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# Effect of tetracycline on pancreas and liver function of adult male albino rats

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## Abstract

The effect of tetracycline, at two doses of 50 and 200 mg $kg^{-1}$ daily, was studied on pancreatic and liver tissue function for 14 and 21 days in adult male albino rats. For pancreatic function the parameters studied were content of amylase and lipase in pancreas, serum amylase and lipase, serum glucose and faecal fat excretion. For liver function, liver specific enzymes in serum, namely alanine amino transaminase, aspartate amino transaminase and lactate dehydrogenase were estimated. In addition, total lipid, antiperoxidative enzymes and lipid peroxidation were measured in pancreas and liver. The content of amylase and lipase in pancreas showed a small but significant decrease in the rats given 50 mg $kg^{-1}$ for 21 days and the decrease was much more significant in those receiving the 200 mg $kg^{-1}$ dose. In pancreas free radical levels show a significant increase and reduced glutathione shows a substantial decrease at the 50 mg $kg^{-1}$ level and a significant change in these parameters was observed at the 200 mg $kg^{-1}$ dose. Antioxidant enzymes, superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase, showed a small but significant decrease in the pancreas of the rats treated with 50 mg $kg^{-1}$ tetracycline. A significant decrease in the antioxidant enzymes level was observed at the 200 mg $kg^{-1}$ dose. In the liver, free radical levels and reduced glutathione were within the normal range at the 50 mg $kg^{-1}$ level and significant changes were observed at 200 mg $kg^{-1}$. The antioxidant status was unaffected in liver after treatment with tetracycline at the 50 mg $kg^{-1}$ level and a significant decrease was observed at the higher dose. Our results reveal the safe nature of tetracycline with respect to the liver at the lower dose tested, whereas, both the higher and lower doses seem to have detrimental effect on the pancreas as revealed by the rise in free radical levels and decrease in the antioxidant enzyme levels.

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## Introduction

Indiscriminate use of antibiotics in aquaculture farms to combat or prevent infections is one of the commonest forms of misuse of drugs. Consumption of fish contaminated with antibiotic residues results in the risk of adversely affecting human health. Hepatotoxicity after administration of large doses of antibiotics, including tetracycline and chloramphenicol, is well established (Schultz et al 1963; De Jonge 1973). However, whether the hepatotoxicity in conjunction with oxidative stress is dose- and duration-dependent has not been documented.

Liver is the major organ involved in metabolizing drugs and also it is well established that most drugs at high doses cause hepatotoxicity (Farrel 1997; Sherlock & Dooley 2002; Bass 2003; Lee 2003; Rashid et al 2004). Antibiotics are reported to have a correlation with diabetes and pancreatic function (Dibas et al 1995; Malaisse et al 1996). There are contradictory reports about the effect of antibiotics on the pancreatic islets, some suggesting their harmless action (White et al 1997), some depicting a beneficial role (Hiramatsu et al 2000) and others indicating deleterious effect (Boschero & Delattre 1985). This investigation was undertaken to study liver and pancreatic damage in rats by administration of tetracycline at two different doses and for two different time periods.

## Materials and Methods

### Chemicals

Tetracycline was obtained from Sigma Chemical Co. (USA) and all chemicals used were of analytical grade.

### Animals

Adult male albino rats, 150–200 g, were used. Rats were housed individually in polypropylene cages at an animal facility in the Institute at constant room temperature (22°C), under a 12-h light–dark cycle. They were maintained on standard diet and water was freely available up to the time of experimentation and weekly body weights were recorded. This study was implemented according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and authorized by the Animal Ethics Committee of the Institute.

### Experimental design

Adult male albino rats were divided into 3 groups. Group 1 (six nos.), 2 (12 nos.) and 3 (12 nos.) rats were fed with standard commercial diet. Groups 2 and 3 received the drug tetracycline (LD50: 318 mg $kg^{-1}$) intraperitoneally, at a dose of 50 and 200 mg $kg^{-1}$ daily, respectively. The drug solution was prepared by dissolving the required amount in normal saline. One half of the rats in groups 2 and 3 were sacrificed after 14 days of drug administration and the second half after 21 days. Group 1 rats served as the control and were sacrificed at the end of 21 days. The rats were treated with the drug for the desired period and the treated rats as well as controls were sacrificed following chloroform anaesthesia, a method approved by CPCSEA. Liver and pancreatic tissue were excised immediately and washed with chilled isotonic saline, frozen, and stored at −20°C for analysis. Faecal matter was collected separately for each group of rats and stored at –20°C until analysis

The liver and pancreatic tissue homogenates prepared in ice-cold 0.1 M Tris-HCl buffer, pH 7.2, were used for the determination of lipid peroxides (LPO) (Okhawa et al 1979), reduced glutathione (GSH) (Ellman 1959), glutathione peroxidase (GPx) [EC 1.11.1.9] (Pagila & Valentine 1967), glutathione-S-transferase (GST) [EC 2.5.1.18] (Habig et al 1974), catalase (CAT) [EC 1.11.1.6] (Takahara et al 1960) and superoxide dismutase (SOD) [EC 1.15.1.1] (Misra & Fridovich 1972). The protein content of the homogenates was estimated (Lowry et al 1951). Liver homogenate was also assayed for aspartate [EC 2.6.1.1] and alanine transaminases [EC 2.6.1.2] (Cheung & Briggs 1974) and lactate dehydrogenase [EC 1.1.1.27] (Bonting et al 1960). Pancreatic homogenate and serum were assayed for pancreatic amylase [EC 3.2.1.1] (Bernfeld 1955) and lipase [EC3.1.1.3] (Roberts et al 1985). Faecal excretion of fat was determined by chloroform–methanol extraction (Schwarz et al 1996). Histological studies of the pancreas and liver were conducted as described earlier (Chowdary at al 1992). Briefly, the pancreas and liver tissue from rats were carefully isolated, trimmed of fat and fixed in 10% buffered formalin. The formalin-fixed specimens were then dehydrated with ethanol and embedded in paraplast. Sections of 5–6 $\mu$m were cut and stained with haematoxylin and eosin.

