily for three days following the method of Ohno et al., (1993).

2.3. Thioacetamide:

Thioacetamide was dissolved in 5 ml saline and injected at 200 mg/kg body weight intraperitoneally three times per week for seven weeks in rats according to the method of Kasahra (1977).

2.4. Sampling:

Blood samples were collected from retro-orbital venous plexus at the medial canthus of the eye by mean of capillary tubes at last day of injection. Whole blood was received on vacuum tubes containing 3.6 mg dipotassium EDTA and used for CBC. blood was collected in plain clean well-dried centrifuge tube and for separation of serum to be used in determination of total protein, albumin, ALT, AST, ALP, GGT, total bilirubin, direct bilirubin, ammonia, creatinine, urea, cholesterol, triglycerides and serum protein electrophoresis. The diagnostic kits of total proteins, albumin, creatinine, urea, ammonia and triglycerides were supplied by Stan Bio- Laboratory, USA. The diagnostic kits of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP) and cholesterol were supplied by Qumica Clinica Aplicada (QCA) (Spain). The rats were sacrificed for kidney specimen at the end of the 4^{th} ,8^{th} and 12^{th} in control and treated gentamicin group while rats were sacrificed for liver specimen at the end of the 5^{th} and 7^{th} week in control and treated thioacetamide. kidney and liver specimens were fixed in 10% neutral buffered formalin for histopathological examination.

2.5. Electrophoretic pattern of serum proteins:

Serum protein electrophoresis was performed according to the method described by Keyser and Watkins (1972) as follows: Serum protein fractions were separated on Cellogel membranes using the electrophoresis tank. A 250 volt, 4 mA power supply was used with Tris hippurate buffer Ph 8.8 for 25 minutes. After separation, the membranes were Stained in Ponceau-S for 5 minutes, decolorized in distaining solution for 5 minutes, cleared in clearing solution for 1 minute, and dried at 100 ^{0}C for 15 minutes. The relative densities of stained protein bands were determined with the microzone densitometer. The absolute values for different protein bands (albumin, alpha, beta, and gamma globulins) were calculated according to the values of the total proteins.

2.6. Histopathological examination:

Autopsy samples were taken from the kidney and liver of rats and fixed in 10% formalin saline for twenty-four hours. Washing was done in tap water then ascending grades of ethyl alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees in hot air oven. Paraffin bees wax tissue blocks were prepared for sectioning at microns by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains for histopathological examination (Banchroft, 1996).

2.7. Statistical analysis

Statistical analysis was performed using the statistical software package SPSS for Windows (Version 16.0; SPSS Inc., Chicago, Ill.). Student's *t*-test was used to determine significant differences between two experimental groups. Results are expressed as the mean ± standard error of mean (SEM). A *P*-value of less than 0.05 was considered significant.

3. RESULTS

3.1. Gentamicin treated group compared to its control group:

Significant decrease in total protein, albumin and A/G ratio were found 4, 8 and 12 days after gentamicin injection. Meanwhile, non - significant

changes in globulin level (Table 1). Our results confirmed that there were no significant changes in α_1 globulin, β globulin and γ globulin at 4,8 and 12 days after gentamicin injection. Meanwhile, α_2 globulin showed significant increase at 4 days after gentamicin injection (Table 2). Furthermore, Urea and creatinine showed significant increase at 4, 8 and 12 days after gentamicin injection (Table 3). cholesterol and triglycerides level showed significant increase at 4 and 8 days after gentamicin injection. Meanwhile, Non - significant changes were found after 12 days of gentamicin injection.

Histopathological examination of kidney revealed Swelling and vacuolization were observed in the lining endothelium of the glomerular tufts at the cortex after 4 days of gentamicin injection Fig. (2) , There were periglomerular and intertubular focal inflammatory cells infiltration with congestion in the cortical blood vessels after 8 days of gentamicin injection Fig. (3) and Edema, inflammatory cells infiltration and congestion in the blood vessels were noticed in the cortical portion between the tubules and glomeruli after 12 days of gentamicin injection Fig. (4).

3.2. thioacetamide treated group compared to its control group:

Total proteins, albumin and globulin at 5 and 7 weeks after thioacetamide were significantly decrease. Concerning to A/G ratio showed non - significant changes at 5 weeks after thioacetamide injection. Conversely, there was a significant increase in A/G ratio at 7 weeks after thioacetamide injection and there were significant increases in cholesterol and triglycerides in thioacetamide groups at 5 and 7 weeks (Table 4). Regarding to concentration of albumin, α_1 globulin, α_2 globulin, β globulin, our results confirmed that There were significant decrease at 5 and 7 weeks after thioacetamide injection. Meanwhile, a non - significant changes in γ globulin was observed in thioacetamide groups (Table5).

