Original article

THE TOXIC EFFECTS OF PROLONGED ADMINISTRATION OF CHLORAMPHENICOL ON THE LIVER AND KIDNEY OF RATS

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The toxic effect of chloramphenicol on the liver and kidney was studied in laboratory Wistar rats. 16 adult rats of both sexes randomly divided into two groups were used. 10 animals in the test group were administered with chloramphenicol orally using rat cannula at human infant recommended dosage of 25mg/kg body weight given once daily for a period of 16 days. The 6 animals in the control group were only administered with 0.9% physiological saline orally over the same period of time. Serum enzymes and levels of serum bilirubin, urea, and creatinine were evaluated to establish any hepatic or renal dysfunction. There was statistically significant increase in aspartate aminotransferase (P<0.05) and alanine aminotransferase (P<0.001) serum levels in the test animals. The increase in serum alkaline phosphatase was not statistically significant (P>0.05). Hyperbilirubinaemia was observed in the rat administered with chloramphenicol, the difference in the mean value of the test and control animals were significant for total and conjugated bilirubin. (Total bilirubin P<0.01; Conjugated bilirubin P<0.05). The average time taken to establish anaesthesia was shorter in the test animals than in animals in the control group, the difference in the mean values was significant (P<0.05). Serum urea and creatinine levels were elevated in the test animals, the increase is only statistically significant for serum urea (P<0.05) but not significant for creatinine (P>0.05). Histopathology revealed vascular congestion and foamy cytoplasm of hepatocytes at the centrilobular region of the liver but did not reveal any damage done to the renal tissue. It was concluded that chloramphenicol may not be nephrotoxic but may have toxic effects on the liver.

Key words: Chloramphenicol, Toxic Effect, Rats.

Chloramphenicol is used extensively in non-industrialised countries for life-threatening infections because it is cheap and effective despite its known haemotoxicity and linkage to fatal aplastic anaemia. The toxic effects have been reported not only from parenteral or enteral route but also from ocular chloramphenicol administration (McGhee and Anasta, 1996; Lazarov and Amichai, 1996; Holt *et al* 1997). Most investigations showed that chloramphenicol-induced toxicity is traceable to bone marrow depression. Robbana-Barnat (1997) claimed that chloramphenicol and it's metabolites inhibited 3H-thymidine incorporation in human bone marrow cells and also induced DNA single stand breaks in the same cells at a dose response relationship. Holt *et al* (1997) further observed that chloramphenicol at sub-therapeutic dose causes apoptosis in heamatopoietic progenitor cells from human neonatal blood in vitro and in vivo. Earlier, Holt *et al* (l993) reported that toxic manifestations of chloramphenicol may be explained by attack of free radicals leading to depletion of cellular anti-oxidants.

In the present study, attempt is being made to investigate possible chloramphenicol-induced toxicity of extramyeloid origin. The liver and kidney so chosen are two important organs in the body because of their involvement in biotransformation and excretion of xenobiotic respectively. There are not much reports of toxicological investigation of chloramphenicol outside its usual or traditional haemotoxic studies. However much work have been carried out on the effect of other antimicrobials on the liver and kidney. Reports available show that aminoglycosides and vancomycin rank the most toxic to these organs especially on the kidney (Pospishil and Anonovich, 1996; Alverez and Gil, 1998; Carrier *et al*, 1998; Malik *et al*, l998). Other drugs such as chloroquine and acetaminophen have been frequently incriminated to elicit hepatotoxic effects. (Bhat *et al*, 1998; Perry and Shannon, 1998). Hepatotoxicity and nephrotoxicity induced by chemotherapeutic agents have been of much concern to many clinicians such that current toxicological studies are aimed at discovering remedies against established cases of hepatic and renal adverse reactions to these antibiotics (Aubrecht *et al*, 1997; Fabrizii *et al*, 1997; Nakamura *et al*, l998).

The findings from this study will serve to widen the scope of understanding of the chloramphenicol –induced toxicity and to establish any hepatotoxic or nephrotoxic effect of chloramphenicol.