### Statistical analysis

Each sample was tested in duplicate. Values are expressed as mean ± s.d. One-way analysis of variance was carried out, and the statistical comparisons among the groups were performed by Student's *t*-test. $P < 0.05$ was considered significant.

## Results

### Liver

The levels of liver-specific enzymes, aspartate and alanine transaminases and lactate dehydrogenase, in serum are given in Table 1. These enzymes were found to be within the normal range in group 1 (control) and group 2 rats, but slightly above normal range in group 3. The rats treated with 200 mg $kg^{-1}$ tetracycline for 21 days had the highest content of these enzymes.

In liver of rats injected intraperitoneally with 50 mg $kg^{-1}$ tetracycline, lipid peroxide levels measured in terms of

**Table 1** Activity of aspartate aminotransaminase, alanine aminotransferase and lactate dehydrogenase enzymes in serum of control and experimental rats

| Parameter | Group 1 | Group 2 | | Group 3 | |
|---|---|---|---|---|---|
| | | 14 days | 21 days | 14 days | 21 days |
| AST | 20.6 ± 4.5 | 23.2 ± 5.2 | 23.9 ± 3.6 | 42.3 ± 6.7* | 59.6 ± 5.8*† |
| ALT | 21.5 ± 4.1 | 24.1 ± 3.9 | 22.4 ± 4.4 | 51.7 ± 6.5* | 66.8 ± 4.9*† |
| LDH | 76 ± 11 | 78 ± 8 | 74 ± 13 | 185 ± 11* | 201 ± 15*† |

Group 1, 2 and 3 rats were fed with commercial diet. Groups 2 and 3 received the drug tetracycline intraperitoneally (LD50: 318 mg $kg^{-1}$) 50 mg and 200 mg $kg^{-1}$ daily, respectively. One half of the rats in groups 2 and 3 were sacrificed after 14 days of drug administration while the other half after 21 days. Values are expressed as mean ± s.d. for six rats and are $\mu$mol pyruvate liberated/hour/litre. *$P < 0.001$, compared with Group 1 control rats; †$P < 0.001$, compared with Group 2 rats.

**Table 2** Levels of lipid peroxides (LPO) and reduced glutathione (GSH) and activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and catalase (CAT) in liver of control rats and rats treated with tetracycline at 50 and 200 mg $kg^{-1}$ daily for 14 or 21 days

| | Group 1 | Group 2 | | Group 3 | |
|---|---|---|---|---|---|
| | | 14 days | 21 days | 14 days | 21 days |
| LPO | 1.188 ± 0.2 | 1.19 ± 0.04 | 2.09 ± 0.09 | 2.54 ± 0.02* | 2.92 ± 0.05*$^{\dagger\#}$ |
| GSH | 381.1 ± 14 | 378.6 ± 12.1 | 379.2 ± 9.2 | 337.0 ± 11.6* | 321 ± 8.3*$^{\dagger}$ |
| SOD | 12.63 ± 0.1 | 11.7 ± 0.3 | 11.03 ± 0.6 | 7.12 ± 0.18* | 5.41 ± 0.26*$^{\dagger}$ |
| GPx | 0.82 ± 0.09 | 0.8 ± 0.07 | 0.78 ± 0.09 | 0.58 ± 0.09* | 0.42 ± 0.04*$^{\dagger}$ |
| GST | 2.94 ± 0.22 | 2.65 ± 0.52 | 2.91 ± 0.24 | 1.36 ± 0.32* | 0.96 ± 0.22*$^{\dagger}$ |
| CAT | 8.18 ± 0.39 | 7.83 ± 0.21 | 7.94 ± 0.35 | 4.95 ± 0.16* | 3.18 ± 0.33*$^{\dagger}$ |

Group designations as for Table 1. Results are mean ± s.d. for 6 rats. Values are expressed as: LPO, ng of thiobarbituric acid (TBA) reactive substances (p/mg protein)$^{-1}$; GSH, $\mu$mol (g wet tissue)$^{-1}$; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation; GPx, nmol GSH oxidized $min^{-1}$ (mg protein)$^{-1}$; GST, mmol 1-chloro-2,4-dinitrobenzene conjugate formed $min^{-1}$ (mg protein)$^{-1}$; CAT, nmol $H_2O_2$ decomposed $min^{-1}$ (mg protein)$^{-1}$. *$P < 0.001$, compared with Group 1 control rats; $^{\dagger}P < 0.001$, $^{\#}P < 0.05$, compared with 14-day tetracycline-treated rats within Group 3.

