EFFECT OF COLEUS FORSKOHLLII EXTRACT ON THE INDUCTION OF HEPATIC CYTOCHROME P450 ISOENZYME CYP 1A IN RATS

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ABSTRACT

Drug metabolism is one of the major determinants of drug clearance, if a cytochrome P450 enzyme is induced by a compound, it may either, increase the metabolism of itself (auto induction), or a concurrent therapy, and so reduce plasma levels, resulting in a potential loss of efficacy. Thus the current study was focused to investigate the induction of CYP1A1 in rat liver by \textit{Coleus forskohlii} root extract (CFE). S9 fraction obtained from Male Wistar rats treated with CFE and \(\beta\)-naphtoflavone (BNF) were used to study the effect on CYP1A1 linked 7-Ethoxyresorufin O-dealkylase (EROD) activity and CYP1A1 mediated mutagenicity of EtBr through AMES assay. EROD activity of S9 obtained from BNF treated was 24.94 folds greater than control. Further studies on mutagenic potential of EtBr in \textit{Salmonella typhimurium} TA 98 and TA100 showed 24.94 and 25.32 folds increase over control whereas S9 fraction obtained from CFE treated animals increased neither EROD nor the mutagenicity of EtBr. Hence on the basis of Ames and EROD assay our study concludes that ethanolic extract of \textit{C. forskohlii} prepared at Vidya herbs Pvt. Ltd did not induce CYP1A1 and so also the genotoxicity.

KEY WORDS: Genotoxicity, Cytochrome P450, Coleus forskolii, Ames test.

INTRODUCTION

P450s constitute an important class of enzymes involved in the phase I metabolism of drugs. These enzymes play critical roles in the biotransformation of drugs, carcinogens, steroid
hormones and environmental toxicants. \cite{1} P450s are responsible for the generation of electrophilic constituents, which may bind to the macromolecules such as nucleic acids and proteins resulting in cytotoxicity and carcinogenicity. \cite{2} Among P450 enzymes, CYP1A induction has profound influence in broad areas of biomedical science including drug metabolism and drug development, cancer research, pharmacology, toxicology, and safety regulation of therapeutic agents and environmental and occupational chemicals. The induction of CYP1A1/1A2 during the drug metabolism followed by the bioactivation of toxic or carcinogenic compounds has been a concern of safety in drug development. CYP1 inducibility is a limiting factor for the drug development as it leads to possible toxic or carcinogenic effects. \cite{3}

\textit{Coleus forskohlii} is an important medicinal plant used as an Ayurvedic medicine to treat several ailments including hypothyroidism, hypertension, congestive heart failure, eczema, respiratory disorders and convulsions. It is mainly used worldwide for weight loss management. Forskolin is the major active principle which has both cAMP-dependent and cAMP-independent activities. Residual organic solvent used in herbal formulations may induce drug metabolizing enzymes. \cite{4} The present study evaluates the effect of \textit{C. forskohlii} extract from Vidya herbs Pvt. Ltd. on the induction of P450 (CYP1), the hepatic drug metabolizing enzyme which is responsible for the bioactivation of toxic compounds.

\textbf{MATERIALS AND METHODS}

\textbf{Chemicals}

β-naphtoflavone (BNF), Phenobarbital (PB), 7-ethoxyresorufin (7-ER) and were purchased from Sigma Chemical Co. (St Louis, MO). Ethidium Bromide (EtBr), Glucose 6-phosphate, Dicumarol, glucose 6-phosphate dehydrogenate NADPH and NADP were purchased from Himedia.

\textbf{Bacterial Strains}

The \textit{Salmonella typhimurium} strains TA98 and TA100 were obtained from LGC Prochem, Bangalore, India -ATCC cultures.

\textbf{Preparation of Plant Extract}

Freshly collected roots of \textit{C. forskolin} were shade dried and then powdered using a mechanical grinder. 100 g of pulverized plant part was extracted with 500 ml of ethanol using a soxhlet apparatus. At the end of extraction, the extracts were filtered under vacuum using
whatmann No. 1 filter paper and the process repeated until all soluble compounds had been extracted. The filtrate obtained was concentrated in vacuo using a rotavapor. The extracts were stored at 4 °C in an air-tight bottle until further use.

