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HEPATOTOXIC EFFECT OF CHLORAMPHENICOL ADMINISTRATION TO ALBINO RATS

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ABSTRACT:

There are many concerns about safety of food contaminated with antibacterial residues of veterinary drugs in meat, milk and their products. Chloramphenicol is a potent and broad spectrum antibiotic used in veterinary practice and it is used extensively in nonindustrialized countries, thus the need to design this experiment to explore the dose and time response effects of chloramphenicol administration on several parameters in either prophylactic or therapeutic dose for 12 weeks on liver enzymatic activities as well as histopathological changes. A total number of 240 male albino rats were divided into four equal groups. Solutions of chloramphenicol succinate were diluted in distilled water and administered to rats by gavages at a constant dose volume of 10 ml/kg body weight. Chloramphenicol was administered to the first and second groups in a dose of 25 and 50 mg/kg body weight daily for 12 weeks. Rats in the third group (considered as a control group of the group one) and fourth group (considered as a control group of the group two) were given distilled water in a volume of 10 ml/kg body weight.10 rats from each group were scarified under anesthesia after 2, 4, 6, 8, 10 and 12 weeks post dosing. Samples of blood were obtained without anticoagulant for determination of serum AST, AP, Gamma-GT and LDH. Liver samples were taken for histopathological examination. Results of liver enzymatic activities including serum AP and γ-GT revealed a significant increase in both prophylactic and therapeutic doses of chloramphenicol treated-rats compared with control.

Histopathological study revealed degenerative changes of hepatic parenchymal cells corroborated to serum enzymatic activity. Serum LDH concentrations showed a significant increase after 2nd week of exposure until the end of experiment at 12th week. Changes in the serum levels of AST were not statistically significant in both prophylactic and therapeutic dose administered groups. Hepatotoxic effect of chloramphenicol is attributed to inhibition of hepatic cytochrome P_{450}. High rate of tissue turnover with destroyed cells leading to an elevated serum LDH. Therefore, increase the level of this enzyme in this study may be attributed to cytotoxic effect induced by chloramphenicol on multiple cellular compartments including hepatic tissue breakdown. These lesions varied greatly in its severity and distribution according to the duration of exposure as well as the dose of chloramphenicol. Hydropic degeneration with hypertrophy of the kupffer cells associated with the loss of ribosomes in rough endoplasmic reticulum was recorded. Mitochondria appeared slightly swollen, the bile canaliculi dilated and some areas of necrosis was the most common histopathological change. Swelling in hepatic cells recorded in this study besides congestion of the hepatic vasculature, accumulation of fat globules and presence of bundles of collagen fibers in between the hepatic cells and in the Disse space represented chronic reaction to chloramphenicol toxicity or side effects. Such reaction could be considered as secondary for hepatocellular degeneration and necrosis.

INTRODUCTION:

Veterinary drugs are widely used in domestic animals for prevention and treatment of infectious diseases and for growth promotion. However, inappropriate use and abuse of these drugs may bring about risk of serious affection, drug resistance and even acute or chronic toxic or allergic reactions by eating the editable tissues of poultry or livestock previously treated with such drugs. Veterinary drug residues in aquatic products, meats and milk products are much more prevalent and risky especially in developing countries.

Chloramphenicol is a potent and broad-spectrum antibiotics used in veterinary practice, including treatment of aquaculture species and livestock husbandry for several decades (Ramos *et al.*, 2003). Chloramphenicol is rapidly absorbed and distributed in organs and edible tissues after oral and parenteral administration (Anadon *et al.*, 1994). Most symptoms are always characterized in dysbacteriosis (Yunis, 1989 and Holt *et al.*, 1998), aplastic anemia (Yunis, 1989 and Diskin, 2005), agranulocytosis disease (Wiholm *et al.*, 1998) and grey baby syndrome (Kasten, 2005) with long-term diet or long period of medical treatment.

There are accumulating reports indicating undesirable effects of chloramphenicol in humans such as aplastic anemia, which has resulted to its limited usage. Even many countries including USA, Canada, Australia and EU member states have banned its use in food animals (JECFA, 1994). Recently some honeys on the international market have been found contaminated with chloramphenicol residues (Rodziewicz and Zawadzka, 2007). The EU prohibits the use of chloramphenicol as a veterinary drug for food producing animals. There are, however, great concerns regarding the human consumption of food products contaminated with drug residues especially in developing countries (Kanarat *et al.*, 2008). Drug residues may contribute in the development of resistant bacteria strains and consequently lead to more serious health problems (Ferguson *et al.*, 2005). Recently, the detection of chloramphenicol residues in imported products to EU including poultry has been reported. Investigation of shrimps and honey reported also the presence of chloramphenicol residues that causing a huge economical impact (Stolker and Brinkman, 2005). Chloramphenicol is still used extensively in many parts of the developing world (Trevett *et al.*, 1992; Durosinmi and Ajaya, 1993; Kumar and Verma, 1993; and Kushwaha *et al.*, 1994). Fewer records have been indicated the hepatotoxic effects resulted in chloramphenicol therapy (Saba *et al.*, 2000). The therapeutic dose is generally 50 mg/kg daily, in divided doses, although higher levels have frequently been used; the time periods of treatment vary, but are often 10±5 days (Chaplin, 1986). In Veterinary Medicine, chloramphenicol is highly used either as a therapy against many diseased conditions or as a growth promoter administered in subtherapeutic dose for long period (European Committee for Veterinary Medical Products, 1994 and Stolker and Brinkman, 2005). Attempts are being made to investigate possible chloramphenicol-induced toxicity of extramyeloid origin. There are not

much reports of toxicological investigation of chloramphenicol outside its usual or traditional haemotoxic studies. Chloramphenicol had earlier been reported only as hepatic cytochrome P_{450} inhibitor (Robillart *et al.*, 1998). During prolonged chloramphenicol administration, concurrent administration of other drugs must be done with caution since the onset of action is shorter and high concentration of drugs persist longer in the plasma than usual, especially if the drugs share the same metabolic pathway with chloramphenicol (Saba *et al.*, 2000).