**Table 3** Levels of total lipid in liver and pancreas of control and experimental rats

| Organ | Group 1 | Group 2 | | Group 3 | |
|---|---|---|---|---|---|
| | | 14 days | 21 days | 14 days | 21 days |
| Liver | 42.93 ± 2.06 | 43.40 ± 3.71 | 41.81 ± 4.33 | 89.24 ± 3.52$^{\dagger}$ | 101 ± 4.75$^{\dagger\ddagger}$ |
| Pancreas | 24.14 ± 1.29 | 29.19 ± 3.36* | 32.21 ± 4.11$^{\dagger\#}$ | 41.32 ± 4.58$^{\dagger}$ | 53.37 ± 2.61$^{\dagger\ddagger}$ |

Group designations are as for Table 1. Results are mean ± s.d. for 6 rats. Values expressed as mg (g tissue)$^{-1}$. *$P < 0.05$, $^{\dagger}P < 0.001$, compared with control; $^{\#}P < 0.05$, $^{\ddagger}P < 0.001$, compared with 14-day study within tetracycline-treated groups.

malondialdehyde (MDA) and GSH did not exhibit significant changes (Table 2). However, the levels of these parameters showed a significant ($P < 0.001$) change in the liver of rats that received 200 mg $kg^{-1}$ tetracycline. Also, the change in the levels of these parameters in this group seems to be dependent on duration of administration of the drug, with the change being considerably higher for the 21-day study than the 14-day study. This indicates that substantial increase in lipid peroxidation has occurred in the liver at the higher but not the lower dose.

Antioxidant enzymes, superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase, remained unaffected and in some cases marginally lower in liver of rats after treatment with tetracycline at the 50 mg $kg^{-1}$ level whereas their concentration was significantly ($P < 0.001$) decreased in rats treated with 200 mg $kg^{-1}$ (Table 2).

Total lipid levels in liver of rats given 50 mg $kg^{-1}$ tetracycline remained almost as in the control group (Table 3), while significant ($P < 0.001$) increase was observed in the rats that received 200 mg $kg^{-1}$. The fat content was significantly more in the 21-day study ($P < 0.001$) (Table 3) in the group that received 200 mg $kg^{-1}$ when compared with the rats that received the drug for 14 days.

Histopathology of liver of control rats and rats treated with tetracycline at 50 and 200 mg/kg body weight for 21 days are shown in Figure 3. Steatosis or fat accumulation is observed in B which is seen to worsen in C.

## Pancreas

The pancreatic and serum content of amylase and lipase are shown in Table 4. The levels of amylase in pancreas showed small but significant ($P < 0.05$) decrease in group 2 rats treated with tetracycline 50 mg $kg^{-1}$ for 21 days and no significant decrease was observed in rats treated for 14 days. Rats in group 3 showed a very significant ($P < 0.01$) decrease in amylase over the control group. The decrease in the levels of lipase in pancreas was not significant in group 2 over the control group. However, a significant ($P < 0.01$) decrease in lipase levels was observed in group 3. There was dose- and duration-dependent increase in the levels of serum amylase and lipase. In group 2 rats treated with the drug at the lower dose for 14 and 21 days, the levels were significantly increased ($P < 0.05$ and $P < 0.01$, respectively) over the control group. In group 3 rats treated with tetracycline at the dose of 200 mg $kg^{-1}$, the increase in these enzymes was much more significant ($P < 0.001$) over the control group.

Figure 1 shows the level of serum glucose in rats. In group 2 rats and 14-day treated rats in group 3, the levels of serum glucose remained comparable with the control group.

**Table 4** Levels of amylase and lipase in serum and pancreas of control and experimental rats

| | Group 1 | Group 2 | | Group 3 | |
|---|---|---|---|---|---|
| | | 14 days | 21 days | 14 days | 21 days |
| Amylase S[a] | 72.4 ± 9 | 83.2 ± 6 | 89.5 ± 8* | 162 ± 17† | 214 ± 13† |
| Amylase P[b] | 32.6 ± 5 | 30.5 ± 7 | 26.6 ± 3* | 17.3 ± 6† | 11.4 ± 5† |
| Lipase S[a] | 34.7 ± 5 | 39.4 ± 4 | 51.6 ± 9* | 170 ± 12† | 191 ± 15† |
| Lipase P[b] | 95.5 ± 10 | 93.1 ± 6 | 96.4 ± 9 | 73.2 ± 6† | 55.7 ± 6† |

Group designations are as for Table 1. Values expressed as mean ± s.d. for six rats. [a]IU $L^{-1}$; [b]U (mg protein)$^{-1}$ (mg pancreas)$^{-1}$. One unit is the amount of enzyme that releases one micromole of products at 37°C under standard assay conditions. *$P < 0.05$, †$P < 0.001$, compared with control rats.

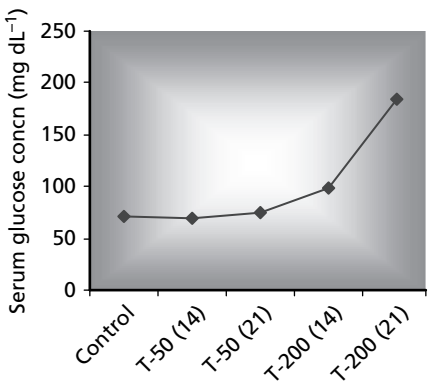


**Figure 1** Levels of serum glucose in control and experimental groups of rats. Group designations are: T-50 (14), rats fed with 50 mg $kg^{-1}$ tetracycline for 14 days; T-50 (21), rats fed with 50 mg $kg^{-1}$ tetracycline for 21 days; T-200 (14), rats fed with 200 mg $kg^{-1}$ tetracycline for 14 days; T-200 (21), rats fed with 200 mg $kg^{-1}$ tetracycline for 21 days. Values expressed as mean ± s.d. for six rats.