Serum ALT, AST, ALP, GGT, total bilirubin, direct, indirect bilirubin and ammonia concentration showed significant increase at 5 and 7 weeks after thioacetamide injection (Table 6). Histopathological examination of liver revealed fibroblastic cells proliferation and massive inflammatory cells infiltration originating from the portal area and dividing the hepatic parenchyma into nodules. The hepatocytes showed karyocytomegalic activation Fig. (5.6).

Table (1): Total protein, albumin, globulin, cholesterol and triglycerides at 4, 8 and 12 days after subcutaneous injection of gentamicin (100 mg/kg, B.Wt./day for three days) in male rats compared with their corresponding control groups.

Groups	T. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	Cholesterol (mg/dl)	Triglycerides (mg/dl)
CN $_{(D4)}$	8.23±0.47	4.99±0.50	3.24±0.73	1.73±0.43	51.7±3.61	99.66±8.83
Neph $_{(D4)}$	6.72±0.18*	3.34±0.29*	3.38±0.39	0.98±0.26*	65.36±0.58*	116.83±7.13*
CN $_{(D8)}$	8.33±0.42	5.38±0.19	2.95±0.31	1.85±0.17	52.00±2.14	105.33±2.66
Neph $_{(D8)}$	6.23±0.29*	3.20±0.38*	2.83±0.23	1.13±0.30*	66.43±1.10*	120.66±2.31*
CN $_{(D12)}$	8.13±0.17	4.93±0.15	3.19±0.32	1.58±0.20	49.43±1.18	96.53±3.21
Neph $_{(D12)}$	6.43±0.08*	3.17±0.28*	3.16±0.21	1.00±0.18*	50.73±1.68	95.03±5.12

Results are expressed as mean ±SEM (N=5). *Significant difference between gentamicin-treated group and its corresponding control group at $p \leq 0.05$.

Table (2): serum proteins electrophoresis (Albumin, α_1 globulin, $\alpha 2$ globulin, β globulin, γ globulin) at 4, 8 and 12 days after subcutaneous injection of gentamicin (100 mg/kg, B.Wt./day for three days) in male rats compared with their corresponding control group.

Groups	Albumin (g/dl)	Alpha 1 globulin (g/dl)	Alpha 2 globulin (g/dl)	Beta globulin (g/dl)	Gamma globulin (g/dl)
CN (D4)	4.99±0.32	0.22±0.01	0.57±0.05	1.52±0.12	0.88±0.11
Neph (D4)	3.24±0.22*	0.26±0.02	0.72±0.03*	1.41±0.22	1.08±0.13
CN (D8)	5.31±0.14	0.23±0.01	0.58±0.04	1.47±0.15	0.94±0.10
Neph (D8)	3.30±0.24*	0.25±0.02	0.51±0.02	1.22±0.18	0.85±0.08
CN (D12)	4.90±0.20	0.21±0.01	0.55±0.07	1.41±0.23	0.94±0.07
Neph (D12)	3.37±0.11*	0.22±0.2	0.57±0.04	1.27±0.32	0.80±0.10

Results are expressed as mean ±SEM (N=5). *Significant difference between gentamicin-treated group and its corresponding control group at $p \leq 0.05$.

Table (3): Urea and creatinine levels at 4, 8 and 12 days after subcutaneous injection of gentamicin (100 mg/kg, B.Wt./day for three days) in male rats compared with their corresponding control groups.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
CN (D4)	29.06±0.59	0.48±0.03
Neph (D4)	42.70±1.31*	0.84±0.03*
CN (D8)	30.30±1.24	0.51±0.01
Neph (D8)	46.16±0.83*	0.91±0.01*
CN (D12)	30.66±1.21	0.48±0.00
Neph (D12)	47.46±0.41*	0.89± 0.04*

Results are expressed as mean ±SEM (N=5). *Significant difference between gentamicin-treated group and its corresponding control group at $p \leq 0.05$.

Table (4): total protein, albumin, globulin, cholesterol and triglycerides at 5 and 7 weeks after intraperitoneal injection of thioacetamide (200 mg/5ml saline/kg, B.Wt. three times per week for 7 weeks) in male rats compared with their corresponding control.

