Assessment of cytochrome P450 enzyme activity in diabetic rat treated with *Costus speciosus* (Koen. ex Retz.) Smith plant extract

[1,2]Bindu C, [3]Rama Bhat P, [4]Mahesh Kumar K S

[1]Affigenix Biosolutions Pvt. Ltd., Bangalore -560099, Karnataka, India,[2] Research & Development Centre, Bharathiar University, Coimbatore, Tamil Nadu,[3]PG Department of Biotechnology, Alva's College, Moodbidri- 574 227, Karnataka, India. [4]Syngene International, Bangalore, India.

Abstract: Diabetes mellitus is known to affect many and varied parameters in rat liver. Insulin, biguanides and sulphonylureas are known anti-diabetics in diabetic treatments. In this study, *Costus speciosus* extract was tested for its effect on liver microsomes of streptozotocin induced diabetic rats. In particular, the changes in the enzyme activities of cytochrome P450 (2E1, 2B and 2C) on androst-4-ene-3, 17-dione metabolism reversal. Metabolism of endogenous substrates, as well as exogenous xenobiotic is crucially mediated by cytochrome P450. The *in vivo* effects of *C. speciosus* extract on internal parameters like enzymatic activities of cytochrome P450 using separated microsomes from liver was estimated. The administration of streptozotocin caused a significant increase ($p<0.01$) in the enzyme activity of liver microsomes.

Index Terms: Diabetes, *Costus speciosus*, cytochrome P450

I.INTRODUCTION

Cytochrome P-450 plays an important role in the metabolism of endogenous substrates, as well as exogenous xenobiotics. The biological effectiveness is responsible for the hydroxylation or oxidative dealkylation of various drugs by liver microsomes (Cooper *et al.,* 1965). The cytochrome P450 are a group of haemoproteins and are located in almost every tissue, and in many subcellular membrane fractions including the endoplasmic reticulum (Brodie *et al.,* 1955) and the mitochondria of the adrenal gland (Harding *et al.*, 1964). In mammals, cytochrome P450 is found at varying concentration in microsomes from liver, kidney, small intestine, lung, adrenal cortex, skin, testis, placenta, and other tissues (Omura and Sato, 1964). Hepatocytes contain different constitutive forms of cytochrome P 450, some of these forms of cytochrome P450 varying in content on challenge of the animal with xenobiotics. The metabolism of xenobiotic is affected by many factors (Gibson and Skett , 1994), one of them being hormones. The first report suggesting that diabetes mellitus may affect drug metabolism showed an overall decreased hepatic microsomal metabolism following acute treatment with the β-cell toxin, alloxan, and that this effect was suppressed by insulin. It was reported that activities of drug-metabolizing enzymes in liver microsomes, such as hexobarbitone hydroxylation were markedly decreased by alloxan-induced diabetes rats. The aim of this study was to evaluate the effect of *Costus speciosus* extract on induced diabetic rat liver cytochrome P450 activity.

II.RESEARCH METHODOLOGY

Preparation of liver microsomes

In order to measure the liver parameters, microsomes were prepared and frozen quickly to prevent damage to the tissue and its enzymes. The liver from plant extract treated rat was homogenized in 5mL of phosphate buffer containing 1.15% potassium chloride in a disposable homogenizer with a Teflon pestle (for 1g of liver, 5m1 of buffer was used). The homogenate was then centrifuged in plastocraft refrigerated centrifuge for 15 minutes at 10000 rpm to sediment the mitochondria and nuclei. The post-mitochondrial supernatant then had calcium chloride (1M) added to it to bring the supernatant to 8mM with respect to calcium chloride (i. e., 0.1 ml of 1M calcium chloride (SD fine Chemicals) for every 12.5 ml of supernatant). The solution was then mixed and left to stand on ice for 5 minutes. The supernatant was then centrifuged again this time at 15000 rpm for 20 minutes to sediment out the microsomes. The supernatant was poured off and the pellet was resuspended in the phosphate buffer. For every 50 volumes of the original homogenate, the sediment was resuspended in 20mL buffer and 5mL glycerol. The microsomes were then frozen at about < -20°C. The microsomes were used later to measure cytochrome P450 activity.

Assay for aniline 4- Hydroxylase activity

This assay acts as a measure predominantly of the enzyme activity of cytochrome P450 2E1 (aniline is hydroxylase to 4-aminophenol) as per Gibson and Skett (1994) as mentioned in figure 1.

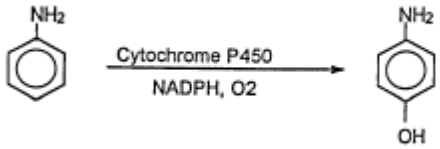

Figure 1: The hydroxylation of aniline

The following was added to each test tube in sequence:
Microsomes: 1 mL
0. IM Magnesium Chloride (SD fine Chemicals): 1 mL
0.5M Nicotinamide (Sigma Chemical Co. Ltd.): 0.1 mL
Tris buffer pH 7.4 0.05M: 0.59ml
0.05M Aniline (MP biomedical): 0. lml
Co-factor mixture: 0.11 ml

The above mixture were incubated at 37°C for 30 minutes in a shaking water bath before the reaction was stopped by adding 1 ml of 20% trichloroacetic acid (SD fine Chemicals), which precipitated the protein present in each incubation. This was mixed well and allowed to stand for 5 minutes at 4°C. The precipitate was then spun down using table top centrifuge for 15 minutes. Volume of 1 ml of the supernatant was then taken to be assayed for 4-aminophenol. To the l ml supernatant was added l ml of 1% phenol in 0.5M sodium hydroxide solution (Juniper) and mixed with 1 ml of sodium carbonate (4% w/v) solution. The solution was then mixed and the resultant solution was left to stand for 30 minutes at room temperature before the optical density was measured at 630nm in Molecular devices spectrophotometer. The measurement obtained was compared to the standard curve to find the concentration of 4-aminophenol formed per minute per mg protein. The standard curve was prepared by using 0.2 to l ml aliquots of fresh 4- aminophenol solution (10µM) (SD fine Chemicals) made up to 1ml with distilled water. The optical density was again measured at 630nm and a standard curve was drawn from the values obtained.

