



Effect of Inorganic Mercury on Drug Metabolizing Enzymes of Promethazine-Pretreated and Phenobarbitone–Pretreated Male Rabbit

Farah Anjum,¹ Farah R. Shakoori,¹ K.A. Al-Ghanim² and Abdul Rauf Shakoori^{1,3,4,*}

¹Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan

²Department of Zoology, College of Science, P.O. Box 24555, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

³School of Biological Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan

⁴Visiting Professor, Department of Zoology, College of Science, P.O. Box 24555, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

ABSTRACT

Effects of inorganic mercury have been studied on the drug metabolizing enzymes (DME) of phenobarbitone- (PB-) and promethazine- (PM-) pretreated male New Zealand white rabbit, *Oryctolagus cuniculus*. After PB treatment, the activities of cytochrome p-450 (CP), aniline hydroxylase (AH), acetanilide hydroxylase (AAH), benzphetamine demethylase (BD), aminopyrine demethylase (APD) and N,N dimethyl aniline demethylase (DAD) increased 214, 266, 207, 108, 81 and 83%, respectively. Cytochrome-c-reductase (CCR) also increased 109%. However, the activities of cytochrome b5 (CB) and N,N dimethyl aniline N-oxide (DAO) decreased 76% and 38%, respectively. PM administration had no effect on any of the DMEs except for AH, AAH and BD, which showed 60, 5 and 16% increase, respectively. HgCl₂ administered at a dose of 1mg/kg body wt per day for 5 days did not affect the CP, CB, APD and CCR. The activities of AH and AAH increased 32 and 33%, respectively, whereas those of BD, DAD, and DAO decreased 35%, 30% and 32%, respectively. When administered to PB-pretreated animals CP, AH, AAH, BD, APD, DAD, CCR, and DAO were inhibited 57, 58, 42, 31, 56, 55, 76 and 59%, respectively. Activity of CB increased 351%. HgCl₂ had no effect on CP and CCR when administered to PM-pretreated animals. AH, AAH and CB increased 17, 135 and 30%, respectively. BD, APD and DAD were reduced 47, 10 and 19, respectively. Formation of DAO was also inhibited 52%. To conclude, mercury drastically interferes with the DME. The metabolism of PB is inhibited, whereas that of PM is variably affected – hydroxylases are up-regulated and demethylases are down-regulated. CB is however, induced 351% after mercury treatment.

Article information

Received 1 January 2016

Revised 1 June 2016

Accepted 13 June 2016

Available online 1 August 2016

Authors' Contributions:

ARS conceived and designed the study and supervised the work. FA executed the experimental work. ARS and FA wrote the article. FRS and KAG analyzed the data.

Key words:

Inorganic mercury, drug metabolizing enzymes, promethazine, phenobarbitone, Cytochrome p450, Cytochrome b5, flavin monooxygenase system.

INTRODUCTION

Mercury is not an essential element to man or animals and is not required for any specific biochemical or physiological function in living tissues. It is however, extensively used in industry especially in electrical and scientific laboratory equipment, and as fungicide, herbicide, insecticide, bactericide, disinfectant and antiseptic (Ozuah, 2000). Some mercury salts are used in therapeutics, as a cathartic, diuretic and antisyphilitic (Brieger and Rieders, 1959; Cook and Yates, 1969).

Mercury is a widespread environmental pollutant which poses serious health hazards (Ozuah, 2000; Chen *et al.*, 2012; Ratcliffe *et al.*, 1996, Kojima *et al.*, 1989). It exists in three major forms – mercury vapor, inorganic

bivalent mercury and organic mercury (Clarkson, 2002). Important human exposure sources are mercury vapors inhaled after release from dental amalgam, methyl mercury (MeHg) from fish and ethyl mercury (EtHg) present in vaccines and some pharmaceutical products (Barregard *et al.*, 2011; Risher *et al.*, 2002; Clarkson and Magos, 2006; Ishitobi *et al.*, 2010). Inorganic mercury, which is both toxic and corrosive, exists in two oxidation states – mercurous and mercuric. Mercuric chloride was used as an antiseptic and is now being used as wood preservative, photographic intensifier, dry battery depolarizer, tanning agent for leather, catalysts in the manufacture of chemicals such as vinyl chloride and disinfectant. G. I. tract and the skin are the common

Abbreviations used: AH, Aniline hydroxylase; AAH, Acetanilide hydroxylase; APD, Aminopyrine demethylase; BD, Benzphetamine demethylase; CB, Cytochrome b5; CCR, Cytochrome C reductase; CP, Cytochrome P₄₅₀; DAD, N-N dimethyl aniline demethylase; DAO, N-N dimethyl aniline N-oxide; DME, Drug metabolizing enzymes; PB, Phenobarbitone; PM, Promethazine.