Further findings were recorded by Saba *et al.* (2000) according to histopathology carried out indicated that there were degenerative changes of hepatic parenchymal cells. This is well corroborated by significant increase in serum activity of aspartate aninotransferase and alanine aminotransferase (Aubrecht *et al.*, 1997 and Bhat *et al.*, 1998). Cornelius, (1989) attributed icterus of hepatic origin to the dysfunction of biliary canaliculi and defective hepatic uptake of bile pigment during hepatotoxicity which results into regurgitation of conjugated bilirubin and varying increase amount of unconjugated pigment into the plasma respectively. Hepatobiliary dysfunction was incriminated as adverse reaction consequent to the administration of minocycline (Bhat *et al.*, 1998) and meropenem by Alverez and Gil, (1998). Hepatotoxicity induced by chemotherapeutic agents have been of much concern to many clinicians such that toxicological studies were aimed at discovering remedies against established cases of hepatic adverse reactions to these antibiotics (Aubrecht *et al.*, 1997; Fabrizii *et al.*, 1997; Nakamura *et al.*, l998).

Esimone *et al.*, (2007) documented that the histopathological changes observed in the liver sections of rats, 21 days after chloramphenicol treatment were degeneration and necrotic changes in the liver. The sections were moderately to severely hyperaemic and with widespread haemorrhages. There was moderate phagocytosis of erythrocytes by Kupffers cells, while the sinusoids were moderately to severely distorted. The hepatocytes had cytoplasms that were either granular or vacuolated while the nuclei were pyknotic or at the stage of karyorrhexis; these changes were most severe in the portal areas.

The aim of this study was to investigate the possible risk of serious adverse effects attributable to chloramphenicol administration in either prophylactic or therapeutic dose for long-term on liver enzymatic activities and histopathological picture. As there are not much reports of toxicological investigation of chloramphenicol outside its usual or traditional haemotoxic studies; attempt is being made in this study to investigate possible chloramphenicol induced toxicity of extramyeloid origin. This study was designed to explore the variable dose and time-response effect of chloramphenicol administration on the liver.

MATERIALS AND METHODS:

Animals and experimental design:

Total of 240 male albino rats weighted 150-200 g were divided into four equal groups. Rats were caged in groups of five per cage on wood shavings with diet, and mains drinking water, *ad libitum*. A temperature of 22±5ºC was maintained, with a relative humidity of 55±5%, and a light/dark cycle of 12 hours. Animals were acclimatized for 7 days before the start of experiment and observed daily for signs of ill-health during the time of drug administration, and three times each week in the post-dosing periods. Body weights were determined at appropriate times for the adjustment of the given dose. All animal procedures followed the Home Office Code of Practice for the Housing and Care of Animals used in Scientific Procedures. For the first group, chloram-phenicol was administered in a dose of 25 mg/kg body weight (half of therapeutic dose). For the second group, chloramphenicol was administered in a dose of 50 mg/kg body weight (therapeutic dose) according to Chaplin, (1986). Rats in the third and fourth groups were used as a parallel control and were given distilled water in a volume of 10 ml per kg body weight daily parallel to the treated ones daily for 12 week dosing period.

Chloramphenicol administration:

Chloramphenicol, 2,2-dichlor-N-[(aR,bR)-hydroxy-a-hydroxymethyl-4-nitriphenethylacet-amide with trade name chloramphenicol 20% produced by EL Nasr Pharmaceutical Chemical Company Egypt. Solutions of chloramphenicol succinate (CAPS; Sigma Chemical Co Ltd, Poole, Dorset, UK) in distilled water, was administered to rats with the aid of special stainless steel gavages for rats at a constant dose volume of 10 ml/kg body weight. After preparation, the drug was kept in suspension during the dosing period by constant mixing on a rotary mixer; and a special wide-bore gavages needle was used to administer the solution. Control animals were given distilled water at the mentioned dose volume.

Collection of Samples:

In all experimental groups, the rats were scarified under anesthesia after 2, 4, 6, 8, 10 and 12 weeks post dosing. Whole blood with anticoagulant was collected and serum was harvested and stored at -20ºC. Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated according to the method of Tietz, (1994). Gamma-GT (GGT) was measured according to Vassaultt, (1982) and lactate dehydrogenase (LDH) was measured as described by Mathieu *et al.*, (1982) using commercial assay kits. Liver samples were obtained from all groups and fixed in 5% cold buffer glutradehyde for histopathological studies (Bancroft and Stevens, 1982 and Gupta, 1983). The samples were processed in E.M. Units of Assiut University, then sectioned and photographed. Semithin sections were examined and photographed by Olympus microscope. The ultrathin section were examined and photographed by Jeol 100 CXII transmission electron microscope.

Statistical analysis:

Results were expressed as the mean± standard deviations of the mean, significant difference between means were determined by the student's *t*-test. Differences are considered significant at p<0.05 level (Bailey, 1992). Data were analyzed using one-way analysis of Variance (ANOVA) with statistical significance P<0.05 (Oyeka, 1996).

RESULTS:

In a dose of 25 mg/kg body weight (half the therapeutic dose) chloramphenicol treated rats; the obtained results revealed a significant increase of AP after 10th and 12th week and γ-GT after 12th week compared with control. There was a significant increase in serum LDH concentration after 4th week of exposure which continued until the end of experiment as presented in table 1.

In therapeutic dose of chloramphenicol treated rats; serum levels of AP activity, showed a significant increase in comparison with the control group after 6th week of exposure while γ-GT revealed a significant increase after 8th week of exposure. Serum LDH concentrations showed a significant increase after 2nd week of exposure until the end of experiment as shown in table 2.