Groups	T. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	Cholesterol (mg/dl)	Triglycerides (mg/dl)
CC $_{(W5)}$	7.99±0.04	4.07±0.04	3.92±0.08	1.04±0.03	62.50±2.39	89.64±9.38
Cirrh $_{(W5)}$	7.44±0.11*	3.74±0.04*	3.49±0.13*	1.13±0.04	242.40±15.18*	147.20±15.07*
CC $_{(W7)}$	8.35±0.18	4.07±0.04	4.27±0.19	0.95±0.04	64.25±6.15	95.56±8.83
Cirrh $_{(W7)}$	6.50±0.20*	3.38±0.12*	3.12±0.09*	1.08±0.02*	281.33±17.07*	164.66±10.74*

Table (5): Serum proteins electrophoresis (Albumin, α_1 globulin, α_2 globulin, β globulin, γ globulin) at 5 and 7 days after intraperitoneal injection of thioacetamide (200 mg/5ml saline/kg, B.Wt. three times per week for 7 weeks) in male rats compared with their corresponding control.

Groups	Albumin (g/dl)	Alpha 1 globulin (g/dl)	Alpha 2 globulin (g/dl)	Beta globulin (g/dl)	Gamma globulin (g/dl)
CC $_{(W5)}$	4.20±0.5	0.19±0.01	1.85±0.12	1.29±0.24	0.97 ±0.10
Cirrh $_{(W5)}$	3.70±0.71*	0.14±0.01*	1.26±0.14*	1.15±0.19*	1.06 ±0.08
CC $_{(W7)}$	4.10±0.34	0.18±0.01	1.68±0.24	1.21±0.17	0.89±0.07
Cirrh $_{(W7)}$	3.50±0.18*	0.15±0.01*	1.21±0.21*	1.13±0.14*	0.88±0.11

Table (6): Liver function tests (ALT, AST, ALP, GGT, Total bilirubin, direct bilirubin and Ammonia) at 5 and 7 weeks after intraperitoneal injection of thioacetamide (200 mg/5ml saline kg, B.Wt. three times per week for 7 weeks) in male rats compared with their corresponding controls.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Indirect bilirubin (mg/dL)	Ammonia (µg/dL)
CC $_{(W5)}$	54.75±3.22	65.25±3.11	104.25±5.33	17.75±1.49	1.06±0.11	0.28±0.04	0.78±0.12	33.41±3.23
Cirrh $_{(W5)}$	196.60±2.50*	235.60±5.8*	261.40±17.77*	67.60±3.70*	3.10±0.17*	1.68±0.15*	1.42±0.22*	136.02±12.84*
CC $_{(W7)}$	46.50±3.01	60.50±2.95	98.25±6.88	10.42±0.32	1.14±0.03	0.40±0.04	0.74±0.05	29.15±1.24
Cirrh $_{(W7)}$	237.33±17.34*	250.33±9.12*	399.33±10.65*	84.33±3.52*	4.75±0.20*	2.05±0.04*	2.70±0.24*	220.32±21.12*