* Corresponding author: arshaksbs@yahoo.com, arshakoori.sbs@pu.edu.pk
0030-9923/2016/0005-1485 \$ 8.00/0
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routes of entry of inorganic mercury and have been shown to be preferentially deposited in the kidney. Mercury also induces metallothionein which binds with the metal and enhances the excretion in a bound and toxicologically inactive form in the urine of the individual (Bryn *et al.*, 1974; Lauwerys *et al.*, 1987). Excretion of mercury compounds is also faecal (Nielsen and Andersen, 1989). All forms of mercury produce varying degrees of toxic effects in many organs or systems such as respiratory, hepatic and renal (Goyer and Clarkson, 2001).

Inorganic mercury can inhibit enzymes containing reactive thiol groups in or near the active site (Bridges and Zalups, 2005; Bozcaada *et al.*, 2007; Draper and Hammock, 1999; Rangunathan *et al.*, 2010). At low concentrations, mercuric ions affect carbohydrate metabolism by blocking glucose entry and by complexing with phosphate ligands or by increasing passive alkali ions permeability and then entering the cell and accumulate in liver lysosomes (Vives-Rego *et al.*, 1986). At higher concentrations, these ions rupture lysosomes and release destructive acid hydrolases (Dalston *et al.*, 1986). High mercury content in cattle food had caused leukemia in men and cattle (Janicki *et al.*, 1987). Biochemical studies reveal significant decrease in the enzyme activities like glucose-6-phosphatase, alkaline phosphatase, ATPase and succinic acid dehydrogenase, aminolevulinic acid dehydratase and cholinesterase activities and moderate increases of acid phosphatases activity in the liver, kidneys and brain of laboratory animals and malonic dialdehyde levels in the kidneys of rats after mercury intoxication (Taylor *et al.*, 1973).

Little is known about the effect of mercury on drug metabolism. The previous investigators used excessively high doses of this metal (Xiao and Lui, 1988). Mostly mercury exposure in the environment is of low level and long term, thus cumulative effect becomes important while its higher concentration is only accidental. Mercury decreases the activity of the liver microsomal detoxification system and indirectly potentiates the harmful effects of other toxicants (Ando and Wakisaka, 1989). Keeping in view the above importance of metal pollution, in present investigation sublethal dose was used to see its adverse effects on different components of drug metabolism.

MATERIALS AND METHODS

Chemicals

All the reagents, chemicals and biochemical used were of highest quality and purchased from BDH Chemicals Ltd. Poole, UK. Promethazine hydrochloride

and Phenobarbital disodium were bought from Aldrich Chemicals Company Gillingham, Dorset, UK.

Animals

Male New Zealand white rabbits *Oryctolagus cuniculus* weighing nearly 1.5-2.0 Kg were used. They had access, at all times, to water and green fodder. Animals were kept under constant conditions of temperature (28-30°C) and humidity (45-50%). The total body weights of the rabbits were recorded daily before and during the experiments. There was a regular gain in the body weight and increase in the food consumption, indicating that they were well adjusted to the given experimental conditions.

Experimental design

Six groups, each of three male New Zealand white rabbits were treated as follows:

Group 1: Aqueous solution of mercuric chloride was injected intraperitoneally (*ip*) at a dose of 1 mg/kg body weight/ day for five days. Animals were sacrificed on the sixth day.

Group 2: Aqueous solution of PB was injected *ip* at a dose of 5 mg/kg body weight/day for 5 days followed by mercuric chloride treatment at 1 mg/kg/day for next five days. Animals were sacrificed on day 11.

Group 3: Aqueous solution of PM was injected *ip* at a dose of 5 mg/kg body weight/day for 5 days followed by mercuric chloride treatment at 1 mg/kg/day for next five days. Animals were sacrificed on the 11th day.

Group 4: Aqueous solution of PB was injected *ip* at a dose of 5 mg/kg body weight/day for 5 days followed by no treatment for next five days. Animals were sacrificed on the 11th day.

Group 5: Aqueous solution of PM was injected at dose of 5 mg/kg body weight per day for 5 days followed by no treatment for the five days. Animals were sacrificed on the 11th day.

Group 6: This group remained untreated and was kept as control.

All the animals were killed by cervical dislocation 24 h after the final dose, following overnight starvation to reduce glycogen. On stipulated day the animals were slaughtered, their livers taken out and microsomes prepared for the estimation of activities of various membrane bound DME such as cytochrome P450 (CP), cytochrome b₅ (CB), cytochrome-c-reductase (CCR), aniline hydroxylase (AH), acetanilide hydroxylase (AAH), benzphetamine demethylase (BD), aminopyrine demethylase (APD), N.N. dimethyl aniline demethylase (DAD), and N.N. dimethyl aniline N-oxidase (DAO).