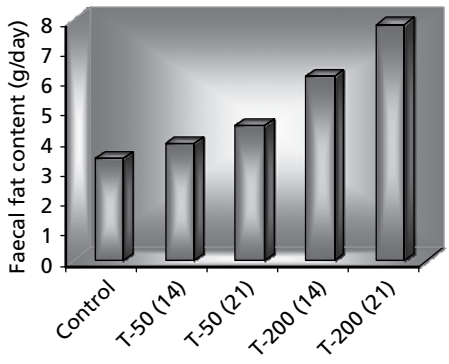


**Figure 2** Faecal fat in control and experimental groups of rats. Group designations are: T-50 (14), rats fed with 50 mg $kg^{-1}$ tetracycline for 14 days; T-50 (21), rats fed with 50 mg $kg^{-1}$ tetracycline for 21 days; T-200 (14), rats fed with 200 mg $kg^{-1}$ tetracycline for 14 days; T-200 (21), rats fed with 200 mg $kg^{-1}$ tetracycline for 21 days. Values expressed as mean ± s.d. for six rats.

The rats treated for 21 days in group 3 had serum glucose levels that were very high ($P < 0.001$) compared with the control rats. Figure 2 shows the fat excretion in control and tetracycline-treated rats. Fat excreted in group 2 rats was comparable with fat excreted in the control group, whereas there was a significant ($P < 0.001$) rise in fat content of excreta in group 3 rats especially in rats treated with tetracycline for 21 days.

Levels of MDA and GSH showed small but significant ($P < 0.05$) change in pancreas of rats treated with 50 mg tetracycline per kg body weight for a 14-day period, when compared with controls, indicating that a significant increase in lipid peroxidation (Table 5) occurred in the pancreas at this dose. The values of MDA and GSH in the pancreas of rats treated with 50 mg tetracycline per kg body weight varied significantly ($P < 0.001$) with the duration of treatment, with levels being significantly higher for the 21-day period. A much higher rise and a significant drop ($P < 0.001$) was observed in the levels of MDA and GSH, respectively in the pancreas of rats administered 200 mg $kg^{-1}$ tetracycline (Table 5), suggestive of occurrence of significant oxidative stress in the organ at this dose. Oxidative stress was decidedly more significant ($P < 0.001$) in the pancreas of the rats sacrificed at the end of the 21-day period (Table 5) when compared with that in the pancreas of rats sacrificed at the end of the 14-day period at both the doses studied.

The antioxidant status decreased significantly ($P < 0.05$) in the pancreas of the rats treated with 50 mg $kg^{-1}$ tetracycline for the 14-day period, when compared to the controls. At the same dose, the rats treated for 21 days showed greater decrease ($P < 0.001$) in antioxidant enzyme levels. A significant decrease ($P < 0.001$) in the antioxidant enzymes levels was observed in the pancreas of the rats treated with 200 mg $kg^{-1}$ of tetracycline (Table 5). The decrease was distinctly prominent in the pancreas of the rats sacrificed at the end of 21-day study for both the doses tested.

Total lipid levels in pancreas of rats given 50 mg $kg^{-1}$ tetracycline for 14 days showed a marginal increase when compared with the control group (Table 3), while significant ($P < 0.05$) increase was observed in the rats that received the lower dose for 21 days. The rats treated with 200 mg $kg^{-1}$ of the drug had significant ($P < 0.001$) increases in lipid content when compared with the control group. In the group that received 200 mg $kg^{-1}$, the fat content was significantly greater for the 21-day study ($P < 0.001$) (Table 3) than for the 14-day study.

Histopathology of pancreas of control rats and rats treated with tetracycline at 50 and 200 mg/kg body weight for 21 days are shown in Figure 4. Ultrastructural changes are observed

**Table 5** Levels of lipid peroxides (LPO) and reduced glutathione (GSH) and activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and catalase (CAT) in pancreas of control rats and rats treated with tetracycline at 50 or 200 mg $kg^{-1}$ daily for 14 or 21 days

| | Group 1 | Group 2 | | Group 3 | |
|---|---|---|---|---|---|
| | | 14 days | 21 days | 14 days | 21 days |
| LPO | 1.27 ± 0.4 | 1.65 ± 0.2* | 1.93 ± 0.4$^{\dagger\#}$ | 2.27 ± 0.6$^{\dagger}$ | 2.98 ± 0.2$^{\dagger\#}$ |
| GSH | 356.4 ± 3.80 | 342.2 ± 4.12* | 334.2 ± 3.7$^{\dagger\#}$ | 312.4 ± 5.7$^{\dagger}$ | 298.4 ± 6.8$^{\dagger\#}$ |
| SOD | 3.81 ± 0.03 | 2.90 ± 0.3* | 1.968 ± 0.09$^{\dagger\#}$ | 1.45 ± 0.15$^{\dagger}$ | 0.93 ± 0.07$^{\dagger\#}$ |
| GPx | 0.041 ± 0.004 | 0.033 ± 0.002 | 0.02 ± 0.005$^{\dagger\#}$ | 0.017 ± 0.001$^{\dagger}$ | 0.012 ± 0.002$^{\dagger\#}$ |
| GST | 0.49 ± 0.03 | 0.4 ± 0.03* | 0.29 ± 0.02$^{\dagger\#}$ | 0.21 ± 0.022$^{\dagger}$ | 0.16 ± 0.01$^{\dagger\#}$ |
| CAT | 0.54 ± 0.036 | 0.41 ± 0.039* | 0.35 ± 0.02$^{\dagger\#}$ | 0.27 ± 0.02$^{\dagger}$ | 0.21 ± 0.05$^{\dagger\#}$ |