**Animal Experiment**

Animal experiments were carried out taking appropriate measures to minimize pain or discomfort in accordance with the guidelines laid down by the NIH (USA) regarding the care and use of animals for experimental procedures and with due clearance from the Animals Ethical Committee (Registration no 1687/PO/c/13/CPCSEA). Healthy inbred male rats of Wistar strain 200-250 g were housed in polypropylene cages and kept on a 12 h light/dark cycle in an animal care facility. The animals were allowed free access to laboratory rodent chow and tap water. Animals were closely observed for any infection; those which showed signs of infection were separated and excluded from the study. Acute toxicity study was performed by UDP method (OECD guideline no 425). 80 mg/kg was taken as the therapeutic dose of extract.

**Experimental Design**

The animals were treated daily for 3 days orally with 80 mg/kg BNF and C. forskohlii extract respectively. The control animals were untreated.

**Preparation of S9 Fraction**

S9 fractions were prepared from pooled liver samples of each treatment groups. The samples were minced and homogenized in 0.01 M phosphate buffer (pH 7.4) containing 1.15 % KCl (3 ml/g liver wet wt). The homogenates were centrifuged at 9000 g for 30 min at 4 °C. The supernatants were stored at -70 °C until use. The protein content was determined by the method of Lowry’s method\(^5\) using bovine serum albumin as standard.

**7-Ethoxyresorufin O-dealkylase (EROD) Activity**

7-Ethoxy- O-dealkylation was determined according to Burke et al. \(^6\) with some modifications. Resorufin formation was monitored using spectrofluorimeter (Perkin Elmer) with excitation 550 and emission 590 nm. S9 fractions prepared from the liver of treated or untreated animals were used instead of microsomes, when S9 fractions were the metabolic activating system in the mutagenesis assays. The reaction mixture consisted of 0.1M Tris HCl (pH 7.4), 10 mM substrate, 10 mM dicumarole, 0.5 mM NADPH, 10 mM glucose 6-phosphate, 2 mM MgCl₂, 3 U/ml glucose 6-phosphate dehydrogenase and 0.5 mg/ml S9
fraction in a final volume of 1 ml. The reaction lasted for 5 min and was terminated by the addition of 2 ml of ice-cold methanol. The increase in fluorescence due to resorufin formation was recorded with an X-Y plotter. The enzyme activities were determined by comparing the rate of increase in relative fluorescence to the fluorescence of the resorufin standards and expressed as pMmin⁻¹mg⁻¹ protein.

**Cytochrome P450 Induction Assay (CYPIA test)**

A CYP1A assay was performed as described by Yahagi et al. [7]. The *S. typhimurium* strains TA98 and TA100 were used for showing the BNF-type of cytochrome P450 induction. Mutagenicity of the EtBr was determined using a preincubation modification of the standard plate incorporation assay as described by Ames. [8] Briefly, 0.5 ml of S9 mix (or 0.1 M phosphate buffer, pH 7.4), 0.1 ml of bacterial culture and 0.05 ml of test solution (or solvent) were added to each tube. The mixture was vortexed, and then allowed to incubate at 37 °C with shaking for 30 min. Following the preincubation period, 2.0 ml of molten top agar (45 °C) supplemented with histidine and biotin (0.5 mM) was dispensed into the tubes, which were immediately vortexed and the contents poured onto the surface of bottom minimal glucose agar. When the agar overlay had solidified, the plates were inverted and placed in a 37 °C incubator. After incubation for approximately 48 h the revertant colonies were counted. Each S9 fraction was examined in the absence of mutagen to rule out the possibility of his⁺ reversion being caused by the mutagenic inducer or metabolite remaining in the preparation.

**RESULTS AND DISCUSSION**

*Coleus forskohlii* is a natural plant and the extract components may vary due to the extraction methods, which may give rise to different experimental results. Previous studies report that residual organic solvent used in herbal formulations may induce drug metabolizing enzymes. [12] In order to address these possibilities the present study was focused on whether *C. forskohlii* root ethanol extract induce CYP450 and hence leading to genotoxicity.

Treatment of rats with 80 mg/kg.bw of *C. forskohlii* extract and standard BNF were performed in order to evaluate the extent of cytochrome induction specifically that of the hepatic CYP1A1 through EROD and AMES assay. After 3 consecutive days of treatment, animals were sacrificed following the last injection. Liver S9 fraction obtained from all the groups was used for assay.