Changes in the serum levels of AST were statistically nonsignificant in both prophylactic and therapeutic dose administered groups.

Gross and histopathological examination of the liver of the first group which received low doses of chloramphenicol did not showed any significant changes during the first 4 weeks either by light or by electron microscopy comparing with the control group. Then a gradual increase of the pathological changes from mild to moderate degree of degenerative changes could be observed at the end of the 6th week till the end of the 12th week. The degenerative changes began to appear from the end of the six week, in the form of mild hydropic degeneration where the hepatic cells became swollen and foamy. Mild congestion of the vasculature and hypertrophy of the kupffer cells could be identified (Fig. 1). Ultrastr ucturally, the changes manifested by presence of variable intercommunicating areas of light electron dense which is representing the hydropic degeneration of the hepatic cells. The kupffer cells appeared having long processes and its cytoplasm contains mitochondria, rough endoplasmic reticulum (RER) and lysosomes (Fig. 2). At the end of the eight week, the degenerative changes of hydropic type in the hepatic cells, congestion of the hepatic vasculature and the hypertrophy of the kupffer cells were increased more than that could be detected at the end of the six week (Fig. 5). Ultrastructurally, these changes appeared in the form of vasculature of the cytoplasm, the rough endoplasmic reticulum lost its ribosomes and the mitochondria appeared slightly swollen. Also the bile canaliculi in between the hepatic cells mostly dilated (Fig. 6). At the end of the tenth week, the hepatic changes either by light or electron microscope were more or les similar to that which observed at the end of the eighth week.

At the end of the experiment after 12 weeks, the hepatic cells showed moderate degree of

degenerative changes of hydropic type, where the hepatic cells appeared swollen and some of them showed chromatolysis of the nucleus and others showed necrobiosis (Fig. 9). Also congestion of the vasculature could be observed. Ultrastructurally, the intercommunicating variable shaped and sized light electron dense areas representing hydropic degeneration could be observed. Peculiar to this duration, bundles of collagen fibers appeared ultrastructurally in between the hepatic cells (Fig. 10).

Animals in therapeutic dose of chloramphenicol treated did not showed any significant changes either by light or electron microscopy two weeks of treatment comparing with control group.

The pathological changes began to appear at the end of the fourth week. These changes gradually increase from mild to moderate degenerative changes. At the end of the fourth week these changes were in the form of moderate degree of hydropic degeneration of the hepatic cells with presence of solitary fat globules in some hepatic cells. Also chromatolysis as well as pyknosis of some hepatic cell nuclei could be observed. Congestion of the vasculature and hypertrophy of the kupffer cells were also observed (Fig. 3). Ultrastructurally, the hepatic cells showed fat globules; intercommunicating light electron dense areas and electron density of the most of their mitochondria with loss in its cristae could be observed. Also some of the mitochondria of the hepatic cells became light electron dense and lost its cristae (Fig. 4).

At the end of the sixth week, liver showed moderate degree of fat degeneration of the hepatic cells beside moderate degree of hydropic degeneration which lead to swelling of the hepatic cells. Pyknosis of the nucleus with coagulate on of the cytoplasm of solitary hepatic cells and congestion of the vasculature could be observed. Ultrastructurally, numerous fat globules in the cytoplasm with wide areas of intercommunicating light electron dense areas representing hydropic degeneration of the cells as well as fat degeneration.

At the end of the eighth week, the pathological findings of the liver became more sever in degree. These changes were in the form of hydropic degeneration with presence of a number of variable sized fat globules in the hepatic cells. Chromatolysis of the nucleus of some hepatic cells and congestion of the hepatic vasculature could be observed (Fig. 7). Ultrastructurally, most of the mitochondria of the hepatic cells lost its cristae with presence of number of fat globules in the hepatic cells could be observed (Fig. 8).

At the end of the tenth week as well as the end of the twelve week, the pathological changes in the liver were more or less similar. These changes were in the form of moderate degree of fatty degeneration of the hepatic cells which is manifested by presence of number of fat globules in their cytoplasm. Also hydropic degeneration of the hepatic cells with congestion of hepatic vasculature could be observed (Fig. 11). Ultrastructurally, peculiar to this period most of the mitochondria lost its cristae and became more electron dense as well as

dilatation of bile canaliculi and presence of bundles of collagen fibers in between the hepatic cells and in the Disse space. Also the light electron dense, intercommunicating areas representing hydropic degeneration could be observed (Fig. 12).

Table 1: The effect of chloramphenicol on liver enzymes in a dose of 25 mg/kg body weight (half the therapeutic dose) - treated rat group

Week \ Parameter		AP (U/l)	AST (U/l)	γ-GT (U/l)	LDH (U/l)
2nd	Dosed	47.03±11.31	22.41±3.12	22.33±2.02	112.10±8.91
	Control	53.90±10.84	21.16±2.95	24.01±1.97	98.25±9.66
4th	Dosed	57.03±12.42	19.45±3.91	19.87±2.24	227.50±10.10 **
	Control	62.71±9.91	20.29±2.19	22.15±3.01	101.20±9.55
6th	Dosed	56.95±8.20	23.54±3.01	21.33±2.14	566.20±18.20 ***
	Control	49.86±9.18	19.25±2.99	23.25±1.98	105.20±10.20
8th	Dosed	66.80±12.40	23.15±1.98	24.22±2.64	733.50±58.12 ***
	Control	56.40±9.29	21.19±1.94	22.19±2.14	97.14±8.47
10th	Dosed	69.21±8.94 *	23.94±2.11	24.47±1.99	1047.40±102.10 ***
	Control	51.13±10.02	24.08±3.18	21.29±2.18	104.60±10.20
12th	Dosed	72.12±10.11 **	22.98±1.99	26.14±1.99 *	1125.50±115.40 ***
	Control	49.54±12.07	24.54±2.18	22.01±2.01	100.50±9.87