Preparation of microsomes

Livers were rapidly removed, blot dried, weighed and then immediately placed in ice-cold 0.25 M sucrose to wash off excess of blood and to cool the tissue. Livers were perfused with ice-cold saline solution to wash away clotted blood in the veins and arteries. Small pieces of livers were finely chopped with surgical scissors and then homogenized very finely using Sorvall omni-mix homogenizer for 5 x 5 seconds.

Microsomes were prepared by modification of the calcium precipitation method described by Gibson and Skett (1986). The method was based on the calcium-dependent aggregation of endoplasmic reticulum fragments and subsequent low speed centrifugation of the aggregated microsomal particles. The homogenates were centrifuged in an ultra-refrigerated J2-21 Beckman centrifuge, at the speed of 10,000xg for half an hour to isolate subcellular fractions. 0.2 ml of 88 mM CaCl₂ solution was added per ml of supernatant. The mixture was then left standing in ice for five minutes. The mixture was again centrifuged for 15 min at 15,000xg. The supernatants were discarded and the pellets obtained were suspended in ice-cold 0.15 M KCl (2 ml/g of original tissue) to remove either adventitious protein or excess CaCl₂. The suspensions were centrifuged at the speed of 15,000xg for 15 min. The pellets formed were again suspended in phosphate buffer pH 7.4 (2 ml/g of original tissue).

Preparation of co-factors

Following ingredients were used to prepare cofactor solution per flask: 2 μmol of NADP, 10 μmol of glucose-6-phosphate, 1.4 μl of glucose-6-phosphate dehydrogenase and 20 μmol of 50% (w/v) aqueous magnesium chloride were dissolved in 2 ml of 0.2M phosphate buffer pH 7.4.

Estimation of cytochrome b₅ (CB) and cytochrome P-450 (CP)

The method used was based on the procedure described by Omura and Sato (1964). Microsomal suspension (equivalent to 0.5 g original liver) were diluted 1:10 with 0.2M phosphate buffer (pH 7.4). Baseline was recorded from 500-400 nm on a Kontron Uvicon 860 spectrophotometer. Approximately 2-3g sodium dithionite was added to the content of the sample cuvette which reduces cytochrome b₅. The spectrum was recorded between the same wave lengths against reference cuvette which had oxidized cytochrome b₅. Cytochrome b₅ (molar extinction co-efficient 424-490 nm is 112 nmol⁻¹cm⁻¹ absorption maximum 424 nm when reduced) in the original solution was calculated by the following formula:

$$\frac{\text{Absorbance difference at 490-424 nm} \times \text{dilution factor} \times 1000}{112 \times \text{tissue protein concentration mg ml}^{-1}}$$

Where 112 is the molar extinction co-efficient. This gives the concentration in nmoles/mg protein.

Approximately the same amount of sodium dithionite was added to the reference cuvette. CO was bubbled into the sample cuvette at the rate of one bubble/second for 20 seconds and the UV scan repeated. The reduced, CO difference spectrum of cytochrome P-450 absorbs maximally at around 450 nm and the extinction co-efficient for the wave length couple 450-490 nm had been accurately determined to be 91 nmol⁻¹ cm⁻¹. The concentration of this cytochrome in the original suspension was calculated using the formula.

$$\frac{\text{Absorbance difference (450-490 nm)} \times \text{dilution} \times 1000}{91 \times \text{tissue protein concentration mg ml}^{-1}}$$

This gives the concentration in nmoles mg⁻¹ protein.

Determination of different isoenzymes of cytochrome P-450

Aniline hydroxylase (AH) and acetanilide hydroxylase (AAH)

The assay used is based upon the method of Schenkman *et al.* (1967) and Mazel (1972) in which 0.5 ml of 10 mM aniline (or acetanilide) hydrochloride was mixed with 1.0 ml of co-factor solution and incubated for 5 min at 37°C. Reaction was initiated by adding 0.5 ml of microsomal reaction and incubation was continued for 30 min at the same temperature. Reaction was stopped by the addition of 1.0 ml of ice-cold 20 % trichloroacetic acid (TCA). The mixture was centrifuged to get clear supernatant. 1.0 ml of supernatant was mixed with 1.0 ml of 1% phenol and 1.0 ml of 1 M sodium carbonate. The reaction mixture was left for 30 min. Absorbance at 630 nm was recorded against blank which was prepared by using the same procedure but replacing aniline (or acetanilide) with 0.5 ml of water.

