INHIBITORY EFFECT OF 'RHEUM EMODI WALL' ON HEPATIC CYTOCHROME P450 ENZYMES

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Background: This study was carried out to assess the inhibitory effect of aqueous extract of Rheum emodi wall on hepatic microsomal P450 enzyme. **Materials and Methods:** Four groups of rats were made, comprising of 20 rats in each group. Two groups were studied for the pentobarbital-induced sleeping time and rest of the two groups were studied for the lethal effects of strychnine. Normal saline and aqueous extract of a plant 'Rheum emodi wall' were given to first two groups prior to intramuscular injection of pentobarbital. Sleeping time was then noted in both groups for the next two hours. Strychnine lethality was observed in the other two groups of rats in which one group received olive oil and the other group received plant extract before injecting strychnine. The rats were monitored for the next two hours to count mortalities. **Results:** Plant aqueous extract caused significant prolongation ($p<0.001$) in pentobarbital-induced sleeping time in aqueous extract treated group as compared to group receiving normal saline along with pentobarbital. Increased lethality by strychnine was observed in the group getting plant aqueous extract than the group in which olive oil was administered along with injection of strychnine. **Conclusion:** The increased effects of pentobarbital on sleeping time and strychnine on the lethality of rats conclude that crude aqueous extract of 'Rheum emodi wall' inhibits hepatic microsomal P450 enzyme.

Keywords: Rheum emodi wall, pentobarbital, strychnine

INTRODUCTION

Hepatic microsomal cytochrome P450 (CYP450) enzymes, a unique family of proteins, catalyze the oxidation of almost all the compounds of our environment, i.e., xenobiotics.[1] These are mainly metabolized in liver and there are three stages for their detoxification, termed first, second and third phases. Cytochrome P450 1A2 (CYP1A2) is a major enzyme responsible for first stage detoxification reactions.[2] Xenobiotics are the drugs, food additives, pollutants etc. to which humans are exposed in this modern age. They also include chemical carcinogens such as polychlorinated biphenyls (PCBs) and certain insecticides. More than 200,000 manufactured environmental chemicals exist. In some cases their products are mutagenic or carcinogenic.[3] Cytochrome P450 3A (CYP3A) is involved in biotransformation of more than half of all drugs currently available.[4] Liver contains highest amounts of CYP450 among all the tissues of the body. Hepatic microsomal P450 enzymes have shown the great diversity of sizes, shapes, and modes of binding of the substrate binding sites of these mammalian cytochromes P450 (mainly from human liver). These active sites are conformationally very flexible that can adapt themselves to the xenobiotic structure for the best possible efficacy of substrate oxidation catalysis.[1] They may also change nonreactive substance to a reactive substance which may be harmful, e.g., CCl_4 to its reactive species which damages the liver.[5,6]

Cytochrome P3A2 is one of the most abundantly expressed cytochrome P-450s in the rat liver and almost identical to and functionally equivalent to human Cytochrome P3A4, which metabolizes numerous drugs including barbiturates which also induce them.[7] Pretreatment with the extract a herbal plant Ginkobiloba potentiated acetaminophen toxicity in cultured rat hepatocytes by inducing CYP3.[8] Gama aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian central nervous system (CNS). Barbiturates like pentobarbital potentiate the effect of GABA by binding at the $GABA_A$ receptor thereby inducing sleep.[9]

Strychnine is a very toxic poison, which is metabolized by CYP450[10] and is believed to be a competitive antagonist of the inhibitory neurotransmitter glycine at receptors in the spinal cord, brain stem and higher centres.[11] It results in increased neuronal activity and excitability, leading to enhanced muscular activity leading to death.

In our previous work we established that crude aqueous extract of a Himalayan plant 'Rheum emodi wall' affords protection against CCl_4-induced liver damage in rats when given before the metabolic activation of the toxin.[12] Inhibitory activity of hepatic microsomal P450 enzymes is reported to be common

in certain medicinal plants. The use of the extracts of the some medicinal plants has shown hepatoprotective and cancer chemoprotective properties.[13]

The present study was done to assess the inhibitory effect of the aqueous extract of 'Rheum emodi wall' on hepatic microsomal P450 enzymes.

MATERIALS AND METHODS

The roots of Rheum emodi wall commonly known as Revand chini were purchased from an herbal dealer locally called as pansari.

One-hundred grams of powdered form of roots was obtained mechanically with a china herb grinder. It was macerated in 500 ml of water for 24 hours with occasional shaking to prepare the aqueous extract. The filtrate was obtained and dried in petri dishes and concentrated by heating at 40^0C under reduced pressure in an oven (Toyo vacuum drying oven, Seisakusho Co., Japan). The dried aqueous extract yield so obtained was 9 grams which was stored in a refrigerator and dissolved in distilled water just before administration to the rats. Young Healthy young adult male Sprague Dawly's Albino rats (n= 80) weighing between 150–200 g, were obtained from the animal house of the National Institute of Health Islamabad and kept under standard conditions. Food and fresh water was available ad libitum.