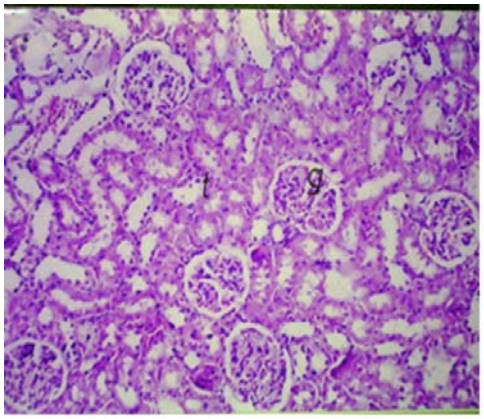

Fig. 1

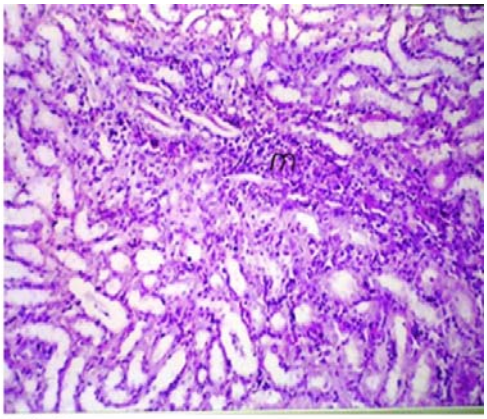

fig. 2

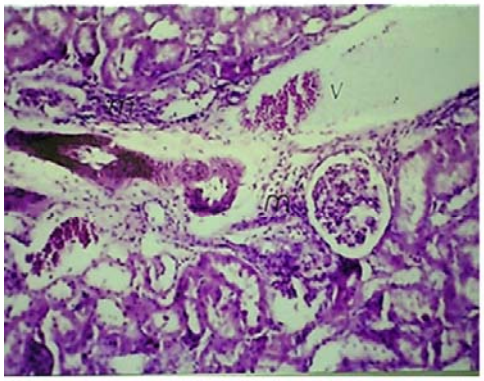

Fig. 3

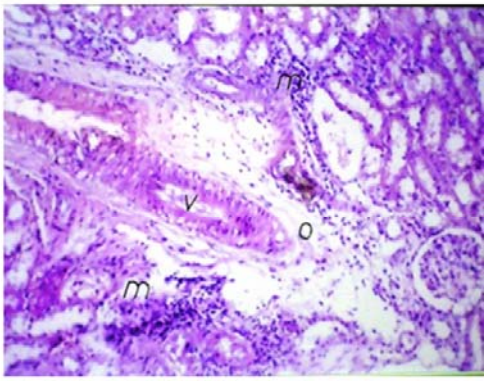

Fig. 4

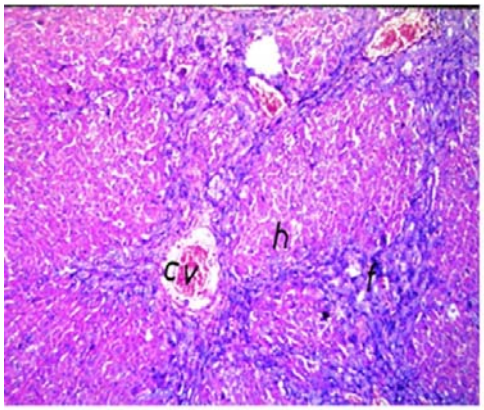

Fig. 5

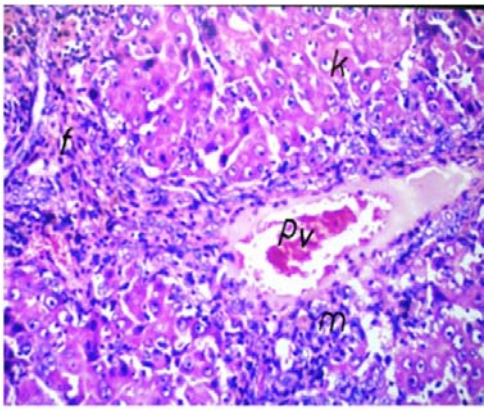

Fig. 6

Figure (1): Kidney of rats kept as control showing normal histological structure of the glomeruli (g) and tubules (t) of the cortex. Fig (2): Kidney of rats after subcutaneous injection of gentamicin (100 mg/kg, B.W/day for three days) and sacrificed after 4 days showing focal inflammatory cells infiltration (m) in between the degenerated tubules at corticomedullary portion. Fig (3): Kidney of rats after subcutaneous injection of gentamicin (100 mg/kg, B.W/day for three days) and sacrificed after 8 days showing periglomerular and intertubular focal inflammatory cells infiltration with congestion in blood vessels (v). Fig (4): Kidney of rats after subcutaneous injection of gentamicin (100 mg/kg, B.W/day for three days) and sacrificed after 12 days showing oedema (o), inflammatory cells infiltration (m) in between the tubules and glomeruli with dilatation in blood vessels (v). Fig. (5): Liver of rats after administration of thioacetamide and sacrificed after 5 weeks showing fibroblastic cells proliferation (f) with inflammatory cells infiltration originating from the portal area and dividing the parenchyma into nodules (n). Fig. (6): Liver of rats after administration of thioacetamide and sacrificed after 7 weeks showing fibrosis (f), inflammatory cells infiltration (m) and congested portal vein (pv).