Table 2: The effect of chloramphenicol on liver function in therapeutic dose -treated rat group

Week \ Parameter		AP (U/l)	AST (U/l)	γ-GT (U/l)	LDH (U/l)
2nd	Dosed	51.12±10.22	23.14±2.35	23.61±1.89	140.20±9.44 *
	Control	49.88±9.85	22.51±3.46	22.97±2.01	99.81±10.89
4th	Dosed	58.98 ±11.58	21.59±2.48	24.04±2.61	316.51±25.40 ***
	Control	52.88 ±10.81	22.94±3.05	23.44±2.89	104.30±8.47
6th	Dosed	69.91 ±10.28 *	24.02±2.18	24.51±3.12	657.90±22.82 ***
	Control	52.22±8.94	22.54±3.15	23.67±2.11	98.70±9.49
8th	Dosed	68.55±11.28 *	23.64±2.50	25.14±1.84	955.40±64.80 ***
	Control	52.91±8.94	22.42±2.88	23.51±3.24	102.30±9.64
10th	Dosed	82.66±10.12 **	24.19±1.94	26.84±2.01 *	1411.20±112.5 ***
	Control	48.93±12.62	23.61±2.61	22.01±2.34	94.80±11.40
12th	Dosed	91.22±13.81 **	25.19±2.41	28.48±2.18 *	1794.40±153.8 ***
	Control	51.36±11.29	23.44±3.11	21.91±1.99	97.20±8.99

,**, Significant difference between the control and treated group ($P<0.05$, $P<0.01$ and $P<0.001$ respectively)**

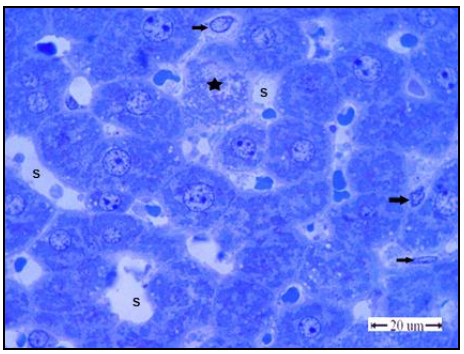

Fig. 1: Semithin section of liver of group one at the end of the six week showing mild congestion of the hepatic sinusoids (s), hypertrophy of the kupffer cells (arrows) and mild to moderate hydropic degeneration of the hepatic cell cytoplasm (star). Toluidine blue stain.

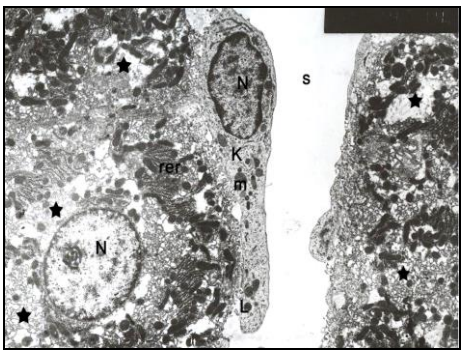

Fig. 2: Transmission electron micrograph of liver of group one at the end of the six week showing intercommunicated light electron dense areas (star) representing hydropic degeneration. Hepatic cells contain rough endoplasmic reticulum (rer) in groups. The kupffer cells (K) have long processes contain mitochondria (m) and lysosomes (L). sinusoidal lumen (s). Mag. 6000 X.

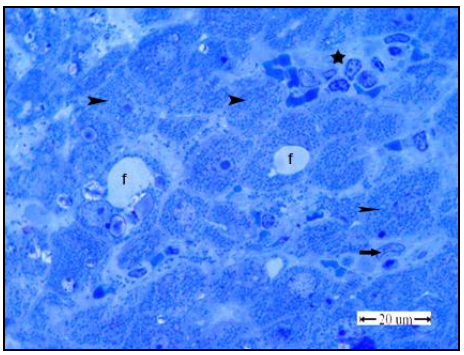

Fig. 3: Semithin section of liver of group two after four weeks of the experiment showing moderate degree of hydropic degeneration of the hepatic cells with presence of solitary large fat globules (f) in its cytoplasm. Some of hepatic cells showed pyknosis or chromatolysis of the nucleus (arrowheads) as well as mild congestion and mononuclear cell aggregation (star), and hypertrophy of the kupffer cells (arrow). Toluidine blue stain.

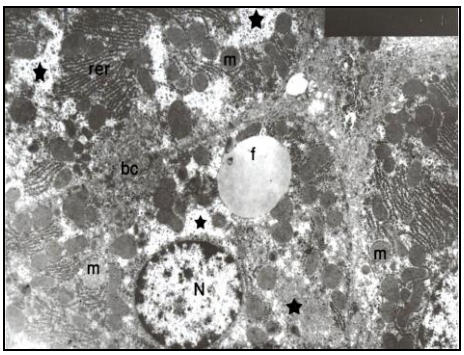

Fig. 4: Transmission electron micrograph of liver of group two at the end of the fourth week showing the hepatic cells contain large fat globules (f), vacuolation of the cytoplasm (stars), rough endoplasmic reticulum (rer), mitochondria (m), and compressed bile canaliculi (bc). Mag. 8800 X.

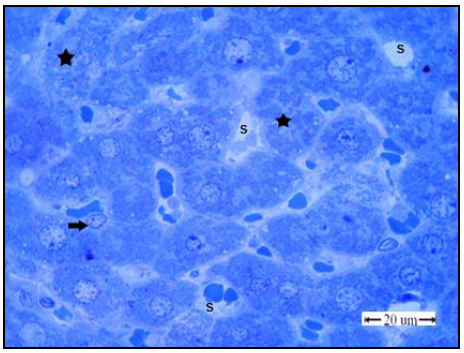

Fig. 5: Semithin section of liver of group one after eight weeks of treatment showing congestion of the hepatic sinusoids (s), hypertrophy of the kupffer cells (arrow) and hydropic degeneration of the hepatic cells (stars). Toluidine blue stain.