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MATERIALS AND METHOD
Experiment Animals
16 adult laboratory Wistar rats of both sexes were used. The animals were kept on commercially prepared rat cubes (Pfizer, Nigeria) and fresh water without restriction. The animals were randomly divided into two groups; A and B. Group A is the test group containing 10 rats while the control group B has 6 rats. Each animal was weighed daily and average daily feed consumed per animal was also determined.

Administration of Drug
Each of the 10 rats in group A was administered with (chloramphenicol palmitate BP, Bombay, India) orally once daily for 16 days with the aid of rat cannula at a recommended dosage for human infant (25mg/kg body weight). However, the rats in group B received 0.9% physiological saline orally for the same period of time. The rats in both groups were closely observed during this period.

Collection of Serum and Sample Analysis
On the 17th day, each rat was weighed and then placed in anaesthetic jar containing ether-soaked cotton wool. The time taken for each rat to be fully anaesthesized was recorded, complete anaesthesia was considered accomplished when the pedal movements and eyelid reflex were abolished, and the animal become recumbert while still breathing. The rats were opened up, blood was obtained by cardiac puncture. The blood collected was transferred into labelled bijou bottle.
The serum was later harvested from the coagulated blood.   Activities of serum aspartate aminotranstrase (AST)   and alanine aminostransferase (ALT) were determined colorimetrically according to Mohun and Cook (1957), alkaline phosphatase (ALP) according to the method of king and Armstrong (1984). The diacetyl monoxine method described by Faucett and Scot (1960) and modified by Kaplan Szabo (1979) was used to determine urea. Serum createnine (SC.) level was determined by the method of Slot (1965) and serum bilirubin level by the Michealson, (1961)
Samples of the liver and kidney were taken for histopathology. Histologic slides were prepared by conventional procedures and stained with haemotoxylin and eosin. Results are expressed as the mean + standard deviation of the mean, significant difference between means were determined by the student's test. Differences are considered significant at p<0.05 level (Bailey, 1992).

RESULTS
There was increase in the serum activities of ALP, AST and ALT in the test animals compared to the animals in the control group. The differences in the means values are statistically significant for AST (P<0.05); ALT (P<0.001) but not significant for ALP (P>0.05) (Table 1). There were significant increase in total bilirubin level (P<0.01) and conjugated bilirubin (P<0.05) in the test animals. However, the mean values of unconjugated bilirubin levels was approximately the same for animals in the test and control group (Table 2). The serum urea and creatinine increased in the test animals compared to the animals in the control group, the difference is significant for serum urea (P<0.05) but not significant for serum creatinine (P>0.05) (Table 2). The time taken for complete anaesthesia was longer in the control group than in the group administered with chloramphenicol, the difference in the mean values is statistically significant (P<0.05) (Table 1).

Histopathology revealed foamy cytoplasm of hepatocytes and vascular congestion at the centrilobular region, in the test animals. There were no pathological changes observed in the kidneys in the test animals or in the liver and kidney of animals in the control group.   The clinical observations, daily feed intake and body weight recorded during the period of study are shown in table 3, figures 1 and 2 respectively

Table 1:
The mean values of serum enzymes and time taken to achieve anaesthesia.

GROUP	NUMBER OF ANIMALS	ALP IU/L	AST IU/L	ALT IU/L	TIME TAKEN TO ACHIEVE ANAESTHESIA (s)
A	10	65+8.18[a]	77.9+1.05[a]	122.5+2.50[c]	125.9+0.27[a]
B	6	61+6.50[a]	68+2.75[b]	55.5+2.65[d]	126.8+0.15[b]