EROD activity describes the rate of CYP1A mediated deethylation of the substrate 7-
ethoxyresorufin (7-ER) to form the product resorufin. The catalytic activity towards this substrate is an indication of the amount of enzyme present and is measured as the concentration of resorufin produced per mg protein per minute (mol/mg/min). The S9 fraction obtained from BNF treated rats resulted in 18.13 fold increase in the level of EROD activity over the control group. In contrast there was no significant difference in CYP1A1 induction among control and C. forskohlii extract treated rats, i.e. 63.18 & 62.28 resorufin pM/mg per minute respectively (Fig. 1).

Mutagenic potential of EtBr was measured using S9 fraction obtained from treated and control group. EtBr showed low mutagenic activity with control S9 but using S9 prepared from BNF treated-rats mutagenicity was greatly increased (24.95 fold over control). When the number of revertant colonies of S. typhimurium per plate with EtBr as mutagen was two folds less than S9 mix control (without EtBr) the response was considered as non significant. [2] C. forskohlii administered at 80 mg/ kg b.w for 3 days resulted in CYP1A induction at same level as that of control group, corresponding to 64.75±3.32 and 66.25±6.65 his+ revertants at 10 µg EtBr /plate respectively (Fig. 2). Hence the response was considered non-significant. On the other hand, the same amount of S9 fraction tested against TA 100 resulted in low mutagenicity of EtBr but the extent of induction was similar i.e., (Std BNF 24.95 and 25.23 folds over control). Fig. 3 shows that mutagenicity of S9 from control and C. forskohlii administered group resulted in non significant induction corresponding to 2 revertants his+/10 µg EtBr.

We showed that C. forskohlii ethanol extract administered to rats did not markedly induce hepatic drug metabolizing enzyme CytP450 CYP1A1. The study comprised of AMES and EROD assays to assess the ability of the extract to induce CYP450 enzymes. The CYP1A induction through AMES assay measures the mutagenic activation by a liver S9 preparation of EtBr into mutagenic metabolites. These are specifically generated by cytochrome p450 monooxygenases induced by MC and PB like compounds. In the present study BNF has been replaced for 3-MC, a potent inducer of CYP1A1. [9]

There is a satisfactory agreement between the Ames test (in presence of activation system) and enzyme induction studies in some cases. [10] For example, Ames test was employed to assed the induction capability of 3MC, and the results revealed a correlation between CYP1A1 enzyme activity and increase mutagenicity of 3MC. In accordance with this, our findings revealed a correlation between EROD activity and Mutagenic activity of animal
treated with BNF and CFE. We found that treatment of rats with BNF at repeated doses (80 mg/kg b.w) resulted in an increase in CYP1A1 induction measured as increase in EROD activity and mutagenic activity of EtBr. However, CFE at the repeated dose of 80 mg/kg b.w resulted in EROD activity at the same level as control group. Hence the response was considered as non significant. Similarly in Ames assay it was observed that S9 fraction obtained from untreated animal did not increase the mutagenicity of EtBr corresponding to decreased CYP1A1 induction. Whereas in case of BNF treated rats enzyme activity was markedly increased resulting in a high mutagenic potential of EtBr. It was almost 25 folds higher than that of the control. At the same time CYP1A1 activity was very low in CFE treated S9 fraction which resulted in loss of mutagenic potential of EtBr.[4]

Figure 1. Effect of C. forskohlii CYP1A1 induction in rats by using (EROD) assay. (a) represents significant difference in regard to control group, (b) represents significant difference in regard to the BNF treated group. p<0.05, Kruskal Wallis and Dunnett non-parametric tests.

Figure 2. Mutagenicity of EtBr for S.typhimurium TA 98 in the presence Of S9 fraction from rat liver after oral dosage of BNF , C. forskohlii .The results are expressed as a mean of four different experiments. p<0.05 in regard to control (Dunnett's multiple Comparison test).
CONCLUSION
This study from Vidya Herbs Pvt Ltd. claims that our CFE preparation is not an inducer of CYP1A1. Use of Ames and Enzyme activity assay provided us a useful tool to measure the CytP450 inducing ability of herbal drug. Further research in this regard using protein biochemical techniques are being carried out by us.

Conflicts of Interest
The authors declare that there are no conflicts of interest.

REFERENCE