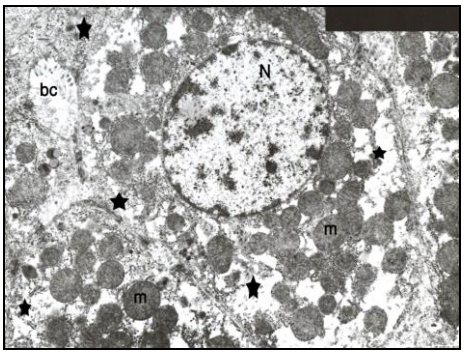

Fig. 6: Transmission electron micrograph of liver of group one at the end of the eight week showing vacuolation of the cytoplasm (star), normal nucleus (N), the rough endoplasmic reticulum lost its ribosomes, the bile canaliculi (bc) are dilated and the mitochondria of the hepatic cells (m) appeared slightly swollen. Mag. 8800 X.

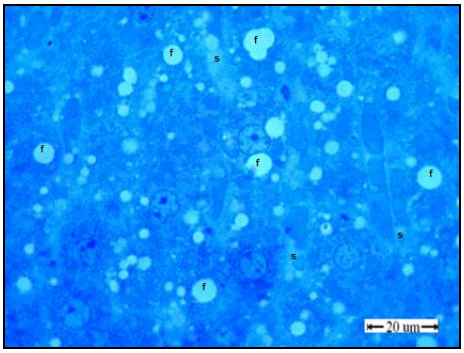

Fig. 7: Semithin section of liver of group two after eight weeks showing prominent large fat globules (f) in most of the hepatic cells, hydropic degeneration of the hepatic cells with chromatolysis of the nucleus of most hepatic cells with congestion of the sinusoids (s). Toluidine blue stain.

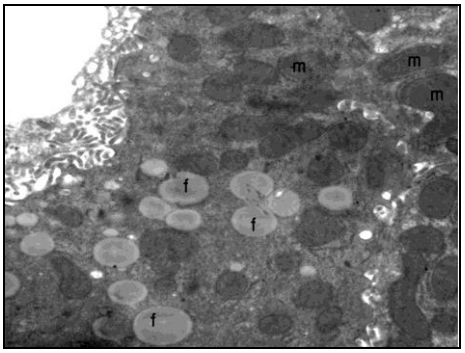

Fig. 8: Transmission electron micrograph of liver of group two at the end of the eight week showing presence of numerous fat globules (f) in the hepatic cells with swelling in mitochondria (m) and loss of cristae. Mag. 11000 X.

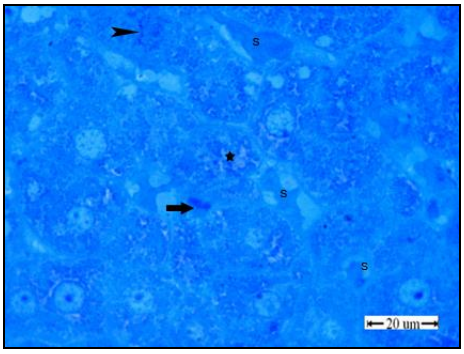

Fig. 9: Semithin section of liver of group one at the end of the experiment showing moderate degree of hydropic degeneration, appearance of some vacuoles in the hepatic cells seems to be fat droplets, chromatolysis of some nuclei (star) as well as shrinkage and corrugation of some hepatic cell nuclei (arrow head) and some hepatic cells in state of mitosis (arrow). Toluidine blue stain.

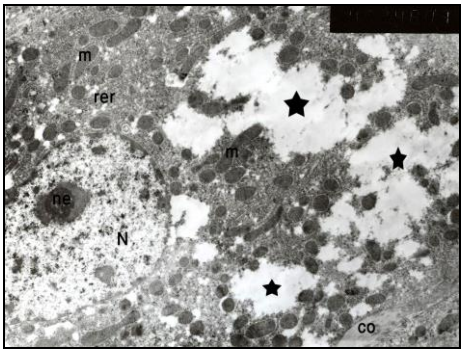

Fig. 10: Transmission electron micrograph of liver of group one at the end of the twelve week showing presence of bundles of collagen fibers (co), vacuolation of the cytoplasm (stars), the mitochondria (m) decreases in its population as well as the rough endoplasmic reticulum (rer). Mag 8800 X.

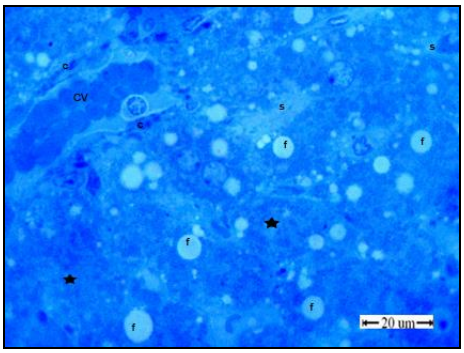

Fig. 11: Semithin section of liver of group two after ten weeks of the experiment showing sever congestion of the central vein CV), presence of collageous fibers (c) surrounding the central vein, presence of numerous variable size fat globules in the hepatic cells (f) and hydrpic degeneration of their cytoplasm (stars). Toluidine blue stain.

