

EXPERIMENT NO.: 7

DATE:

AIM: TO STUDY OF EFFECT OF HEPATIC MICROSOMAL ENZYME INDUCERS ON THE PHENOBARBITONE SLEEPING TIME IN MICE.

REQUIREMENTS:

- Mice (20-25g), Syringe, Needle (22-24 Gauge), Stop watch

DRUGS:

- Pentobarbital sodium (45 mg/kg, ip), Phenobarbitone Sodium (50 mg/kg)

Principle:

The drugs which induce hepatic microsomal enzyme system enhance the metabolism of other drugs. As a result in the presence of an enzyme inducer the duration of action drug will be reduced. This has significant clinical relevance because when more than one drug is administered at a time one drug may modify the action of another through the microsomal enzyme inducing property.

Theory:

Microsomal enzymes are typically found in the endoplasmic reticulum of hepatocytes. Microsomes are fragments of endoplasmic reticulum and attached ribosomes.

There are several microsomal enzymes, including flavin monooxygenases, cytochrome P450, NADPH cytochrome c reductase, UDP Glucuronosyl transferases (UGT), glutathione-S-transferases, epoxide hydrolases, etc. Cytochrome P450 and NADPH cytochrome c reductase are the two main enzymes in this system.

Microsomal enzyme inducing drugs such as phenytoin, phenobarbital and carbamazepine, and also alcohol, influence serum lipid and apoprotein concentrations. The inducers increase the concentrations of hepatic microsomal enzyme and apo A-I mRNA, and also proteins and phospholipids. Phenobarbitone is a cytochrome P450 inducer, and is used to reduce the toxicity of some drugs.

PROCEDURE:

1. Weigh and number the mice, divide them in to 2 groups and each group consist three mice.
2. To the 1st group inject phenobarbitone sodium once daily for 5 days.
3. To the 2nd group inject distilled water, similarly, for 5 days.
4. One hour after the last dose of phenobarbitone sodium on the 5th day, inject pentobarbital sodium to both the groups.

5. Note the onset and duration of sleep due to pentobarbitone sodium in both groups.
6. The time of the onset of action is loss of righting reflex in mice i.e. animal fails to uphold its normal position or falls asleep (hypnosis) is recorded for each animal.
7. The animals are placed on their back leaving sufficient space in between two animals.
8. Next, the time of recovery from sleep is recorded.

OBSERVATION TABLE:

Group	Drugs	Dose (mg/kg, i.p.)	Animals with Body Weight (g)	Onset of action (min)	Duration of action (min)	% reduction in sleep time
I	Pentobarbital	45	1.			
			2.			
			3.			
			MEAN			
II	Pentobarbital + Phenobarbitone	45 50	1.			
			2.			
			3.			
			MEAN			

CALCULATION:

$$\begin{aligned}
 & \frac{\text{Duration of action without Phenobarbitone} - \text{Duration of action with Phenobarbitone}}{\text{Duration of action without Phenobarbitone}} \times 100 \\
 & \text{\% Reduction in Sleep}
 \end{aligned}$$

RESULTS:

The % reduction in group II [Pentobarbital + Phenobarbitone] is _____.

DISCUSSION:

Phenobarbitone is a microsomal enzyme inducer which increases metabolism of Pentobarbital.

TEACHER'S SIGNATURE