The Effects of Sub-chronic Piperazine Treatment on the Liver Status of a Rat Model

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Abstract: Background: Piperazine reputed to be a safe drug was evaluated for hepatotoxic effects in the rat following sub-chronic administration. Transaminases, alkaline phosphatase and bilirubin levels were measured by different methods. **Results:** The aspartate transaminase values with piperazine 30, 60 and 100 mg kg^{-1} were 22.8±1.43, 27.6±1.08 and 38.4±3.75 i.µ L^{-1}, respectively. Compared to the control value of 12.8±1.32 i.µ L^{-1}, the changes were statistically significant (p = 0.0009, <0.0001 and 0.0002, respectively). The alanine transaminase levels in the groups that were treated with piperazine 30, 60 and 100 mg kg^{-1} also showed dose dependent statistically significant rises of 17.0±0.77, 18.2±0.92 and 24.6±1.50, respectively compared to the control. This was also the case with the result of alkaline phosphatise. When compared with the control value of 49.6±10.36 i.µ L^{-1} the groups of rats administered with piperazine 30, 60 and 100 mg kg^{-1} had alkaline phosphatase values of 96.2±0.49, 132.8±12.06 and 314.8±38.46 i.µ L^{-1}, respectively. Results seen with total and conjugated bilirubin levels varied with doses of piperazine: whereas there was no statistically significant difference in the 30 mg kg^{-1} group compared to the control, the group that received piperazine 100 mg kg^{-1} showed significant increases. In the case of the 60 mg kg^{-1} group, the total bilirubin level increased significantly compared to the control while conjugated bilirubin did not. The liver histology revealed extensively deranged morphology. **Conclusion:** It is concluded, therefore, that chronic high doses of piperazine would be inimical to the liver and therefore, compromise the normal liver function.

Key words: Piperazine, liver function test, liver histology, rat

INTRODUCTION

Piperazine is a useful and inexpensive anthelmintic agent active against *A. lumbricoides* and *E. vermicularis* (Tracy and Webster, 2001). Apart from its effects on the worm, piperazine has been demonstrated to have various actions on isolated tissues and organs of the mammal: It has a direct non-specific, non-vascular smooth muscle relaxant action as it inhibits barium chloride, histamine, 5HT and acetylcholine-induced contractions in the guinea-pig ileum and rabbit duodenum by a direct smooth muscle depressant action (Onuaguluchi, 1966, 1981, 1984). It also antagonized the effect of adrenaline on the guinea-pig vas deferens and oxytocin induced contractions in the rat uterus. Also, piperazine was shown to decrease the rate and force of contraction of the isolated frog heart and the rabbit heart Langendorff preparation (Onuaguluchi, 1966).

It has also been established that piperazine has definite antiarrhythmic properties (Onuaguluchi and Ghasi, 2006; Ghasi and Onuaguluchi, 2007; Ghasi, 2008). It was postulated, based on the electrocardiographic patterns of piperazine, that the major mechanism of the antiarrhythmic action of piperazine among others was prolongation of the cardiac action potential as a result of K$^+$ channels blocking activity of the drug (Onuaguluchi and Ghasi, 2006; Ghasi and Onuaguluchi, 2007). These acute studies of the electrocardiographic changes induced by piperazine citrate in the conscious human subjects as well as in anaesthetized rats showed that piperazine caused dose-dependent prolongation of P-R, Q-T, and J-T intervals with little or no effects on the QRS complex. In contrast, piperazine caused a dose-dependent decrease in heart rate in both studies.

Adverse events attributable to piperazine administration even at the usual oral dose of 75 mg kg^{-1} body weight have been shown to be very rare. Most of the recorded toxic effects were related to the neuromuscular system (Schuch *et al.*, 1966; Parsons, 1971; Bomb and Bedi, 1976). The relative safety of piperazine as a drug compared to many other drugs is understandable in view of the very high LD$_{50}$ (3.548 g kg^{-1} i.p.) of the drug in mice (Onuaguluchi and Igbo, 1985). In the same study, the LD$_{50}$ and therapeutic index (TI) of piperazine in the toad were given as 12.59 g kg^{-1} and 8.5, respectively. Interestingly, the LD$_1$ and ED$_{99}$ for

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piperazine in the same animal model were 2.63 and 1.862 g kg^{-1}, respectively (Onuaguluchi and Igbo, 1985), giving a Certain Safety Factor (CSF) of 1.4. In other words, increasing the dose expected to give about 100% therapeutic effect by just 40% could sometimes lead to emergence of serious toxic effects.

