INTRODUCTION

Paracetamol is widely used as antipyretic and analgesic agent that effectively relieves mild to moderate pain. It is remarkably safe. It is cheap and easily available drug. Its popularity become gradually increased due to low incidence of adverse effect and to its analgesic effect comparable to that of aspirin. Its is an over the counter (OTC) drug. So it is used indiscriminately by the common people for its analgesic and antipyretic effects.

Overdoses of paracetamol causes acute liver necrosis in man is first reported by Davidson and Eastham. 10-15 gm of paracetamol causes serious intoxication in adult with normal liver function. Hepatic necrosis produced by paracetamol overdose due to increased formation of a highly reactive intermediate which is produced by oxidation of paracetamol through the cytochrome P-450 mixed function oxidase system. The reactive metabolite is N-acetyl-P-benzoquinimine. These reactive metabolite is normally detoxified by endogenous glutathione. In toxic doses of paracetamol, glucuronide and sulfate conjugation are saturated then the drug is metabolised by cytochrome P-450 system to the reactive metabolite. The reactive metabolite deplete the hepatic store of glutathione and covalently binds to tissue macromolecules and cause irreversible damage.

Phenobarbitone is a stimulator of hepatic microsomal enzyme system (MES) result in increased formation of toxic metabolites of paracetamol which causes the increased hepatotoxic effect of paracetamol in phenobarbitone treated rats. The hepatotoxicity produced by paracetamol in phenobarbitone pretreated rats in excess to those treated with paracetamol alone. Pretreatment of rats with inducer of drug metabolizing enzymes such as phenobarbitone increased the production of reactive metabolite and enhance hepatotoxicity in paracetamol treated rats.

But simultaneous administration of MES inhibitors markedly decreases the metabolism of paracetamol covalent binding and the hepatic necrosis.

The present study was carried out to find the protective effect of cimetidine and also enzyme inducing
Paracetamol induced hepatotoxicity in rats, using serum ALT, AST, Alk. phos. levels.

MATERIALS AND METHODS

The whole experiment was carried out on a total number of 24 adult rats of either sex, of Long Evans Norwegian Strain.

The rats were 3-4 months old, weighing between 150-250 gm on standard laboratory diet and were allowed to drink water ad libitum.

Paracetamol in pure powder form obtained from Beximco Pharmaceuticals limited. For chronic treatment, fresh suspension of paracetamol was prepared in distilled water in a concentration of 40 mg/ml.

Cimetidine obtained from Beximco Pharmaceuticals limited. Fresh suspension of cimetidine was prepared in distilled water in a strength of 30 mg/ml. Phenobarbitone was supplied in white powder form by pharmacology laboratory of IPGM & R. Fresh suspension of phenobarbitone also prepared in distilled water in a concentration of 5 mg/ml.

The rats were divided into four groups comprising each of six (6) rats and given the following treatment. Group I received laboratory diet and water orally daily for 21 days. Group II received paracetamol suspension 150 mg/kg b.w. orally daily for 21 days. Group III received paracetamol suspension + double doses of cimetidine suspension twice daily for 21 days.

Group IV was given single dose of paracetamol suspension 500 mg /kg b.w. orally. Group V was given pretreatment with phenobarbitone suspension 20 mg/ kg b.w. orally daily for 17 days + simultaneous administration of single dose of paracetamol suspension 500 mg/kg b.w.

On the 22nd day, all the rats were sacrificed after 12 hours fasting. Blood was collected by cutting the carotid artery at the neck and serum was separated for biochemical estimation of ALT, AST and Alk. phos. levels. The significance of differences between the two groups were calculated using unpaired student’s ‘t’ test.

RESULTS

Paracetamol treated group produced highly significant (P<0.001) rise of serum ALT, AST & alkaline phosphatase level as compared to control group and is shown in table-I.

Whereas paracetamol and cimetidine treated group significantly decreased (P<0.001) serum ALT, AST and alkaline phosphatase levels as compared to paracetamol group and is shown in table-II.