The amount of metabolite formed was calculated by direct reference to the standard curve constructed by using known concentration of 4 amino phenol. The enzyme activity was quantitatively calculated by the following formula.

$$\text{Enzyme activity (nmoles mg}^{-1}\text{protein)} = \frac{4}{\text{total protein in 2 ml}}$$

Aminopyrine N-demethylase (APD), Benzphetamine demethylase (BD) and N.N. dimethylaniline demethylase (DAD)

1.0 ml of modified co-factor solution (prepared by replacing 0.15 MgCl₂ with 0.15 M MgCl₂/0.1 M semicarbazide) was mixed with 0.5 ml of 20 mM

aminopyrine (or benzphetamine or N.N. dimethylaniline) and incubated for 5 min at 37°C in a shaking water bath. Enzyme reaction was initiated by adding 0.5 ml of microsomal fraction and terminated after 30 min incubation, by adding 0.5 ml of 25% ice-cold ZnSO₄. Later 0.5 ml of ice cold super saturated barium hydroxide was added. Mixture was centrifuged for 15 min to get clear supernatant. 1.0 ml of the clear supernatant was mixed with 2 ml of Nash reagent and incubated for 30 min at 60°C in shaking water bath. Tubes were then cooled and absorbance was noted at 415 nm. Values were read on a standard curve of formaldehyde which was constructed by using stock solution of 0.2 mM formaldehyde. The enzyme activity was expressed in nmoles/min/mg protein.

N.N. dimethyl aniline N-oxide (DAO)

Method used was slightly modified from the one described by Ziegler and Pettit (1964). 0.5 ml of N.N. dimethyl aniline was mixed with 2 ml of co-factor and incubated for 5 min at 37°C. 1.0 ml of microsomal fraction were added to initiate the reaction and incubated for 30 min. Reaction was stopped by adding 1.0 ml of terminator 0.3 M perchloric acid. Mixture was then centrifuged and 3 ml of deproteinized supernatant solution was adjusted to pH 9.4-10 by adding 1.0 ml of 2 M NaOH. Mixture was washed and extracted 3-4 times with 5.0 ml of diethyl ether to remove all the unchanged or demethylated N.N. dimethyl aniline. 2 ml of 3M TCA was added to reduce pH 2.5 to 3 approximately. 1.0 ml of 0.009 M NaNO₃ was added and reaction mixture incubated at 60°C for 5 min. Absorbance was read at 420 nm against water. The amount of metabolite formed was calculated by using extension coefficient 8.2/cm. The enzyme activity was expressed in nmoles per mg protein.

NADPH-cytochrome C (P-450) reductase (CCR)

In 3 ml sample cuvette 0.4 ml aqueous solution of cytochrome c (5 mg/1 ml), 2.0 ml of phosphate buffer (pH 7.4) and 0.1 ml of liver microsomal suspension were mixed. The reference cuvette contained all the same solutions in the same amount except for co-factor solution (0.5 ml) and phosphate buffer (2.5 ml.). The reaction was initiated by adding 0.5 ml of co-factor solution to the sample cuvette. The absorbance change at 550 nm was recorded as a function of time for the linear period of the reaction (about 3 min).

Enzyme activity was calculated by the following formula.

$$\frac{\text{Absorbance change (550 nm) per minute (linear portion)}}{19.6 \text{ m}^{-1} \text{ Cm}^{-1} \times \text{protein concentration per mg}}$$

whereas 19.6 mM⁻¹Cm⁻¹ was a extinction co-efficient. This gives the concentration in μmol cytochrome reduced/ min/ mg protein.

Determination of protein

Protein was determined by the colorimetric method of Lowry *et al.* (1951).

RESULTS

Table I shows the effect of mercuric chloride on DME in the male New Zealand white rabbits. Mercuric chloride administration (1 mg/kg for 5 days) did not affect CP, CB, CCR and APD activities, though AH and AAH activities increased 32 and 33%, respectively. The BD (35%) and DAD (30%) activities, and formation of DAO under flavin containing monooxygenase were, however, significantly inhibited (Fig. 1).

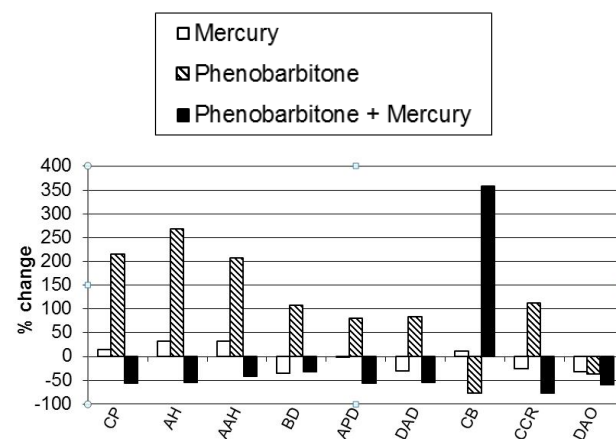


Fig. 1. Effect of mercuric chloride (1 mg/kg body weight for 5 days) on the % increase (+) or decrease (-) in drug metabolizing enzymes of phenobarbitone (5 mg/kg for 5 days) pretreated rabbits.