Group designations are as for Table 1. Results are mean ± s.d. for 6 rats. Values are expressed as: LPO, ng of TBA reactive substances (mg protein)$^{-1}$; GSH, $\mu$mol (g wet tissue)$^{-1}$; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation; GPx, nmol GSH oxidized $min^{-1}$ (mg protein)$^{-1}$; GST, mmol 1-chloro-2,4-dinitrobenzene conjugate formed $min^{-1}$ (mg protein)$^{-1}$; CAT, nmol $H_2O_2$ decomposed $min^{-1}$ (mg protein)$^{-1}$. *$P < 0.05$, $^{\dagger}P < 0.001$, compared with Group 1 control rats; $^{\#}P < 0.001$, compared with 14-day tetracycline-treated sub-group within Groups 2 and 3.

in the parenchyma of pancreas in B. Extensive parenchymal necrosis and fibrosis is seen in C.

## Discussion

In this study it was shown that tetracycline did not cause increase in lactate dehydrogenase in serum at a dose of 50 mg $kg^{-1}$ but it did significantly at a dose of 200 mg $kg^{-1}$. The increase was also duration dependent. This observation implies that tetracycline cause liver cell death at the higher dose, which results in leakage of the liver cell marker enzyme into the blood stream (Makarenko et al 1994). Evidence that tetracycline also causes significant parenchymal cell damage is given by the increased levels of serum ALT and AST (Navarro & Senior 2006). On the other hand, tetracycline seems to have caused injury to the pancreas at both the lower and higher doses, as implied by the significant rise in serum levels of amylase and lipase (Nicolau et al 1991). In addition, the lowered levels of pancreatic amylase and lipase give further indication that the drug causes pancreatic cell damage (Tucker & Webster 1972). Fat excretion studies carried out to assess exocrine function suggest that pancreas remains essentially functional when tetracycline is administered at the lower dose, but at the higher dose loss in function occurs, as suggested by the increased amount of fat excreted in the faeces. Several studies have been reported wherein tetracycline has been found to cause acute pancreatitis leading to exocrine dysfunction. The amount of fat excreted also increases with the duration of treatment with tetracycline. Glucose concentrations in serum register a significant increase in rats treated with higher dose of tetracycline, indicating a loss in endocrine function of pancreas. Studies report that tetracycline administration causes hyperglycaemia (Storozhuk & Shamsutdinova 1972). The increase is far larger in the rats treated with tetracycline for 21 days than in those treated for 14 days; no significant change was observed in serum glucose, in group 2.

Tetracycline at the doses tested, caused oxidative stress in the liver and pancreas to varying degrees, by reducing the levels of antioxidant enzymes and GSH and by elevating the levels of lipid peroxides. Also it was demonstrated that the oxidative stress is dose and duration dependent and that the two organs studied, liver and pancreas, respond differently to the drug. Many studies have demonstrated the ability of antibiotics to facilitate the generation of oxygen radicals both in-vivo and in-vitro, and this process plays an important role in antibiotic-induced tissue injuries, like nephrotoxicity and ototoxicity (Conlon et al 1999). Use of high efficiency of antioxidants, such as tocopherol acetate, in alleviating hepatotoxicity is indirect evidence of the role of the free radicals in initiation of lipid peroxidation in tetracycline affectations of the liver (Oleinik & Ovsiannikova 1983). Tetracycline is also known to cause hepatic dysfunction in man by inducing steatosis. Fat droplets, 0.1–0.5 $\mu$m, accumulate in the space of Disse of hepatocytes in rats treated intraperitoneally with 300 mg $kg^{-1}$ tetracycline hydrochloride (Jack et al 1974). Increased fat accumulation occurred in the liver of rats treated with the higher dose of the antibiotic and not the lower dose in this study, and this could apparently provide more substrate for lipid peroxidation and in turn lead to enhanced generation of reactive oxygen species (ROS). Hepatic fat content affects ROS production because the cell membranes of parenchymal cells, where fat droplets are located, are metabolically active and exposed to the extracellular space, where antioxidant enzymes like superoxide dismutase and catalase are very low; therefore, these cells are particularly susceptible to oxidative stress and to generation of ROS. Fat droplets in the liver parenchymal cells may necessarily mean that increased substrate would be available for peroxidation and free radical formation (Zhong et al 1998). ROS are toxic to cells because they can react with most cellular macromolecules, including proteins, lipids and DNA. It is evident from this study that there is a significant increase in the ROS formation measured in terms of MDA in the liver of rats treated with 200 mg $kg^{-1}$ tetracycline. It follows from the above discussion that this rise in the levels of MDA is by virtue of tetracycline causing hepatic accumulation of fat as well as through production of hydroxyl free radicals. Decreased hepatic GSH level was