This observation was found to be true during our investigation of the electrocardiographic patterns of piperazine in the rat (Ghasi and Onuaguluchi, 2007). It was found that piperazine 100 mg kg^{-1} given intravenously via the external jugular vein sometimes caused serious aberrations in some of the animals. This finding necessitated further studies on the possible toxic effects of piperazine especially at cellular level.

Elevation of the serum enzymes could point to some disease states. Recently, a piperazine derivative, M-chlorophenylpiperazine was shown to affect some brain hormones (Hatzidimitriou *et al.*, 2002). This may arise as a consequence of histological effect of the drug as some piperazine derivatives have been demonstrated to cause lesions (De Deurwaerdere and Chesselet, 2000; Eilon *et al.*, 2000; Van-Bogaert *et al.*, 2001). Since some piperazine derivatives have equally been found to have protective effects (Merz *et al.*, 1998; Minato *et al.*, 1996, 1997; Williams *et al.*, 1996), it is suggested that sub-chronic study of pathologic effects of piperazine be undertaken in which case effects of piperazine are evaluated to identify any possible pathological changes. This is especially important since the morphological evidence of a pathological process is the most consistent of the changes that can be identified as the result of a toxic process.

In the present study, we investigated the effects of sub-chronic treatment of albino rats with piperazine on the liver enzymes and morphology.

MATERIALS AND METHODS

Forty Albino Wistar rats weighing originally between 175 and 250 g were divided into 4 groups of ten sex-matched rats each. Each group of rats received orally one dose of piperazine, 30, 60 or 100 mg kg^{-1} given two times daily along with standard diet. The fifth group of rats served as the control and was fed orally with the standard diet only. The total period of treatment was 16 weeks.

At the end of the 16 weeks, some of the animals were sacrificed under chloroform anaesthesia. The liver was harvested and fixed in 10% formalin for 24 h. The tissues were processed using an automatic tissue processor, embedded in paraffin wax and thin sections (about 4-5 mm thick) cut using a rotary microtone. The sections were stained by haematoxylin and eosin (H and E) method,

examined and photographed independently by two histopathologists using a light microscope.

At the end of treatment, the rats were anaesthetized with thiopentone sodium (50 mg kg^{-1} i.p.) and blood samples collected by cardiac puncture for biochemical studies. The results were expressed as Mean±SEM and the significance of difference between treated and control determined using unpaired Student's t-test. Differences between the various treated groups were then evaluated using one-way ANOVA and when found statistically significant, Bonferron's Multiple Comparison Test was done to determine patterns of significance on group-to-group basis.

Transaminases estimation: The method of Reitman and Frankel (1957) was employed for this study. The amino transferases are metabolic enzymes which catalyse transamination reaction in the body and they include aspartate tranaminase (formerly known as serum glutamic oxaloacetic transaminase, SGOT) and alanine transaminase (formerly called serum glutamic pyretic transaminase, SGPT). In this method the oxo acids produced are estimated by their coupling reaction with 2,4-dinitrophenyl hydrazine to form an oxo acid hydrazone which is reddish brown in alkaline medium.

Alkaline phosphatase estimation: Alkaline phosphatases are a group of phosphomonoester enzymes that have optimal activity when the pH is around 9.8. They act on large variety of physiological or non-physiological substrate. The method of Kind and King (1954) was employed. In the above method, disodium phenyl phosphate is used as the substrate. Hydrolyses of this substrate by the enzyme then liberates phenol and phosphoric acid. The phenol is estimated by its reaction with 4-aminoantipyrene to produce a colour in the presence of alkaline oxidizing agent, alkaline ferricyanide.

Serum bilirubin estimation: The serum bilirubin estimation was carried out using the method of Powell (1944). Bilirubin is a breakdown product of haemoglobin. It is insoluble in water. It is conveyed from the reticulo-endothelial system to the liver attached to plasma albumin. In the parenchymal cells of the liver it is conjugated by glucuronyl transferase 3 glucuronic acid and excreted in the bile largely as the indirect reaction while, the unconjugated bilirubin is estimated in the direct reaction. Both bilirubin and the diglucuronide (conjugated bilirubin) are capable of coupling with diazotised sulphanilic acid to form colored compounds known as Azo-bilirubin (Purple colour). In order that the water insoluble bilirubin may couple with

the diazonium salt, an accelerator (or solubilizing agent) must be added. In this procedure sodium benzoate-urea solution is used.