After PB treatment (5 mg/kg for 5 days) all the enzymes were induced except for CB which was inhibited 76%. The activities of CP, AH, AAH, BD, APD, DAD and CCR increased 215, 266, 207, 108, 81, 83 and 109%, respectively. DAO formation was decreased 38% (Table I, Fig. 1).

Mercuric chloride administration (1 mg/kg for 5 days) to PB- pretreated animals inhibited the activities of all the enzymes studied except cytochrome b5 (CB) which was increased 351%. The activities of CP, AH, AAH, BD, APD, DAD and CCR decreased 57, 58, 42, 31, 56, 55 and 76%, respectively. DAO decreased 59% (Table I, Fig 1).

Table I.- Effect of mercuric chloride (1 mg/kg body weight per day for 5 days) on drug metabolizing enzymes in the liver of phenobarbitone (5 mg/kg body weight per day for 5 days) pretreated and promethazine (5 mg/kg body weight per day for 5 days) pretreated rabbits.

Parameters	Control (n=3)	Mercury treated (n=3)	Phenobarbitone-treated (n=3)	Mercury treatment to phenobarbitone pretreated (n=3)	Promethazine treated (n=3)	Mercury treatment to promethazine pretreated (n=3)
Cytochrome P-450 (nmoles/mg protein)	1.02±0.06	1.17±0.15	3.21±0.21***	1.39±0.19**	1.25±0.27	1.34±0.18
Aniline hydroxylase (nmoles/mg protein)	5.99±0.48	7.92±0.34*	22.04±3.25**	9.9±0.30**	9.6±0.23***	11.23±0.50*
Acetanilide hydroxylase (nmoles/mg protein)	3.89±0.52	5.16±2.77*	11.95±0.52***	6.95±1.38*	6.1±0.23*	14.34±0.79***
Benzphetamine demethylase (nmoles/mg protein)	119.01±0.09	77.22±8.81***	247.3±5.9***	169.81±4.01***	139.1±6.21*	74.62±5.05***
Aminopyrine demethylase (nmoles/mg protein)	152.09±12.05	149.75±7.16	275.23±4.33***	121.85±4.85***	155.2±2.37	139.93±3.05*
N.N.dimethylamine demethylase (nmoles/mg protein)	112.26±8.12	78.08±3.56*	205.59±10.15***	93.53±3.12***	132.62±2.49	107.56±1.95**
Cytochrome b5 (nmoles/mg protein)	0.51±0.08	0.57±0.10	0.12±0.001**	0.55±0.04***	0.53±0.02	0.69±0.01**
Cytochrome-c-reductase (μmole cytochrome reduced/min/mg protein)	0.08±0.01	0.06±0.006	0.17±0.02*	0.04±0.01**	0.07±0.06	0.06±0.004
N.N. dimethyl aniline N oxide (nmoles/mg protein)	52.99±3.08	35.95±1.10***	33.08±0.31**	13.48±2.01***	60.23±2.39	28.61±2.00***

Mean ± SEM; student's 't' test; *P<0.05, **P<0.01, ***P<0.001.

For statistical significance the mercury-treated, the phenobarbitone-treated and the promethazine-treated group was compared with the control, while mercury-treated phenobarbitone group has been compared with phenobarbitone-treated group, and mercury-treated promethazine group has been compared with promethazine-treated group.

PM treatment (5 mg/kg for 5 days) affected only AH, AAH, and BD activities which were increased 60, 57 and 16%, respectively. All the rest of the enzymes remained unaffected after PM treatment (Table I, Fig.2).

Mercuric chloride administration (1 mg/kg body weight for 5 days) to PM-pretreated rabbits did not affect CP and CCR. AH, AAH and CB levels increased 17, 135 and 30%, respectively, whereas those of BD, APD and DAD decreased 46, 10 and 19%, respectively. Formation of DAO was decreased 52% (Table I, Fig. 2).