4. DISCUSSION

Gentamicin, an aminoglycoside class of bactericidal antibiotic, is effective against Gram-negative bacterial infections. In spite of inducing nephrotoxicity, gentamicin is used clinically due to its wide spectrum of activities against gram-negative bacterial infections caused by Pseudomonas, Proteus, and Serratia *(Balakumar et al., 2010)*. Concerning to serum proteins in the current study; there was significant decrease in total protein; albumin and A/G ratio at 4, 8 and 12 days after gentamicin injection, meanwhile, there were non - significant changes in globulin level compared with their corresponding control groups. These results agree with *Vicente et al. (2013)* who stated that the urinary excretion of some proteins was significantly increased in gentamicin nephrotoxicity in rats compared to control ones, namely albumin, hemopexin and transferrin. These proteins are serum-borne proteins whose increased urinary excretion has been widely reported in renal diseases coursing alterations in the sieving properties of the glomerular filtration barrier *(Mackinnon et al., 2003, Varghese et al., 2007 and Basi et al., 2008)*. Serum electrophoretic pattern showed non-significant changes in α_1 globulin, β globulin and γ globulin in gentamicin groups compared with their corresponding control groups. Meanwhile, Alpha 2 globulin showed significant increase at 4 days after gentamicin injection compared with their control groups. Regarding to the results of cholesterol and triglycerides, there were significant increase at 4 and 8 days of gentamicin injection compared with their corresponding control groups. None significant changes were found after 12 days of gentamicin injection. These results agree with *Banday et al., (2008), Priyamvada et al., (2008) and Ademiluyi et al., (2013)* who reported that serum cholesterol and phospholipids were increased with respect to time of gentamicin administration and peaked after 10 days' treatment. Marked decrease in TCA cycle enzymes indicates an impaired oxidative metabolism of glucose/fatty acids that will lead to lower ATP production most likely due to mitochondrial dysfunction *(Abdel-Gayoum et al., 1994)*. In addition, extensive albuminuria leads to increased cholesterol synthesis by the liver *(Stockham, and Scott, 2008)*. Kidney function tests showed significant increase in serum urea and creatinine levels at 4, 8 and 12 days of gentamicin injection compared with their control groups. These results agree with *Ali (1995), Jose et al. (1996), Paquette et al. (2002) and Com et al. (2012)* who stated that gentamicin induce its toxicity on kidney with increased serum urea and creatinine levels, lowered creatinine clearance than saline treatment; by enhancing the production of hydrogen peroxide by rat renal cortical mitochondria and an increased glomerular NO production *(Ali et al., 2002)*. In addition, it increases in the renal cortical phospholipidosis that cause damage to cell membranes. It has been reported that gentamicin causes significant alterations in the lysosomal enzymes (proteinases, acid phosphatase, and cathepsin B and L) in the proximal tubules *(Ali and Bashir, 1996 and Zeeni et al., 2007)*. Histopathological examination of the kidney revealed swelling and vacuolization in the lining endothelium of the glomerular tufts at the cortex, associated with focal inflammatory cells infiltration in between the degenerated tubules at the corticomedullary portion. In addition, edema and congestion in the blood vessels were noticed in the cortical portion between the tubules and glomeruli. These results agree with *Kumar, et al., (2000) and Ozaki, et al., (2010)* who stated that histopathology of the renal tissues of the rats treated with gentamicin showed necrosis and desquamation of the tubular epithelial cells in renal cortex.

Thioacetamide (TAA) is a potent selective hepatotoxin, *(Bruck et al., 2004)*. Oxidative injury has been recognized as the major mechanism in TAA-induced liver damage. The oxidative metabolism of TAA through the hepatocyte FAD–mono-oxygenase and cytochrome P-450 monooxygenase systems produces reactive oxidative agents, especially the very reactive compound thioacetamide-S-dioxide, which targets tissue lipids, protein, and DNA, leading to tissue oxidative injury and necrosis *(Bruck et al., 2001)*. Intraperitoneal injection of thioacetamide (200 mg/kg, B.W) showed significant decrease in total proteins, albumin and globulin compared with their corresponding control groups. A/G ratio showed non-significant changes in liver $cirrh_{(5W)}$ group when compared to the corresponding control group. Conversely, there was a significant increase in A/G ratio in liver $cirrh_{(7W)}$ when compared with the control group. There was significant decrease in concentration of albumin, α_1 globulin, α_2 globulin, β globulin 5 and 7 weeks after thioacetamide injection compared with their corresponding control groups. On the other hand, there were non-significant changes in γ globulin in thioacetamide groups compared with their corresponding control groups. These results are in accordance with *Alkiyumi et al., (2012) and Mustafa et al., (2013)*. These results indicate

disturbances in protein metabolism induced by TAA intoxication. It is well known that following cellular damage the capacity to synthesize proteins is reduced, and as the extent of damage increases, the levels of these proteins in the plasma will tend to decrease (total protein and albumin levels) *(Woodman 1996)*. Mal-absorption that frequently accompanies cirrhosis, could also contribute to hypoalbuminemia *(Fontana et al., 1996)*. TAA-active metabolites also induce immune suppression with decreased gamma globulin (*Esmat et al., (2013)*.