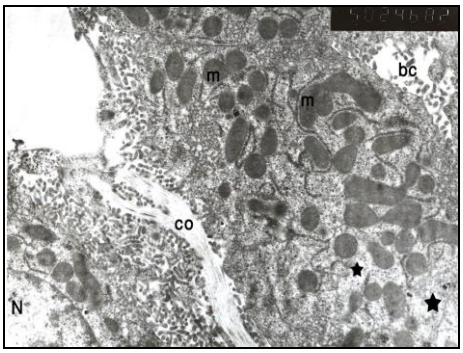

Fig. 12: Transmission electron micrograph of liver of group two at the end of the experiment showing presence of bundles of collagen fibers (co) in between the hepatic cells, mitochondria (m) lost its cristae, dilatation of the bile canaliculi (bc) and variable intercommunicating light electron dense areas (stars). Mag. 11000 X.

DISCUSSION:

The toxic effect of chloramphenicol on the liver was studied. The obtained results of liver enzymatic activities including AP and γ-GT concentrations in serum revealed a significant increase in their activities in both 25 mg/kg body weight (half the therapeutic dose) and therapeutic dose of chloramphenicol treated rats compared with control; while the level of AST revealed nonsignificant changes. This increase in serum level of these enzymes agree with earlier work of Aubrecht *et al.* (1997) on hygromycin B and Bhat *et al.*, (1998) on minocydine and also with advanced work by Effiong *et al.*, (2010). The increased levels of these enzymes in the serum were resulted mostly from the escape of these enzymes from injured liver cells or from the damage of billiary epithelium or from bile duct obstruction. Further findings in the study according to histopathology carried out indicated that there were degenerative changes of hepatic parenchymal cells. This is well corroborated by significant increase in serum activity in the animals administered with chloramphenicol.

The significant increase in the liver enzymes activities was attributed to liver damage as well as cytotoxic effect of chloramphenicol on the liver cells leading to leakage of alkaline phosphates from damaged hepatocytes and γ-GT from the affected billiary epithelium (Tietz, 1994). Cytochrome P_{450s} (CYPs) play a key role in the oxidation of numerous endogenous and exogenous compounds (Porter and Coon. 1991). The hepatotoxic effect of chloramphenicol is attributed to inhibition of hepatic cytochrome P_{450}; this criterion had been reported by Robillart *et al.*, (1998). Numerous studies on rodents have revealed that chloramphenicol is an inhibitor of CYP in liver microsomes (Porter and Coon, 1991; Rendic and Di Carlo, 1997).

In other advanced researches documented that chloramphenicol affects the drug removal mechanism through its inhibition of several enzyme activities, including the hepatic microsomal P_{450} enzymes amidase and esterase (Farombi, 2001; Somjetlerdcharoen, 2002). The inhibitory effect of chloramphenicol on human cytochrome P_{450} isoforms was evaluated with human liver microsomes and cDNA-expressed CYPs (Ji-Young *et al.*, 2003). Nonsignificant changes in AST could be explained according to Jubb and Kennedy, (1995) who stated that AST is no sensitive to chronic exposure of xenobiotics in rats.

Serum LDH concentrations showed a significant increase after 4th week of exposure in prophylactic treated group and after 2nd week of exposure in therapeutic-treated group until the end of experiment at 12th week. Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion of lactate to pyruvate. This is an important step in energy production in cells. Many different types of cells in the body contain this enzyme. Some of the organs relatively rich in LDH are the heart, kidney, liver, and muscle. Elevated LDH levels can be caused by a number of noncancerous conditions, including heart failure, hypothyroidism, anemia, and lung or liver disease (Pagana, *et al.*, 1998). Tissue breakdown

elevates levels of LDH, in medicine; LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis. It is used to follow-up cancer (especially lymphoma) patients, as cancer cells have a high rate of turnover with destroyed cells leading to an elevated LDH activity (Butt *et al.*, 2002). Therefore, increase the level of this enzyme in this study may be attributed to cytotoxic effect induced by chloramphenicol on multiple cellular compartments including hemolysis of red blood cells or hepatic tissue breakdown. Pathological findings recorded in this research confirmed this suggestion.

The toxic effect of chloramphenicol can be induced by different mechanisms. Antibiotics are actively involved in physiochemical and biochemical changes and in the production of free radicals such as reactive oxygen species (ROS) by specific chemical reactions (Teo *et al.*, 1986; Mates and Sanchez-Jimenez, 1999). Chloramphenicol and other ROS-inducing compounds (hydrazine, H_2O_2, etc.) cause structural changes in mitochondria (Wakabayashi and Karbowski, 2001), and in culture cells chloramphenicol is responsible for megamitochondria formation (MG) which appears to provoke apoptosis after long-term exposure (Karbowski *et al.*, 1999). The p-nitro group that characterizes chloramphenicol causes severe damage to DNA such as DNA strand breakage, DNA synthesis inhibition and mutations (Yunis, 1984), but also significant changes to macromolecules which have a role in detoxification and antioxidant mechanisms (Farombi, 2001). Enhanced production of ROS during chloramphenicol exposure is thought to be an important mechanism of pollutant-mediated toxicity in aquatic organisms (Wiesner *et al.*, 1991and Livingstone, 2001).

Histopathological studies in this research revealed degenerative changes were observed in the liver of rats from both groups treated with chloramphenicol. However, these lesions varied greatly in its severity and distribution according to the duration of exposure and the dose of chloramphenicol. Hydropic degeneration with hypertrophy of the kupffer cells associated with the rough endoplasmic reticulum lost its ribosomes, the mitochondria appeared slightly swollen, the bile canaliculi dilated and some areas of necrosis was the most common histopathological changes observed in prophylactic dose-treated rats. Intensity and distribution of these changes were severe and extended to involve a massive area of the hepatic parenchyma in the rats with therapeutic exposure including presence of solitary fat globules in some hepatic cells and the mitochondria lost its cristae. Also chromatolysis as well as pyknosis of some hepatic cell nuclei. Similar lesions were observed in the liver of rats administered chloramphenicol by Esimone *et al.* (2007).