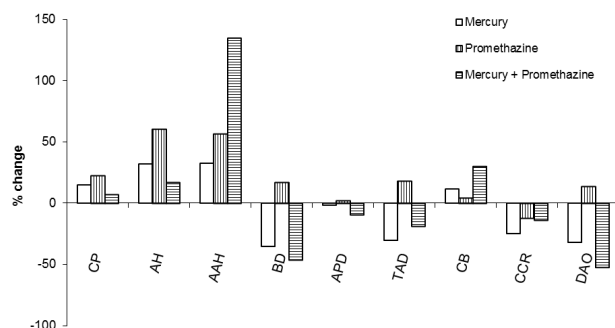


Fig. 2. Effect of mercuric chloride (1 mg/kg body weight for 5 days) on the % increase (+) or decrease (-) in drug metabolizing enzymes of promethazine (5 mg/kg for 5 days) pretreated rabbits.

Figure 3 shows representative spectrophotometric profiles of absorbance of cytochrome P450 and cytochrome b5 at different wave lengths in different experimental groups, whereas Figure 4 shows the same representative profiles of cytochrome c reductase.

DISCUSSION

Cytochrome P-450 dependent enzymes systems

Mercuric chloride administration did not effect the CB level, but when administered to PB-treated rabbits the CB level increased 215%. PB has already been reported to a good inducer of cytochrome P-450 enzyme systems (Nebert, 1979).

CP activity was not inhibited after mercuric chloride treatment, but when mercuric chloride was administered to PB-pretreated rabbits the CP was significantly. PM did not seem to induce CP level in contrast to previously reported data. Different investigators reported inhibition of microsomal CP after mercury treatment *in vitro* (Xiao and Lui, 1988) and in different laboratory animals (Chakrabarti and Brodeur, 1986; Su and Okita, 1986; Takabatake *et al.*, 1986). This characteristic inhibition was not observed in PM pretreatment. Mercury probably, effects the binding sites of the enzyme in PB-pretreated animals.

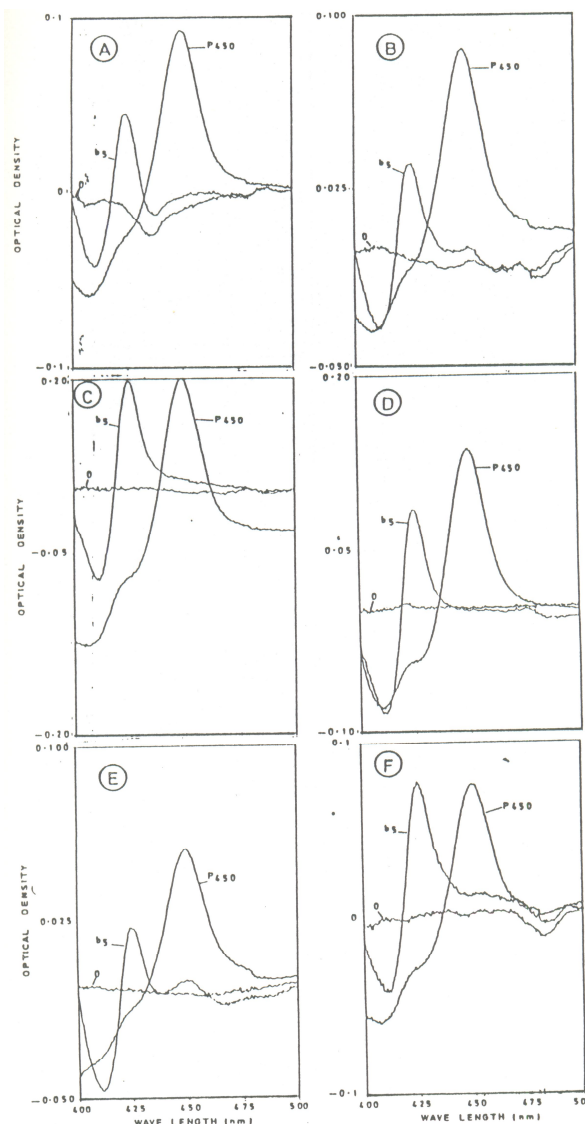


Fig. 3. The representative spectrophotometric profiles of absorbance of cytochrome P-450 and cytochrome b5 at different wave lengths in control (A), mercury treated (B), phenobarbitone treated (C), phenobarbitone + mercury treated (D), promethazine treated (E) and promethazine + mercury treated (F) rabbit liver.

CP is a multigene family, which consists of several related enzymes, including isoenzymes involved in four hydroxylations Lu and West (1980). Although this class has more than 50 enzymes, six of these metabolize 90% drugs with the two most significant being CYP 3A4 and CYP 2D6 (Lynch and Price, 2007). CYP 2C19

metabolizes PB, during which CYP12A and CYP2C9 are induced (Czekaj, 2000). CYP2D6 is responsible for PM metabolism. PM inhibits CYP2D6 (Nakamura *et al.*, 1996). Currently the most relevant DME are CYP2D6, CYP2C9, CYP2C19, thiamine methyltransferase and UDP-glucuronosyltransferase (Sim *et al.*, 2013; Wu, 2011).