N. B   Mean values with different superscripts on vertical column differ significantly at (P<0.05) but c-d at (P<0.001).

Table 2:
The mean values of serum bilirubin, urea and creatinine levels

| GROUP	NUMBER OF ANIMALS	TOTAL BILIRUBIN (mg/dl)	CONJ. BILIRUBIN (mg/dl)	UNCONJ. BILIRUBIN (mg/dl)	SERUM UREA (mg/100ml)	SERUM CREATININE (mg/100ml)
A	10	0.52+0.12[c]	0.42+0.16[a]	0.10+0.08	30.0+2.23[a]	1.5+0.05
B	6	0.38.+0.14[d]	0.25+0.15[b]	0.13+ +0.05	28.5+3.11[b]	1.4+0.08

N.B   *Mean values with different superscripts in vertical column differ significantly at (P<0.05) but c-d at (P<0.01).*

Table 3: Clinical Symptoms observed in the test and control groups

Group	Number of animals	Clinical observations
A	10	The animals were very active and alert for the first seven days. After the 7th day the faeces became soft and later became watery. The animal became increasingly dehydrated and lethargic. There was loss of weight, rough hair coat and unthrifty appearance generally.
B	6	Gradual gain in weight, the animals were active throughout the course of the study.

Figure 1
The Pattern of daily feed intake per average body weight of rats in the test and control group
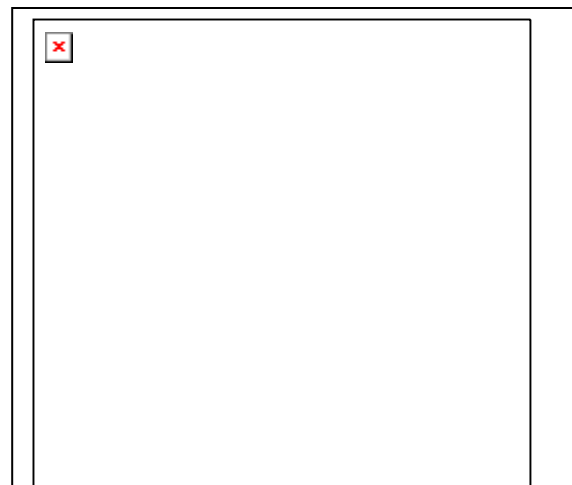
Figure 2
Daily average body weight of rats in the test and control group

DISCUSSION

The results obtained from this study revealed that chloramphenicol does really have toxic effect on the liver. Chloramphenicol had earlier been reported only as hepatic cytochrome p450 inhibitor (Robillart *et al,* 1998). This same reason is assumed to be responsible for the shorter time taken for the test animals to be fully anaesthesized than for the control animals in this study. The rate of hepatic clearance/metabolism of the anaesthetic (ether) in the plasma is slower in the test group than in the control group, with concomitant faster establishment of anaesthesia in the test group than in the control group. In effect, 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.

Further findings in the study according to histopathology carried out indicated that there were degenerative changes of hepatic parenchymel cells. This is well corroborated by significant increase in serum activity of aspartate aninotransferase (AST) and alanine aminotransferase (ALT). These

increases in serum level of these enzymes agree with earlier work of Bhat *et al* (1998) on minocydine and Aubrecht *et al* (1997) on hygromycin B. Hyperbilirubinaemia was also observed in the animals administered with chloramphenicol. 80% of the total bilirubin was conjugated bilirubin which bear strong indication that the hyper bilirubinaemia is most likely to be of hepatic origin. 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 by Bhat *et al* (1998) and another antibiotic meropenem by Alverez and Gil, (1998).

Though chloramphenicol was found to be hepatotoxic, there is no strong evidence to suggest that chloramphenicol is nephrotoxic according to this study, There was no noticeable pathological changes observe from renal histopathology.   There was significant elevation of serum urea, the serum creatinine level was normal in the test animals. Shils (1963) ascribed tetracycline-induced increase in serum urea with unaltered serum creatinine level to increased protein catabolism rather than nephrotoxiocosis. The test animals in this study were observed to be dull, diarrhoeic, dehydrated and anorexic. Finco (1989) observed that starvation or stress among other factors could induce increase protein catabolism, this is believed to be responsible for the significant increase in serum urea level rather than chloramphenicol-induced nephrotoxicity.

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