Hydropic, fatty degeneration and necrosis were the manifestation of the cytotoxic effect of chloramphenicol on hepatic cells as chloram-phenicol metabolites have mainly a cytotoxic effect which was demonstrated by inhibition of incorporation of tritiated thymidine into DNA (Robbana-Barnat *et al.*, 1997). It might also due

to hypoxia result from anemia observed frequently in experimental groups (Turton *et al.*, 2000). It is well known that chloramphenicol as hepatic cytochrome P_{450} inhibitor in the hepatocytes play an important role in the disturbance of the metabolism of the hepatic cells leading to fatty degeneration and necrosis (Robillart *et al.*, 1998). Hepatocellular degeneration, congestion, dilation of hepatic sinusoids and hypertrophy of the kupffer cells were commonly observed in rats sacrificed at an early stage of experimental period. Dilatation of the bile canaliculi, swelling in hepatic cells, and congestion of the hepatic vasculature, accumulation of fat globules and presence of bundles of collagen fibers in between the hepatic cells and in the Disse space represented chronic reaction to chloramphenicol toxicity or side effect. Areas of cellular reaction consist of hypertrophy of Kuppfer cells were observed. Such reaction could be considered as secondary for hepatocellular degeneration and necrosis.

Dilatation of the bile caniculi was constantly observed as well as fibroblastic reaction in between hepatic cells in the portal tract. These two lesions represent chronic reaction process for chloramphenicol toxicity and an attempt for healing by substitution (Jubb and Kennedy, 1995). In the group of therapeutic dose treated rat administrated chloramphenicol for long-period, the mitochondria lost its cristae were frequently observed in the hepatic cells. Interference of chloramphenicol to antioxidants in mouse hepatic cells was documented with Holt *et al.* (1997). Chloramphenicol has a cytotoxic effect on mitochondrial ribosomes (Kucers *et al.*, 1997). Thus chloramphenicol can also inhibit mitochondrial protein synthesis in mammalian cells. The inhibition of mitochondrial protein synthesis suppressed the division of mitochondria and resulted in the formation of megamitochondria (Matsuhashi *et al.*, 1996). It appeared that chloramphenicol has a deleterious effect on oxidative status of the hepatic cell parenchyma and might lead to hepatic dysfunction. The later was manifested by hypoproteinaemia, hypoalbuminemia, and increased activity of some serum enzymes related to hepatic function. Hydropic, fatty degeneration and necrosis were due to direct effect of chloramphenicol on the hepatic cells and were primary in nature representing a disturbance of metabolism of hepatic cells. Some areas of cellular reaction of Kuppfer cells type considered secondary manifestation of hepatocellular degeneration. Fibroblastic reaction was observed in such cases and represents an attempt for healing by reconstitution Needham *et al.* (1996).

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التأثيرات السمية للكلورامفينيكول على الكبد في الفئران البيضاء

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المضادات الحيوية البيطرية كثيرة الاستعمال في الحيوانات الأليفةِ وفي حيوانات المزرعة لمنعِ ومعالجةِ الأمراضِ ولزيادة معدلات النمو أحيانا والاستعمال الغير مناسب وسوء الاستخدام يجلب لتلك الحيوانات أضراراً كثيرة مثل مقاومة الدواء والتسمّم الحادّ أو المُزمن وكذلك أضراراً أشدّ لمستهلكي منتجات هذه الحيوانات مثل ردود أفعال والحسّاسية نتيجة استخدام لحوم الدواجنِ وبيضها أو لحوم الماشيةِ وألبانها. الكلورامفينيكول مضاد حيوي واسع المدى ذو فاعلية على البكتيريا الموجبة الجرام والسالبة الجرام على حد سواء ويؤثر أيضاً على الريكتسيا والبكتيريا اللاهوائية مما يجعله مادة دوائية غير مكلفة وذات قيمة علاجية ووقائية كبيرة.

وحديثاً أوضحت الدراسات أن عدداً من أصحاب المناحل يستخدمون الكلورامفينيكول، دون أن يكونوا على علم بأخطاره على البشر، وأن عسل النحل الموجود بالسوق المصرية يحتوي أغلبه علي مادة الكلورامفينيكول، وكذلك أكدت نتائج الدراسات التي قام بها العديد من الباحثين وجود متبقيات الكلورامفينيكول في لحوم الحيوانات والدواجن الأمر الذي يؤكد أن الكلورامفينيكول لا يزال يستخدم في العلاج على الرغم من التحذير المستمر من استعماله. ومن هنا كانت الحاجة لتَصميم هذه التجربةِ لاستكشاف تأثيرِ هذا الدواء على عِدّة مؤشرات في دم وكبد الفئران البيضاء بعد استخدام جرعة وقائيةَ وأخرى علاجّية لمدة ١٢ أسبوع.

تم استخدام مجموع ٢٤٠ جرذٍ أبيض ذكرٍ تم شراؤها من بيت الحيوان بكلية الطب - جامعة أسيوط قُسّمَت إلى أربع مجموعاتٍ، بواقع ٦٠ فأراً في كُلّ منها. تم استخدام محلول سكسنات الكلورامفينيكول التي تمت إذابتها في الماءِ المُقَطَّرِ، وأُعطيت للفئران باستخدام اللي المعدي في حجم جرعةٍ ثابتٍ بواقع ١٠ ملي لتر/كيلوجرام من وزن الجسم. تم إعطاء جرذان المجموعة الأولي جرعة مِنْ الكلورامفينيكول مقدارها ٢٥ مجم كلورامفينيكول/كيلوجرام من وزن الجسم يومياً (نِصْف الجرعةِ العلاجّية) لمدة ثلاثة شهور. تم إعطاء الجرذان في المجموعة الثانية جرعة مِنْ الكلورامفينيكول مقدارها ٥٠ مجم كلورامفينيكول/كيلوجرام من وزن الجسم يومياً (الجرعة العلاجّية) لمدة ثلاثة شهور. تم إعطاء الجرذان في المجموعتين الثالثة والرابعة ماءَ مُقَطَّرَ بواقع ١٠ ملي لتر/كيلوجرام من وزن الجسم كمجموعتين ضابطين موازيتين للمجموعتين الأولي والثانية.