Liver plays the main role in metabolism of different nutrients, such as carbohydrates, proteins, and lipids; in addition, it shares in clearance of waste products resulting from metabolism and elimination of exogenous drugs and other xenobioc *(Saleem et al., 2010)*. Concerning to the effects of TAA on serum biochemical parameters in relation to liver functions, Serum ALT, AST, ALP, and GGT showed significant increase at 5 and 7 weeks of thioacetamide injection compared with their corresponding control groups. thioactamide injection also significantly increased total bilirubin, direct and indirect bilirubin in both times of serum collection compared to their control group. Serum ammonia concentration also follow liver enzymes and bilirubin and showed significant increased values after 5 and 7 weeks of thioacetamide injection.

Increase levels of ALT and AST may be due to AST present in both mitochondria and cytosol of liver cells, while ALT is found in cytosol only, therefore when liver cells damage release these enzymes into the extracellular fluid and results in increased plasma levels of transaminases activity *(. 2003, Baskaran et al., 2010)*. The ALP is a group of glycoprotein enzymes, which are a membrane bound enzymes. ALP mainly arises from sinusoidal surface of the hepatocyte and the microvilli of the bile canaliculi and ducts *(Johnson 1989)*. The increased level of ALP is a reliable marker for biliary flow alteration *(Sehrawat et al., 2006)*. GGT is an enzyme embedded in the hepatocyte plasma membrane, mainly in the canalicular domain, and its release indicates damage of the cell membrane. GGT was significantly increased and this is in agreement with *Cascales et al., (1991) and Kretzschmar et al., (1991*). So that increase in ALP and GGT levels might be due cholestasis as cholestasis implies impairment of bile flow, which can be caused by obstruction of biliary tract *(Martin and Friedman 1998)*.

There was significant increase in the level of total bilirubin, direct and indirect bilirubin. These results agreed with (Galisteo et al., (2006*)*. The increase in these enzymes and bilirubin confirmed by histopathological examination in which liver showed fibroblasts proliferation and inflammatory cells infiltration were originating from the portal area and dividing the hepatic parenchyma into nodules after 5 weeks from thioacetamide administration. In addition, after 7 weeks, there were fibroblastic cells proliferation and massive inflammatory cells infiltration originating from the portal area and dividing the hepatic parenchyma into nodules in thioacetamide treated group. The hepatocytes showed karyocytomegalic activation. These results agree with *Shaker et al., (2011)*, *Wijesundera et al. (2013)* and *Mustafa et al., (2013)* who recorded that rats intoxicated with TAA for 8 weeks also showed mixed-sized fibrotic nodules associated with apoptotic bodies, moderate vacuolar degeneration and bile duct epithelial proliferation. In comparison to control groups, there were significant increases in cholesterol and triglycerides in thioacetamide groups at 5 and 7 weeks compared with their corresponding control groups. These results agree with *Al-Attar (2011)* and *Mustafa et al., (2013)*. This result indicates disturbances in lipid metabolism induced by TAA intoxication.

5. CONCULUSION

In conclusion, the results of present study confirmed that liver damage induced by both either bacterial infection or chemical treatment could be reflected by alteration in serum proteins which might have diagnostic value.

6. REFERENCES

Abdel-Gayoum, A.A., Ali, B.H., Abdel-Razig, K.M., Bashir, A.A., Ghywarsha, K., (1994): Effect of gentamicin-induced nephrotoxicity on some carbohydrate metabolic pathways in rat renal cortex. Archieves of Toxicology 68: 643–647.

Ademiluyi, A.O., Oboh, G., Owoloye, T.R., Agbebi, O.J., (2013): Modulatory effects of dietary inclusion of garlic (Allium sativum) on gentamycin-induced hepatotoxicity and oxidative stress in rats. Asian Pac J Trop Biomed, 3(6):470-475.

Al-Attar, A.M., (2011): Hepatoprotective Influence of Vitamin C on Thioacetamide-induced Liver Cirrhosis in Wistar Male Rats. *Journal of Pharmacology and Toxicology* 6: 218-233.

Ali, B.H., (1995): Gentamicin Nephrotoxicity in Humans and Animals Some Recent Research. Gen. Pharma. 26(7):1477-1487.