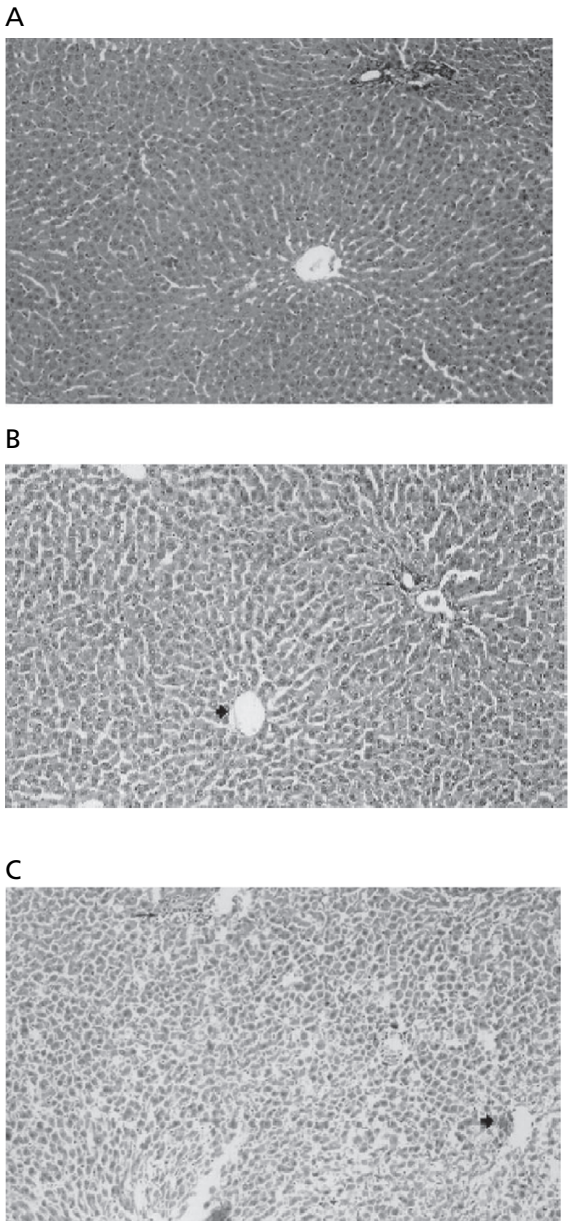


**Figure 3** A. High-power photomicrograph of normal-appearing liver in control rats (haematoxylin–eosin × 100 original magnification). B. Liver of rats treated with tetracycline (50 mg $kg^{-1}$ daily) for 21 days (haematoxylin–eosin × 100 original magnification). C. Liver of rats treated with tetracycline (200 mg $kg^{-1}$ daily) for 21 days (haematoxylin–eosin × 100 original magnification). Thin arrow indicates portal area; thick arrow indicates central vein.

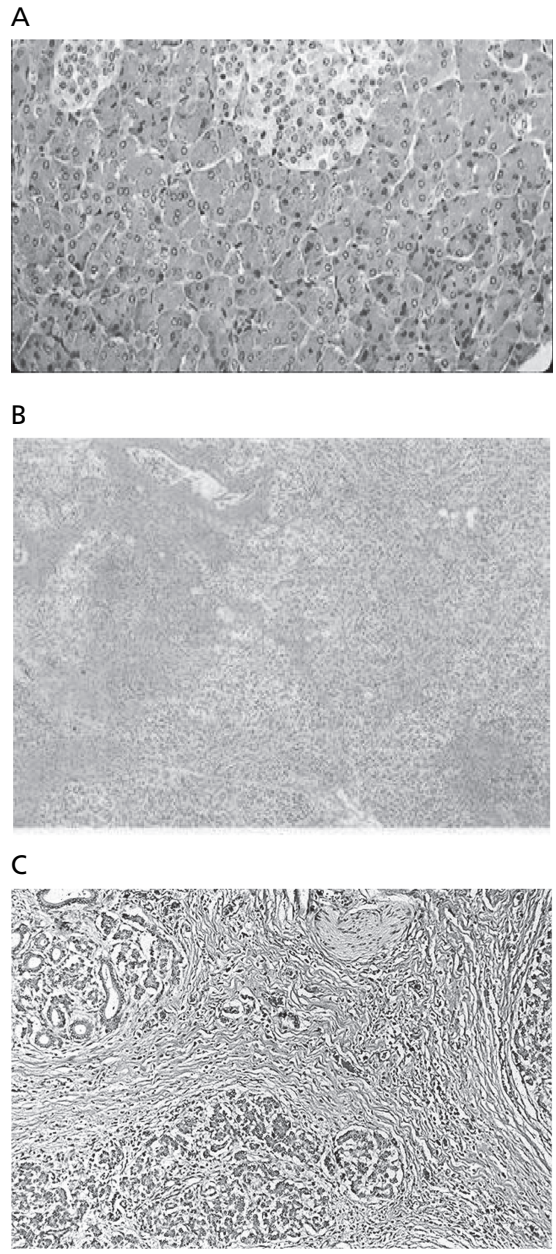


**Figure 4** A. High-power photomicrograph of normal appearing pancreas in control rats (haematoxylin–eosin × 100 original magnification). B. Pancreas of rats treated with tetracycline (50 mg $kg^{-1}$ daily) for 21 days (haematoxylin–eosin × 10 original magnification). C. Pancreas of rats treated with tetracycline (200 mg $kg^{-1}$ daily) for 21 days (haematoxylin–eosin × 40 original magnification).

observed in the group treated with 200 mg $kg^{-1}$ tetracycline and the decline was substantially enhanced with increase in duration of treatment. GSH defends the cell against the toxic effects of hydroxyl radicals and singlet oxygen. Previous studies stated that some antibiotics cause a decrease in renal glutathione levels in rats (Ramasammy et al 1985). In general, when GSH levels decrease, glutathione-related enzymes decrease as well (Casari et al 1985). In the pancreas oxidative stress was distinct in the rats treated with both 50 and 200 mg $kg^{-1}$ tetracycline, as indicated by the significant increases noted in the levels of MDA. Because pancreatic islets naturally posses low antioxidant capacities (Wu et al 2004) they are quite vulnerable to the increased generation of ROS. Superoxide dismutase expression was in the range of 30% of the liver values; the expression of the hydrogen peroxide-inactivating enzymes catalase and glutathione peroxidase was extremely low, in the range of 5% of the liver (Arthur 2000).