RESULTS

Serum transaminases: The transaminases showed significant and dose dependent increases over control values following chronic treatment with various doses of piperazine citrate. The aspartate transaminase values after 4 months of treatment with piperazine 30, 60 and 100 mg kg^{-1} were 22.8±1.43, 27.6±1.08 and 38.4±3.75 i.µ L^{-1}, respectively. When compared to the control value of 12.8±1.32 i.µ L^{-1}, the changes were statistically significant (p = 0.0009, <0.0001 and 0.0002 for the 30, 60 and 100 mg kg^{-1} doses, respectively). All the rats in the treated groups except the group that received piperazine 30 mg kg^{-1} had aspartate transaminase values well above the upper limit of the normal range (5-18 i.µ L^{-1}). It is noteworthy to mention that 3 out of the 10 rats in the 100 mg kg^{-1} group had aspartate transaminase levels well above 40 i.µ L^{-1}. They exceeded the upper limit of normal by 228% in one rat and 256% in the other 2 rats (Table 1).

The result of alanine transaminase determination follows the pattern of aspartate transaminase. The mean control value of 10.2±1.56 i.µ L^{-1} was within the normal range value of 3-15 i.µ L^{-1}. Compared to the control value, the alanine transaminase levels in the groups that were treated with piperazine 30, 60 and 100 mg kg^{-1} showed dose dependent significant rises of 17.0±0.77, 18.2±0.92 and 24.6±1.50, respectively. The increases were statistically significant with p values of p = 0.0045, 0.0022 and 0.0002, respectively. Table 1 shows the values of the serum transaminases in the control group and groups treated with various doses of piperazine.

Alkaline phosphatase: After 4 months of treatment with piperazine, there were concentration dependent significant increases in the levels of alkaline phosphatase in all the piperazine treated groups over the control group. Compared to the control value of 49.6±10.36 i.µ L^{-1}, the groups of rats administered with piperazine 30, 60 and 100 mg kg^{-1} had alkaline phosphatase values of 96.2±0.49, 132.8±12.06 and 314.8±38.46 i.µ L^{-1}, respectively. The differences were statistically significant (p = 0.002, 0.0008 and 0.0002, respectively). One-way analysis of Variance (ANOVA) showed statistically significant difference between the drug treated groups (p<0.0001) (Fig. 1).

Serum bilirubin: Compared to controls there was no statistically significant change in both total and conjugated bilirubin levels except in the group that
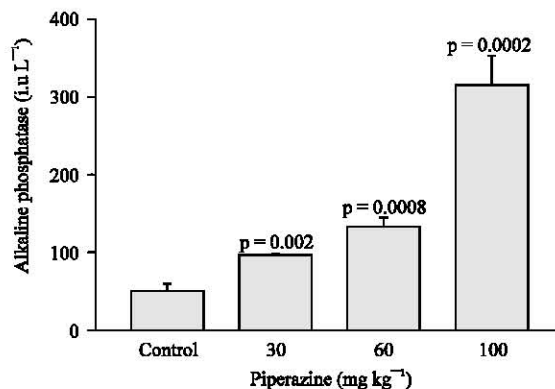

Fig. 1: The effect of piperazine on the serum alkaline phosphatase (ALP) of the Wistar rat. Differences between the control and drug treated groups were statistically significant

Table 1: Aspartate transaminase in the control and piperazine treated groups of rats*

Drug dose	Aspartate transaminase (i.µ L^{-1})		Alaninine transaminase (i.µ L^{-1})	
	Mean±SEM	p-value	Mean±SEM	p-value
Control	12.8±1.32	-	10.2±1.56	-
Piperazine				
30 mg kg^{-1}	22.8±1.43	0.0009	17.0±0.77	0.0045
60 mg kg^{-1}	27.6±1.08	0.00011	8.2±0.92	0.0022
100 mg kg^{-1}	38.4±3.75	0.00022	4.6±1.50	0.0002