Ali, B.H., and Bashir, A.K., (1996): Effect of Superoxide Dismutase Treatment on Gentamicin Nephrotoxicity in Rats. Gen. Pharmac. 27(2):349-353.

Ali, B.H., Al-Qarawi, A.A., Mousa, H.M., (2002): The effect of calcium load and the calcium channel blocker verapamil on gentamicin nephrotoxicity in rats. Food and Chemical Toxicology, 40:1843–1847

Alkiyumi, S.S., Abdullah, M.A., Alrashdi, A.S., Salama, S.M., Abdelwahab, S.I. and Hadi, A.H.A., (2012): Ipomoea aquatica extract shows protective action against thioacetamide-induced hepatotoxicity. *Molecules* 17: 6146-6155.

Balakumar, P., Rohilla, A., Thangathirupathi, A., (2010): Gentamicin-induced nephrotoxicity: Do we have a promising therapeutic approach to blunt it? Pharmacological Research, 62:179–186.

Banchroft, J.D., Stevens, A., Turner, D.R., (1996): 4th Ed. Churchil livingstone, New York, London, San Francisco, Tokyo.

Banday, A.A., Farooq, N., Priyamvada, S., Yusufi, A.N.K., Khan, F., (2008): Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. Life Sciences, 82:450–459.

Baskaran, Y., Periyasamy, V., Venkatraman, A.C., (2010): Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. Toxicology, 268 (2010) 204–212.

Bruck, R., Aeed, H., Avni, Y., Shirin, H., Matas, Z., and Shahmurov, M., (2004): Melatonin inhibits nuclear factor kappa B activation and oxidative stress and protects against thioacetamide induced liver damage in rats. *Journal of Hepatology* 40(1): 86– 93.

Bruck, R., Shirin, H., Aeed, H., Matas, Z., Hochman, A., Pines, M., and Avni, Y., (2001): Prevention of hepatic cirrhosis in rats by hydroxyl radical scavengers. *Journal of Hepatology* 35(4): 457–464.

Cascales, M., Martin-Sanz, P., Craciunescu, D.G., Mayo, I., Aquilar, A., Robles-Chillida, M., and Cascales, C., (1991): Alteration in hepatic peroxidation mechanism in thioacetamide induced tumor in rats. Effect of *rhodium (III)* complex. *Carcinogenesis* 12: 233-240.

Com, E., Boitier, E., Marchandeau, J., Brandenburg A., Schroeder, S., Hoffmann, D., Mally, A., Gautier, J.C., (2012): Integrated transcriptomic and proteomic evaluation of gentamicin nephrotoxicity in rats. Toxicology and Applied Pharmacology, 258:124–133. Elmaouhoub, A., Dudas, J., Ramadori, G., (2007): Kinectics of albumin and Alpha-fraction-production during rat liver development. Histochem Cell Biol 128: 431-443.

Esmat, A.Y., Said, M.M., Soliman, A.A., El-Masry, K.S.H., Badiea, E.A., (2013): Bioactive compounds, antioxidant potential, and hepatoprotective activity of sea cucumber (Holothuria atra) against thioacetamide intoxication in rats. Nutrition 29:258–267.

Fontana, L., Moreira, E., Torres, M.I., Fernandez, M.I., Rios, A., and Sanchez, de., Medina, F., (1996): Serum amino acid changes in rats with thioacetamide-induced liver cirrhosis. *Toxicology* 106:197-206.

Galisteoa, M., Suarez, A., Montillaa, M.P., Fernandez, M.I., Gil, A., and Navarroa, M.C., (2006): Protective effects of Rosmarinus tomentosus ethanol extract on thioacetamide-induced liver cirrhosis in rats. *Phytomedicine* 13: 101–108.

Joliff, C.R., (1992): Analysis of the plasma proteins. J. Clin. Immunoassay, 15:151-161.

Kaneko, J.J., Harvey, J.W., Bruss, ML., (1997): Serum proteins and the dysproteinemias. In: Clinical biochemistry of domestic animals, 5 th ed., Academic Press, San Diego. CA PP,117-138.

Kasahara Y (1997): Spleno-hepatpplasty in rats with chemically-induced cirrhosis of the liver-Archivum Japonicum chirurgicum 46:335-360.

Keyser, J.W., and Watkins, G.L., (1972):" Estimation of serum proteins by electrophoresis on cellulose acetate" Clin. Che., 18(2): 1541-1542.