Antioxidant enzymes render protection against ROS toxicity and the enzymes involved in the elimination of ROS include SODs, catalase and GPx (Rana et al 2002). Previously, studies reported that antibiotics, such as gentamicin, depressed GPx activity in kidney and heart (Ozturk et al 1997). It was stated that oxygen free radicals involved in antibiotic-induced nephrotoxicity and singlet oxygen might directly inactivate GPx activity (Turrens 1991). The decreased levels of GSH observed in the rats treated with tetracycline causes the GSH-dependent enzymes GPx and GST to decrease. When the cell is challenged with oxidative stress, the cell responds by modulating the antioxidative enzyme levels. In both liver and pancreas at the higher dose of tetracycline (200 mg $kg^{-1}$), the activity of the enzymes studied was significantly reduced; the reduction was significant ($P < 0.001$) in the 21-day study when compared with the 14-day study. The lower dose of 50 mg $kg^{-1}$ did not cause any significant decrease in the levels of the antioxidative enzymes in the liver whereas in the pancreas there was significant decrease in the enzyme levels at this dose. Depending on the degree of oxidative stress and resulting tissue damage, the antioxidant enzyme levels progressively decrease (Hill & Singal 1996), as seen in this study.

## Conclusion

This study has shown that tetracycline administration at a dose of 200 mg $kg^{-1}$ caused tissue damage to both liver and pancreas. It has been demonstrated that tetracycline at the higher dose causes damage to liver, causing LDH to spill from the cells, and also caused levels of alanine and aspartate transaminases to increase. Tetracycline proved toxic to the pancreas at the lower dose, as indicated by the rise in pancreatic enzymes in the serum and the reduction in the content of pancreatic enzymes. At the higher dose, endocrine function was found to be affected as demonstrated by the rise in serum glucose levels. Evidence of loss in exocrine function due to tetracycline intoxication was shown by an increase in excretion of fat. Also this study reveals that the increase in lipid peroxides and decrease in reduced glutathione and antioxidant enzymes was clearly dose and duration dependent. It is interesting to note the absence of oxidative stress due to tetracycline administration with respect to the liver at the lower dose whereas it caused apparently small but significant oxidative stress in the pancreas. On the basis of data presented in this paper, it is recommended that judicious use of antibiotics should be made.

## References


Arthur, J. R. (2000) The glutathione peroxidases. *Cell. Mol. Life Sci*. **57**: 1825–1835

Bass, N. M. (2003) Drug-induced liver disease. In: Friedman, S., Grendell, J., McQuaid, K. (eds) *Current diagnosis and treatment in gastroenterology*. McGraw-Hill Professional, New York, pp 664–679

Bernfeld, P. (1955) Amylases alpha and beta. *Methods Enzymol*. **1**: 149

Bonting, S. L., Pollak, V. E., Muehrcke, R. C., Kark, R. M. (1960) Quantitative histochemistry of the nephron. III. Lactic dehydrogenase activity in man and other species. *J. Clin. Invest*. **39**: 1381–1385

Boschero, A. C., Delattre, E. (1985) The mechanism of gentamycin inhibited insulin release by isolated islets. *Arch. Int. Pharmacodyn. Ther*. **273**: 167–176

Casari, M., Gabrielli, G. B., Dusi, S., Nicoli, N., Bellisola, G., Corrocher, R. (1985) Decreased activity of liver glutathione peroxidase in human hepatocellular carcinoma. *Eur. J. Cancer Clin. Oncol*. **21**: 941–994

Cheung, T., Brigs, M. H. (1974) Pyridoxal phosphate and the measurement of aminotransferase activity. *Clin. Chim. Acta* **54**: 127

Chowdary, P., Rayford, P. L., Chang, L. W. (1992) Induction of pancreatic acinar pathology via inhalation of nicotine. *Proc. Soc. Exp. Biol. Med*. **20**: 159–164

Conlon, B. J., Aran, J. M., Erre, J. P., Smith, D. W. (1999) Attenuation of aminoglycoside induced cochlear damage with the metabolic antioxidant alpha-lipoic acid. *Hear. Res*. **128**: 40–44

De Jonge, H. R. (1973) Toxicity of tetracyclines in small-intestinal epithelium and liver. *Biochem. Pharmacol*. **22**: 2659–2677

Dibas, A. I., Yorio, T., Easom, R. A. (1995) Gramicidin D is a potent insulin secretagogue: dependence on extracellular calcium influx. *Biochem. Biophys. Res. Commun*. **212**: 449–454

Ellman, G. L. (1959) Tissue sulfydril groups. *Arch. Biochem. Biophys*. **82**: 70–71

Farrell, G. C. (1997) Drug-induced hepatic injury. *J. Gastroenterol. Hepatol*. **12**: S242–S250

Habig, W. H., Pabst, M. J., Jackoby, W. B. C. (1974) Glutathione-S-transferases: the first enzymatic step in enzymatic stepping mercapturic acid formation. *J. Biol. Chem*. **249**: 7130–7139

Hill, M. F., Singal, P. K. (1996) Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats. *Am. J. Pathol*. **148**: 291–300

Hiramatsu, S., Hoog, A., Moller, C., Grill, V. (2000) Treatment with diazoxide causes prolonged improvement of the $\beta$-cell function in rat islets transplanted to a diabetic environment. *Metabolism* **49**: 657–661