*One-way analysis of Variance (ANOVA) comparing drug treated groups was statistically significant: p = 0.0019 for aspartate transaminase and 0.0008 for alanine transaminase

received piperazine 100 mg kg^{-1}. The total and conjugated bilirubin levels in the 100 mg kg^{-1} group of rats were 16.18±1.34 and 8.80±0.00 µmol L^{-1}, respectively. The differences compared to the control values of 8.12±0.08 and 4.24±0.098 µmol L^{-1} were found to be statistically significant (p<0.0001; p = 0.0002, respectively). For the group that received piperazine 30 mg kg^{-1} the total and conjugated bilirubin levels were 8.32±0.08 and 4.32±0.08 µmol L^{-1}, respectively (p = 0.1151 and 0.5447). In respect of the group that received piperazine 60 mg kg^{-1} the levels were 10.32±0.9 and 4.4±0.00 µmol L^{-1} (p = 0.0406 and 0.1407, respectively). One-way analysis of Variance (ANOVA) showed statistically significant difference of p = 0.0002 for the total bilirubin and p<0.0001 for conjugated bilirubin between drug treated groups (Fig. 2).

Histological studies: From the plates, the control animals showed a normal histological architecture (Fig. 3) whereas in the treated groups of animals, various degrees of histological changes were observed and this was seen to be dose dependent (Fig. 4-6). The liver morphological changes manifested as simply focal areas of frank
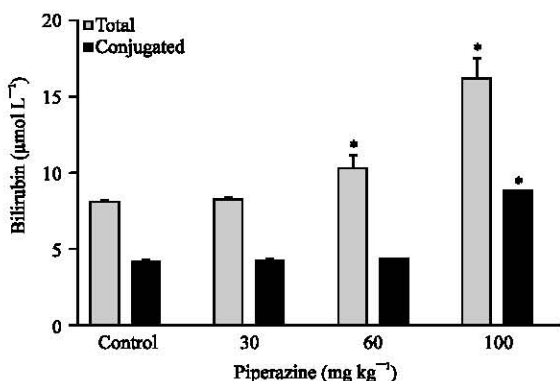
Fig. 2: The effects of piperazine on the serum total and conjugated bilirubin of the Wistar rat *p<0.0001

Fig. 3: Liver section from control rat showing normal architecture. There is no evidence of pathological changes. H and E Stain x100
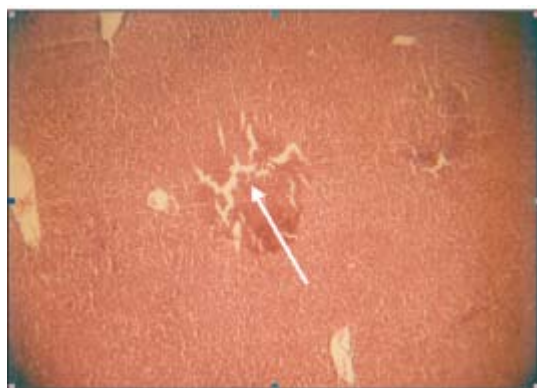

Fig. 4: Liver section of rat sub-chronically treated with piperazine 30 mg kg^{-1} showing focal areas of frank necrosis. H and E Stain x100
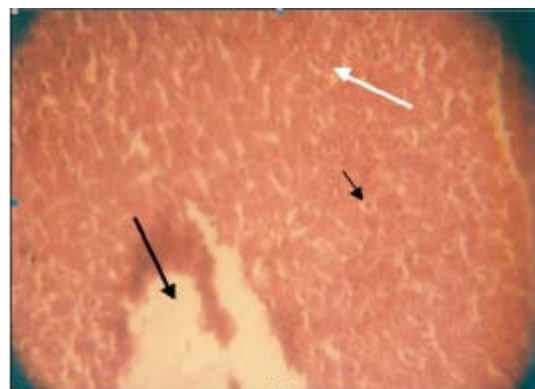