Animals were divided into four groups having 20 rats in each group. Group I received normal saline (10 ml/kg) and group II was given aqueous plant extract (500 mg/kg of rat body weight) as a single oral dose. Intra-muscular injection of Pentobarbital (75 mg/kg) was then administered in the thigh muscle after 1 hour to both groups. These two groups were then observed for two hours for their sleeping patterns. The third group was given olive oil (7.5 mg/kg) followed by a sub-lethal oral dose of strychnine (0.4 mg/kg) after one hour. The animals in fourth group were given similar treatment except that the olive oil was replaced by the aqueous plant extract (500 ml/kg of rat body weight). The animals were monitored for next two hours to count mortalities.

Statistical analysis was made by using the computer programme Statistica. Mean and standard deviation were calculated in the pentobarbital study while in the strychnine lethality study the percentage of mortality was calculated.

RESULTS

The results of the present study were such that the sleeping time was 91±9 minutes in the saline-treated group whereas it was 147±16 minutes in the group receiving plant aqueous extract. The sleeping time was found significantly increased in the aqueous extract-treated group as compared to the group getting pre-treatment with normal saline.

While observing strychnine-induced lethality, it was noticed that 80% animals died in the group receiving plant aqueous extract before getting strychnine. Only 20% albino rat mortality was observed in the group treated with olive oil.

DISCUSSION

It is a well known fact that the hepatic cytochrome P450 enzymes are subjected to induction and inhibition by exposure to a wide variety of xenobiotics. These enzymes are affected by multiple active constituents of medicinal plants or herbs. Many compounds isolated from herbs have been identified as substrates, inhibitors, and/or inducers of various CYP enzymes. For example, St. John's wort induces CYP3A4. It contains ingredients causing inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.[14] Some flavonoid compounds have recently been isolated from plants like hops, Humulus lupulus. Their chemical structures are similar to other plant-derived compounds, many present in the human diet, that have been proved to have cancer chemopreventive properties due to inhibition of cytochrome P450 enzymes that activate carcinogens.[13] Fennel, which is a seed of Foeniculum vulgare, was found having inhibitory effect on CYP 3A4 when its methanolic extract was used.[15] The duration of pentobarbital-induced sleep in intact animals is considered as an index for the activity of hepatic CYPs.[16] In the present study, duration of sleeping time was 147 minutes in the group in which plant extract was administered prior to intraperitoneal pentobarbital injection. The group in which only saline was administered prior to injection of pentobarbital, had the sleep time 91 minutes. The sleeping time was significantly increased in the former group showing the greater effect of pentobarbital. The results of our study are comparable with the results of study conducted by Gilani AH *et al*[17] They observed 117 minutes sleeping time in the study group pretreated with extract obtained from a plant Artimisia absinthium as compared to 81 minutes sleeping time in control group of mice getting saline instead of plant extract. Dose of pentobarbital was exactly same as in our study. In another study crude alkaloid fraction isolated from the leaves of Helieta apiculata was used. A statistically significant decrease in the sleep latency and prolongation of pentobarbital-induced sleep time was observed.[18] Results of our study are in agreement with the results of this study. Goloubkova *et al* in their study observed that crude extract of Heilata apiculata extract had inhibitory effect on cytochrome P450 enzymes leading to potentiation of the central nervous system depressant effect of pentobarbital. In fact pentobarbital is metabolized by hepatic CYPs to inactive metabolites and drugs with inhibitory effect on CYPs, likely to prolong pentobarbital-induced sleeping time.[19]

Sleep potentiality of pentobarbital can also be achieved by central nervous system (CNS) depressing drugs without alteration in cytochrome P450 enzymes activity.[20] A strychnine toxicity test was performed to see whether or not the plant extract mediated potentiation of pentobarbital sleep is due to enzyme inhibitory action or sedative effect. Strychnine is a substrate for cytochrome P450 enzymes[10] and most of the known inhibitors of cytochrome P450 enzymes increase the toxicity of strychnine through potentiation of CNS stimulant activity.[21] In our study, the group which was given normal saline had 20% mortality while 80% mortality was observed in the group, which was administered plant extract. The observed increased mortality of the sub-lethal dose of strychnine in pre-treated group with plant extract confirmed that strychnine could not be metabolized by the hepatic microsomal cytochrome P450 enzymes due to the inhibitory effect of the plant extract. Similar observations were made by Gillani AH *et al*[17] on study of Artemisia absinthium extract in which they observed 60% more mortalities using the same dose of strychnine as in our study. Gilani *et al*[22], on extract of fumaria Parviflora and Janbaz KH *et al*[23] with protopine fraction also observed increased mortalities with sub lethal dose of strychnine when given alongwith plant extracts showing inhibitory effects of tome P450 enzymes.

The results of the present study indicate that crude aqueous extract of the plant significantly increase the pentobarbital sleeping time in rats as well as the lethality induced by strychnine. Hence it is concluded that the crude extract of 'Rheum emodi wall' bears inhibitory effects on hepatic cytochrome P450 enzymes.

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