Jack, E. G., Royal, N. W., Paul, S., John, M., Charles, E. D., Kurt, S. (1974) Effects of tetracycline on ultrastructure and lipoprotein secretion in the rat hepatocyte. *Toxicol. Appl. Pharmacol*. **30**: 317–332

Lee, W. M. (2003) Drug-induced hepatotoxicity. *N. Engl. J. Med*. **349**: 474–485

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with Folin-phenol reagent. *J. Biol. Chem*. **193**: 265–275

Makarenko, T. N., Dudchenko, A. M., Luk'yanova, L. D. (1994) Rat model of acute tetracycline hepatosis and its dynamic predictors. *Bull. Exp. Biol. Med*. **118**: 1294–1296

Malaisse, W. J., Lebrun, P., Pirotte, B., Van Polje, P. D., Vinambers, C. Vollanuevad, Pennacarrilo, M. L., Valverde, I., Gabel, J., Rorsman, P. (1996) The riddle of formycin A insulinotropic action. *Biochem. Mol. Med*. **57**: 47–63

Misra, H. P., Fridovich, T. (1972) The role of superoxide ion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem*. **247**: 3170–3175

Navarro, V. J., Senior, J. R. (2006) Drug-related hepatotoxicity. *N. Engl. J. Med*. **354**: 731–739

Nicolau, D. P., Mengedoht, D. E., Kline, J. J. (1991) Tetracycline-induced pancreatitis. *Am. J. Gastroenterol*. **86**: 1669–1671

Ohkawa, H., Onishi, N., Yagi, K. (1979) Assay for lipid peroxides in animal tissue by thiobabituric acid reaction. *Anal. Biochem*. **95**: 351–358

Oleinik, A. N., Ovsiannikova, L. M. (1983) Mechanism of the prooxidant activity of tetracycline. *Antibiotiki* **28**: 845–848

Ozturk, H. S., Kavutcu, M., Kaçmaz, M., Canbolat, O., Durak, I. (1997) The effects of gentamicin on the activities of glutathione peroxidase and superoxide dismutase enzymes and malondialdehyde levels in heart tissues of guinea pigs. *Curr. Med. Res. Opin*. **14**: 47–52

Pagila, D. E., Valentine, W. N. (1967) Studies on the glutathione characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med*. **70**: 158–169

Ramsammy, L. S., Ling, K. Y., Josepovitz, C., Levine, R., Kaloyanides, G. L. (1985) Effect of gentamicin on lipid peroxidation in rat renal cortex. *Biochem. Pharmacol*. **34**: 3895–3900

Rana, N., Sonali, M., Jawaid, I. (2002) Superoxide dismutase — applications and relevance to human diseases. *Med. Sci. Monit*. **8**: 210–215

Rashid, M., Goldin, R., Wright, M. (2004) Drugs and the liver. *Hosp. Med*. **65**: 456–461

Roberts, J., Stella, V. J., Decedue, C. J. (1985) A colorimetric assay of pancreatic lipase: rapid detection of lipase and colipase separated by gel filtration lipids **20**: 42–45

Schultz, J. C., Adamson, J. S., Workman, W. W., Norman, T. D. (1963) Fatal liver disease after intravenous administration of tetracycline in high dosage. *N. Engl. J. Med*. **269**: 999–1004

Schwarz, M., Lund, E., Setchell, K., Kayden, H., Zerwekh, J., Bjorkhem, I., Herz, J., Russell, D. (1996) Disruption of cholesterol 7$\alpha$-hydroxylase gene in mice. *J. Biol. Chem*. **271**: 18024–18031

Sherlock, S., Dooley, J. (eds) (2002) Drugs and the liver. In: *Diseases of the liver and biliary system*. 11$^{th}$ edn, Blackwell Science, Oxford, UK, pp 335–364

Storozhuk, P. G., Shamsutdinova, V. V. (1976) Effect of certain antibiotics of tetracycline series on the level of blood sugar and the role of insulin in the mechanism of its regulation. *Probl. Endokrinol. (Mosk)*. **22**: 106–110

Takahara, S., Hamilton, B. H., Nell, J. V., Kobra, T. Y., Ogawa, Y., Nishimura, E. T. (1960) Hypocatalasemia: a new genetic carried state. *J. Clin. Invest*. **29**: 610–619

Tucker, P. C., Webster, P. D. (1972) Effects of tetracycline on pancreatic protein synthesis and secretion in pigeons. *Am. J. Dig. Dis*. **17**: 675–682

Turrens, J. F. (1991) The potential of antioxidant enzymes as pharmacological agents in vivo. *Xenobiotica* **21**: 1033–1040

White, S. A., Swann, A., Hughes, D. P., Contractor, H. H. London, N. J. (1997) Different treatment regimens to reduce microbial contamination of purified porcine islets of Langerhans. *Transplantation* **64**: 770–773

Wu, L., Nicholson, W., Knobel, S. M., Steffner, R. J., May, J. M., Piston, D. W., Powers, A. C. (2004) Oxidative stress is a mediator of glucose toxicity in insulin-secreting pancreatic islet cell lines. *J. Biol. Chem*. **279**: 12126–12134

Zhong, Z., Connor, H. D., Mason, R. P., Lemasters, J. J., Thurman, R. G. (1998) Ethanol, not fat accumulation per se increases free radical production in a low-flow, re-flow liver perfusion model. *Transplantation* **66**: 1431–1438