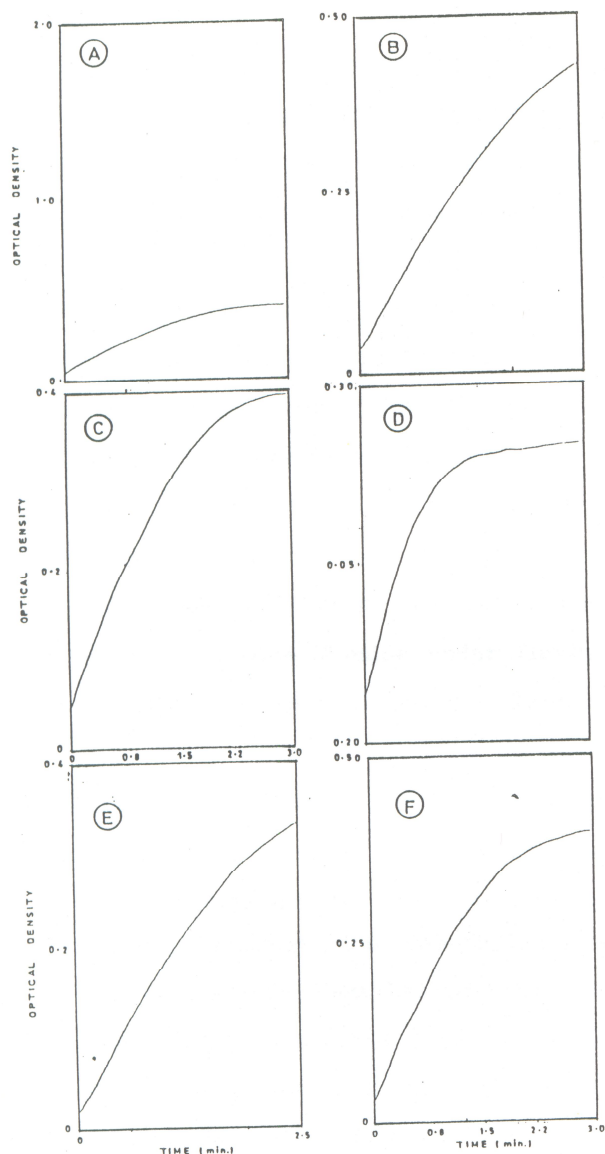


Fig. 4. The representative spectrophotometric profiles of absorbance of cytochrome c reductase at different time (min) in control (A), mercury treated (B), phenobarbitone treated (D), promethazine treated (E) and promethazine + mercury treated (F) rabbit liver.

Two substrates aniline and acetanilide were used to

see the effects of mercuric chloride on hydroxylation. Mercuric chloride induced AH and AAH significantly. PB-treatment induced activity of both the hydroxylases significantly, but after mercuric chloride treatment the AH activity was inhibited 58% and AAH 42%. Promethazine treatment also induced the hydroxylases, but when mercuric chloride was administered to PM-pretreated animals, activities of both these hydroxylases were significantly increased. Xiao and Lui (1988) reported marked inhibition in the activities of AH and hexobarbital hydroxylase with mercuric chloride treatment to PB-pretreated animals. Takabatake *et al.* (1986) also reported inhibition of the AH, which suggested that the hexobarbital hydroxylase reduction was due to CP reduction.

PB induced forms of isoenzymes were probably sensitive to mercuric chloride treatment as is evident from their impaired activities.

Mercuric chloride did not affect AD activity. It inhibited BD and DAD activities significantly. Under PB treatment, all the three demethylases were increased significantly, suggesting PB as a good demethylase inducer. There was significant increase in benzphetamine dimethyl after PM treatment. Mercuric chloride decreased the PB induced demethylation reactions for all the three substrates. Inhibition pattern of mercuric chloride was same for the three demethylases in PM-pretreated animals. Takabatake *et al.* (1986) reported inhibition in the activities of aminopyrine N-demethylase and P-nitroanisole o-demethylase after mercury administration. However, Kaduska *et al.* (1985) reported no effect on the activities of ethyl morphine N-demethylase benzphetamine N-demethylase after mercury treatment. Xiao and Lui (1988) reported marked decrease in the PB induction of demethylation reactions.