Microsomes analysis for P450 activity
Microsome analysis for cytochrome P450 E1, Aniline 4- hydroxylase activity was performed. Since the assay is highly sensitive, activity differences for treatment against the control was recorded immediately.
The amount of metabolite formed was calculated by direct reference to the standard curve.
Amount of product in incubation =X nmoles
Incubation time = 30 minutes
Protein content =Y mg protein
Enzyme activity =X /30Y nmoles/min./ mg protein

III.RESULTS AND DISCUSSION

The in-vivo effects of *C. specious* extract on internal parameters like enzymatic activities of cytochrome P450 using separated microsomes from liver was estimated. Results obtained were represented in table 1 and figure 2. The administration of streptozotocin caused a significant increase ($p<0.01$) in the enzyme activity.

Table 1: The effect of diabetes and *Costus speciosus* extract treatment on Aniline 4- Hydroxylase activity (P450 E1 activity)

Treatment group	Aniline 4- hydroxylase activity (nmol /min/mg)
Normal + Distilled water with 5% Tween 80	0.22 ± 0.01
Normal + Ethanol extract , 500 mg/kg body weight with Tween 80	0.13± 0.02**
Diabetic + Distilled water with 5% Tween 80	0.38 ± 0.02
Diabetic + Ethanol extract, 500 mg/kg body weight with Tween 80	0.21± 0.02**

** = $p< 0.01$ compared to control rat & t= $p<0.05$ compared to diabetic rat. Results are expressed as mean± SD (N=6)

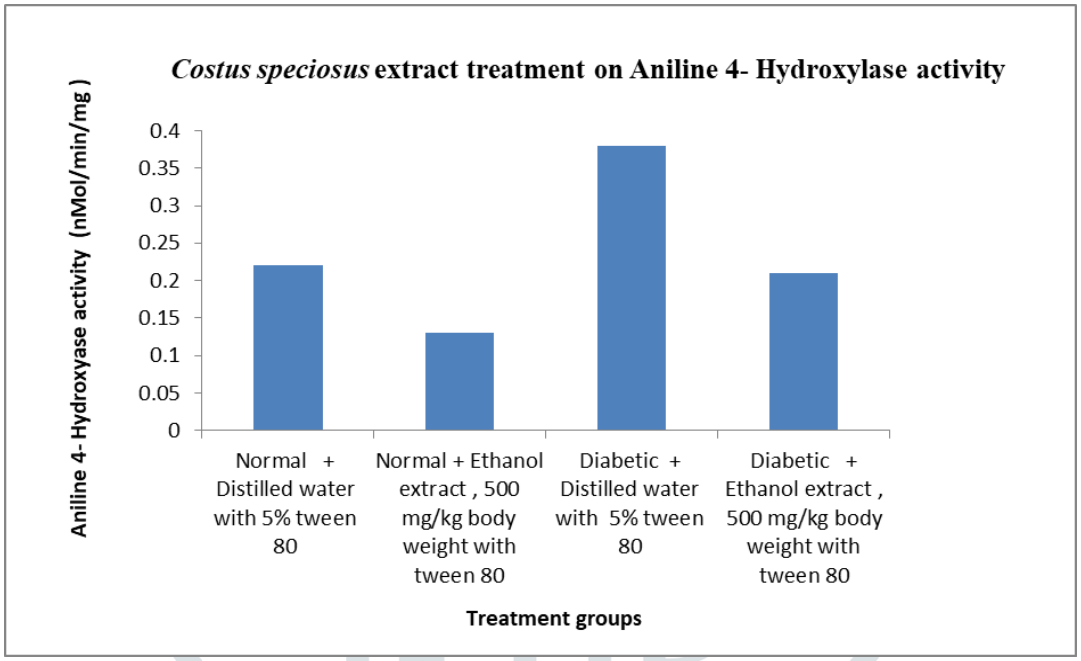

Figure 2: *Costus speciosus* extract treatment on aniline 4-hydroxylase activity

This increase in enzyme activity was partially reversed by the administration of *Costus speciosus* extract to the diabetic rats but there was no significant effect on the enzyme. Similar results were obtained in the studies conducted previously. This suggests that the binding capacities of cytochrome P450 for hexobarbital and aminopyrine, which are normally stimulated by androgen (Schenkman *et al.*, 1990), are decreased in the microsomes of diabetic male rats and similar results were reported by other workers (Weiner *et al.*, 1972).

IV.CONCLUSION

The *in vivo* effects of *Costus specious* extract on internal parameters like enzymatic activities of cytochrome P450 using separated microsomes from liver was estimated. The administration of streptozotocin caused a significant increase ($p<0.01$) in the enzyme activity. This increase was partially reversed by the administration of *Costus speciosus* extract in the induced diabetic rats but had no significant effect on the enzyme.

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