But the effect of paracetamol in rats was significantly increased when the rats were pretreated with phenobarbitone. Paracetamol and phenobarbitone treated group produced highly significant rise of ALT, AST and alkaline phosphatase levels as compared to paracetamol treated group alone and is show in table-III.

Table-I

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Treatment</th>
<th>Mean serum AST level (I.U/ml)±SEM</th>
<th>Mean serum ALT level (I.U/ml)±SEM</th>
<th>Mean serum alkaline phosphatase level (K.A.units)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>Vehicle</td>
<td>19.16±2.23</td>
<td>26.83±2.26</td>
<td>18.65±0.45</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Paracetamol</td>
<td>70.83±3.30***</td>
<td>77.66±2.30***</td>
<td>22.67±0.88***</td>
</tr>
</tbody>
</table>

***=P<0.001
*=P<0.05

Table-II

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Treatment</th>
<th>Mean serum AST level (I.U/ml)±SEM</th>
<th>Mean serum ALT level (I.U/ml)±SEM</th>
<th>Mean serum alkaline phosphatase level (K.A.units)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>6</td>
<td>Paracetamol</td>
<td>70.83±3.30</td>
<td>77.66±2.30</td>
<td>22.67±0.88</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Paracetamol + Cimetidine</td>
<td>34.16±1.40***</td>
<td>39.33±1.98***</td>
<td>22.03±0.25*</td>
</tr>
</tbody>
</table>

***=P<0.001
*=P<0.05
DISCUSSION

The mechanism of paracetamol induced liver necrosis has been extensively studied. At recommended doses it is metabolized by conjugation with glucuronide and sulfate. Hepatic necrosis occurs after paracetamol overdoses due to increased formation of a highly reactive intermediate which is N-acetyl-P-benzoquinonimine produced by cytochrome P-450 mixed function oxidase system. These reactive metabolites is normally detoxified by endogenous glutathione to produce mercapturic acid which is excreted in the urine. When the dose of paracetamol is increased and glucuronide and sulfate conjugation are saturated, larger proportion of the drug is metabolised by cytochrome P-450 system to the reactive metabolite.

The reactive metabolite may deplete the hepatic store of glutathione and covalently binds to tissue macromolecules and thereby causes irreversible damage and cell damage. Pretreatment of rats with inducer of drug metabolising enzymes, phenobarbitone causes increase the production of the reactive metabolite of paracetamol and enhance its hepatotoxicity. But pretreatment with microsomal enzyme inhibitors markedly decreases the metabolism of paracetamol covalent binding and the hepatic necrosis. There is evidence that the role of hepatic microsomal enzyme system and Paracetamol induced hepatotoxicity (PIH) in rat. Toxic doses of paracetamol produced hepatic necrosis which can be increased or decreased by stimulator or inhibitor of MES. Cimetidine is a potent inhibitor of cytochrome P-450 mediated drug metabolism. This inhibitory effects of cimetidine on the cytochrome P-450 system acts as hepatoprotection.

From this study it can be concluded that phenobarbitone stimulates the HMES and potentiate the PIH but cimetidine could be useful agent for protection of PIH. This hepatoprotection is due to prevention of the toxic metabolite formation. When phenobarbitone is used concurrently with paracetamol induced MES which in turn aggravates the PIH.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Treatment</th>
<th>Mean serum AST level (I.U/ml)±SEM</th>
<th>Mean serum ALT level (I.U/ml)±SEM</th>
<th>Mean serum alkaline phosphatase level (K.A.units)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>6</td>
<td>Paracetamol</td>
<td>59.50±2.10</td>
<td>66.16±2.99</td>
<td>22.65±0.52</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>Phenobarbitone + Paracetamol</td>
<td>84.83±3.14***</td>
<td>88.66±2.31***</td>
<td>28.70±0.35******</td>
</tr>
</tbody>
</table>

***P<0.001

Table-III

Showing the effects of paracetamol and paracetamol plus phenobarbitone on serum transaminases (AST, ALT) and alkaline phosphatase levels in rats.

REFERENCES:


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