Kretzschmar, M., Machnik, G., Muller, A., Splinter, F.K., Zimmermann, T., and Klinger, W., (1991): Gamma glutamyl transpeptidase in experimental liver cirrhosis induced by thioacetamide: A biochemical and enzyme histochemical study. *Experimental Pathology* 42: 195-203.

Kumar, K.V., Shifow, A.A., Naidu, M.U.R., carvedjlol, K.S., (2000): a beta blocker with antioxidant property protects against gentamycin induced nephrotoxicity in rats. Life Sciences, 66(26):2603-2611.

Martin, P., and Friedman, L.S., (1998): Assessment of liver function and diagnostic studies In. Handbook of liver Disease. Friedman LS, Keeffe EB and Maddery WC. London, Churchill, Livingstone.

Mustafa, H.N., El Awdan, S.A., Hegazy, G.A., (2013): Protective role of antioxidants on thioacetamide-induced acute hepatic encephalopathy: Biochemical and Ultrastructural study. Tissue and Cell, 45(5): 350–362.

Ohno, I., Shlbasakl, T., Nakano, H., Matsuda, H., Matsumoto, H., Mlsawa, T., Ishlmoto, F., Sakal, O., (1993): effect of gentamicin induces nephrotoxicity in rats Toxicology(G7): 145-147.

Ozaki, N., Matheis, K.A., Gamber, M., Feidl, T., Nolte, T., Kalkuhl, A., Deschl, U., (2010): Identification of genes involved in gentamicin-induced nephrotoxicity in rats–A toxicogenomic investigation. Experimental and Toxicologic Pathology, 62:555–566.

Paquette, M., Plante, I., Labrecque, G., Beauchamp, D., Thibault, L., (2002): Dietary composition alters gentamicin-induced nephrotoxicity in rats. Physiology & Behavior, 77:141-150.

Priyamvada, S., Priyadarshini, M., Arivarasu, N.A., Farooq, N., Khan, S., Khan S.A., Khan, W, Yusufi, A.N.K., (2008): Studies on the protective effect of dietary fish oil on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Prostaglandins, Leukotrienes and Essential Fatty Acids 78:369–381.

Saleem, T.S.M., Chetty, C.M., Ramkanth, S., Rajan, V., Kumar, K.M., Gauthaman, K., (2010): Hepatoprotective herbs—a review. Int. J. Res. Pharm. Sci., 1: 1–5.

Sehrawat, A., Khan, T.H., Prasad, L., and Sultana, S., (2006): Butea monosperma and chemomodulation: protective role against thioacetamide-mediated hepatic alterations in Wistar rats. *Phytomedicine*, 13: 157–63.

Shaker, M.E., Shiha, G.E., Ibrahim, T.M., (2011): Comparison of early treatment with low doses of nilotinib, imatinib and a clinically relevant dose of silymarin in thioacetamide-induced liver fibrosis. European Journal of Pharmacology 670: 593–600.

Vaden, S.L., Knoll, J.S., Smith, S.W.K., Tilley, L.P., (2009): Protein electrophoresis. In: S.L. Vaden, J.S. Knoll, S.W.K. Smith, and L.P. Tilley (eds.) Blackwell's five-minute veterinary consult: laboratory tests and diagnostic procedures. Wiley-Blackwell, Ames, IA, USA, pp. 501-503.

Vicente, L.V., Ferreira, L., Buitragoc, J.M., Francisco, J., Hernandeza, L., Lopez-Novoaa, J.M., and Morales, A.I., (2013): Increased urinary excretion of albumin, hemopexin, transferrin and VDBP correlates with chronic sensitization to gentamicin nephrotoxicity in rats. Toxicology, 304:83–91.

Wijesundera, K.K., Juniantito, V., Golbar, H.M., Fujisawa, K., Miyuu, T., Ichikawa, C., Izawa, T., Kuwamura, M., Yamate, J., (2013): Expressions of Iba1 and galectin-3 (Gal-3) in thioacetamide (TAA)-induced acute rat liver lesions. Experimental and Toxicologic Pathology 65: 799– 808.

Woodman, D.D., (1996): Assessment of hepatotoxicity. In Animal Clinical Chemistry: A Primer for Toxicologist; Evans G.O., Taylor & Francis: London, UK.

Zeeni, N., Selmaoui, B., Beauchamp, D., Labrecque, G., Thibault, L., (2007): Dietary protein level alters gentamicin-induced nephrotoxicity in rats. Physiology & Behavior, 90:760–770.