تم إعدام عدد ١٠ من الجرذان تحت تأثير المخدر في كُلّ المجموعاتِ التجريبيةِ، بعد ٢, ٤, ٦, ٨, ١٠، ١٢ أسبوع من بدء التجربة. تم أخذ عينات الدمِ وفصل المصل منه لإجراء الفحوص البيوكيميائية وتحديد أنشطة الكبد كما تم أخذ عينات الكبد للفحص الهستوباثولوجي. تم قياس الأسبارتيت أمينو ترانسفيريز (AST) والجاما جلوتاميل ترانسفيريز (γ-GT) والفوسفاتيز القاعدي (Alkaline Phosphatase)، وخميرة اللاكتات ديهيدروجينيز (LDH) في مصل الدم لتحديد مدي تأثير الكلورامفينيكول على وظائف الكبد. وتم الفحص بالميكروسكوب الضوئي لنسيج الكبدَ لتحديد التأثيرات الباثولوجية، وتم استكمال فحصها بالميكروسكوب الاليكتروني.

حدثت زيادة معنوية في نشاط خمائر الفوسفاتيز القاعدي (Alkaline Phosphatase, AP) الجاما جلوتاميل ترانسفيريز (γ-GT) التي تقيس نشاط الكبد في الحيوانات التي تعرضت لكلتا الجرعتين الوقائية والعلاجّية من الكلورامفينيكول مقارنة بالمجموعة الضابطة. وتعزي هذه الزيادة في مستويات هذه الإنزيماتِ في المصلِ إلي هروبِ هذه الإنزيماتِ مِنْ خلايا الكبدِ المصابةِ بالتسمم أو مِنْ ضررٍ يكون قد أصاب المرارة أو أدى إلى انسداد القنياتِ المرارية.

أظهرت نتائج الفحص الهستوباثولوجى ما يؤيد هذه النتائج في الدراسةِ حيث أشارتْ إلى وجود تغييراتَ باثولوجية في خلايا الكبد ويمكن تفسير الزيادةِ المعنوية في نشاطِ هذه الخمائر في مصلِ الدم إلى التأثيرَ السامّ للكلورامفينيكول على الكبدَ الذي أدى إلى تثبيط إنزيمات السيتوكروم P_{450}.

ارتفع مستوي (LDH) في مصل الدم ارتفاعاً معنوياً بعد الأسبوع الرابع مِنْ التعرّضِ للكلورامفينيكول في المجموعةِ الوقائيةِ، وبعد الأسبوع الثاني مِنْ التعرّض في المجموعةِ العلاجّيةِ، وظل على هذا الارتفاع حتىَ نهايةِ التجربةِ في الأسبوع الثاني عشر. وتعزى النسبة العالية من (LDH) في المصل للتكسير الحادث في أنسجة الكبد، وتشير زيادةُ مستوى هذا الإنزيم في مصل الدم في هذه الدراسةِ إلى التأثيرِ السام للكلورامفينيكول على خلايا الكبد ذاتها (Cytotoxic effect). كشفت الدراسات التشريحية المرضية في هذا البحث عن تغيرات هستوباثولوجية مختلفة في كبد الفئران. هذه التغيرات تختلف كثيراً في حدتها وتوزيعها وفقاً لمدة التعرض والجرعة من الكلورامفينيكول. ظهر تورم الميتوكوندريا، وكذلك توسع في القنيات الصفراوية، وتورم في خلايا الكبد، واحتقان الأوعية الدموية في الكبد، وتراكم كريات من الدهون مع وجود حزم من ألياف الكولاجين بين الخلايا الكبدية كرد فعل للتسمم المزمن للكلورامفينيكول أو الآثار الجانبية لاستخدامه.

الاختلاف الظاهر في نَتائجِ هذه الدراسة بين الجرعةِ الوقائيةِ والجرعة العلاجّيةِ للكلورامفينيكول هو فقط في وقتْ ظهورِ هذه التغييراتِ وحدتها حيث يسبق ظهورها في الجرعات العالية تلك في الجرعات المنخفضة، وتكون في الحالة الأولي أشد حدة من الثانية. إن وجود متبقيات المضادات الحيوية وفي القلب منها الكلورامفينيكول يشكل مصدر خطر على صحة الإنسان، فقد يظهر حالات حساسية جلدية, غثيان, قيئ, وربما صدمة وربما الموت، كما وجد أن الاستعمال الحكيم للمضادات الحيوية والتوعية الصحية على استعمالها بشكل صحيح والتربية والذبح الصحي في المجازر، وتحت إشراف طبي بيطري كامل سوف يساعد على انقاص مقاومة الأدوية في الإنسان والحيوان. ينصح بعدم استخدام الكلورامفينيكول لفترات طويلة أو متكررة، ولو بجرعات صغيرة لتفادى آثاره الجانبية الضارة على الحيوان والإنسان، وأن يقتصر استعماله في الحالات التي لا تستجيب لأي مضاد حيوي آخر مع الوضع في الاعتبار ألا يكون الحيوان منتجاً للغذاء. وكذلك يجب التفكير في إصدار قراراً يتضمن منع استعمال مادة الكلورامفينيكول في الحيوانات والطيور المنتجة للغذاء في جمهورية مصر العربية حفاظاً علي صحة المستهلكين لهذه المنتجات.