Fig. 5: Liver section of rat sub-chronically treated with piperazine 60 mg kg^{-1} showing eosinophilic nuclei of hepatic cells (Black small arrow). Perinuclear 'haloes' in cells were noted (White arrow) and the liver also shows degeneration of cells around the central vein (Black long arrow). H and E x100
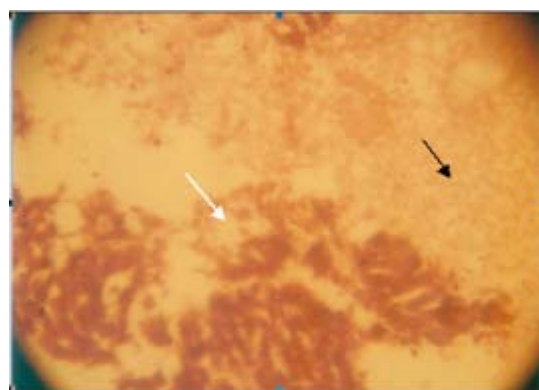

Fig. 6: Liver section of rat sub-chronically treated with piperazine 100 mg kg^{-1} showing parenchymal degeneration, necrosis and exudation (Black arrow). Lymphocytic infiltration, vacuolisation of hepatocytes and nuclear eosinophilia (White arrow) are also seen in this animal. H and E Stain x100

necrosis in the group that received the smallest dose of piperazine to other more serious hepatological derangement including necrosis with exudation, parenchymal degeneration, lymphocytic infiltration, vacuolisation of hepatocytes and nuclear eosinophilia in the groups that were treated with 60 and 100 mg piperazine. Monocellular infiltration, cuffing of the bile duct by inflammatory cells and sinusoidal dilatation were also noted.

DISCUSSION

Effects of piperazine on the serum enzyme were investigated since serum enzyme levels are useful indicators for the assessment of the liver parenchymal function (Linch and Yates, 1986). The liver enzyme levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) showed statistically significant increases over the control values and this was found to be dose dependent using one-way analysis of variance. The enzymes in the treated groups did not only show increases over the control values but their serum levels were found in some instances to be many times higher than the upper limit of normal.

Increases in AST, ALT and ALP are commonly found in liver disease. Elevation of AST is definite indication of hepatotoxicity by toxic agents (Schalm, 1967) while, ALT is raised in myocardial infarction or skeletal muscle dysfunction (Linch and Yates, 1986). An increase in the enzyme alkaline phosphatase (ALP) is indicative of such conditions as obstructive jaundice and various forms of bone disease. As both ALT and ALP in the treated groups were markedly raised compared to the control group levels, it is probable that disease conditions of the myocardium, skeletal muscle and/or bone partly contribute to the high serum enzymes levels.

Ghasi *et al.* (2010) had shown that there was no evidence of cardiac dysrhythmic phenomenon expressed as ECG aberration in rats following treatment with piperazine for 4 months. It was therefore very unlikely that the high serum ALT was due to piperazine-induced myocardial infarction. Some piperazine derivatives have been shown to have cardioprotective effects during ischaemia (Boucher *et al.*, 1994; Clarke *et al.*, 1993). Therefore, one would expect piperazine in the same manner to rather protect the heart, as has been demonstrated in its cardioprotective properties earlier in this study (Ghasi, 2008). Drake-Holland *et al.* (1993) demonstrated infarct-size reduction ability of a piperazine derivative, trimetazidine and showed that this was higher than that of propranolol. The fact that aspartate transaminase (AST) was similarly elevated would appear to suggest that the increase in the serum enzymes studied was primarily a consequence of liver toxicity and if there were no complications of bone or intestinal diseases because of the elevated ALP, it would mean that piperazine induced some degree of cholestasis in the rat. However, the fact that at 100 mg kg^{-1} the ECG showed raised S-T segment at T waves might indicate that at this dose myocardial toxicity may have contributed.

Increase in total and conjugated bilirubin became statistically appreciable only at very high dose of piperazine 100 mg kg^{-1}. Therefore, chronic high doses of piperazine would produce jaundice sometimes obstructive in nature and would therefore compromise the normal liver function.

Piperazine although reputed to be a very safe drug because of its high therapeutic index, has some deleterious effects on the liver and its enzymes when the animals are treated over a period of 4 months. The liver enzymes, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) are markedly elevated by piperazine to levels many times above the upper limit of normal. Total and conjugated bilirubin also increased. These increases are indicative of liver toxicity evidenced also in the liver histology study. The extensive derangement of the liver architecture at all doses shows that liver toxicity is primarily responsible for the elevated liver enzymes.

CONCLUSION AND RECOMMENDATION

Our advice is that piperazine should not be given for an extended period of time when employed for prophylaxis. Intermittent administration not exceeding 1-2 months at a time would suffice.

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