Although sublethal mercury administration did not affect CP level, when injected alone, it affected all the related isoenzymes studied. Hydroxylations, catalyzed by CP were enhanced, while demethylation processes were inhibited. All the isoenzymes along with CP decreased with mercuric chloride treatment, when administered to PB-pretreated rabbits. Likewise mercuric chloride in PM-pretreated rabbits showed enhanced rate of hydroxylation, whereas demethylation of different substrates decreased without affecting CP level. All the above discussed results suggest that mercury administration did not affect total CP level when injected alone. All the hydroxylation and demethylation reactions though CP dependent, all are controlled by different isozymes. Mercury affected all these enzymes differently.

NADPH-cytochrome P-450 reductase or cytochrome-c-reductase (CCR) is an essential component of MFO system responsible for drug oxidation. Mercury

chloride treatment did not affect CCR activity. PB treatment had significantly increased CCR activity, while mercuric chloride treatment had significantly reduced the activity when administered to PB pretreated animals.

There was no effect on the activity of CCR with PM pretreatment. Activity remained unaffected even after mercuric chloride administration to PM-pretreated animals.

Both the drugs affect the CCR differently. PB induces the activity as it is a good inducer for this particular enzyme (Utlely and Mehendale, 1988), while PM did not induce or inhibit the activity. Probably PB induced CCR was more sensitive to mercury treatment than the uninduced forms in mercuric chloride treated and PM pretreatment animals.

Nakahama *et al.* (2001) have reported that mercury was the most potent in reducing CP content and MFO activities including CCR and benzopyrene hydroxylase. Mercury reduces hepatic enzyme activities of cytochrome P₄₅₀ and MF) (Alvares *et al.*, 1972; Abbas, 1980).

Cytochrome b₅

Cytochrome b₅ (CB) is an obligatory component of mono-oxygenase, and it passes the electrons and reducing equivalents to cytochrome P-450 monooxygenase system.

Activity of CB remained unaffected with mercuric chloride treatment. Ribarov and Benov (1981) reported increased content of CB, although in the present study PB treatment did not induce the activity, it had rather inhibited the level significantly. With PM pretreatment, there was no effect on the CB level. However, this level increased with mercuric chloride treatment to PM-pretreated animals. The possible explanation for the increased level is the preferential increase of other proteins by mercury which causes increase in the CB content, or it could be drug metal interaction which probably forms certain metabolites, which could activate the synthesis, causing increased level.

Flavin containing mono-oxygenases

Estimation of N-oxides from tertiary amine generally reflects the oxidation reaction catalyzed by flavin containing mono-oxygenase (Ziegler, 1980). N-oxide formation from N.N. dimethyl aniline were markedly inhibited by mercuric chloride treatment. PB did not induce the activity of flavin containing mono-oxygenase. However, the activity was markedly inhibited by the administration of metal to the PM pretreated rabbits. In all the three cases, activity of flavin containing mono-oxygenase was reduced by mercury treatment, as reflected by reduced formation of N-oxide. As discussed before, this substrate was only metabolized by flavin containing monooxygenase. The present investigation

suggests that the mercury is a strong inhibitor of flavin containing monooxygenase. Its mode of action is quite similar to CP. Sublethal administration of the metal did not affect CP, while it strongly inhibited the flavin containing monooxygenase in mercury treated rabbits.

High doses of mercury has been reported to inhibit CP (Chakrabarti and Brodeur, 1986). In present investigation, CP was inhibited by mercuric chloride in PB-pretreated animals. These different factors produce different changes which directly or indirectly affect drug metabolism.

PB is a hypnotic drug causing sleep in animals when administered, PB induced CP and its related isoenzyme, which in turn metabolize the drug causing sleep in the animal. Like other barbiturates decreased duration of hexobarbital sleeping time is a well known indicator of impaired drug metabolism. Under mercury administration hexobarbital sleeping time was reported to be significantly less (Su and Okita, 1986; Kaduska *et al.*, 1985). Mercuric treatment to animals not only affect phase I drug metabolism but it was also reported to depress the activities of enzymes involved in phase II drug metabolism. Glutathione binds with xenobiotics and helps in their elimination by biliary excretion. Gregus and Varga (1985) reported increased concentration of hepatic glutathione in rats with mercuric chloride treatment, which was then transported from liver to kidney for excretion. PB enhances the biliary excretion of heavy metals by increasing the transport of glutathione.

To conclude, in the present investigation, sublethal dose of mercuric chloride showed inhibitory pattern on DME. The treatment did not affect CP activity, however the observed demethylase were inhibited while hydroxylases were induced. CCR was also inhibited. All these findings suggest that mercury affects some of the drug metabolism pathways and liver microsomal detoxification processes.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the University of the Punjab, and Deanship of Scientific Research, King Saud University for funding this research through the Prolific Research Group No 1436-011.

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