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RESEARCH ARTICLE

EFFECT OF POORNA CHANDROTHAYA CHENDOORAM (METALLIC DRUG) ON LIVER DETOXIFICATIVE ENZYMES AND SERUM MET HEMOGLOBIN LEVELS IN YOUNG MALE RATS

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Abstract: The effect of the Poorna Chandrothaya Chendooram was studied in male albino rats to explore its activities on liver. Poorna Chandrothaya Chendooram (3 mg/ml/100 gm) was given orally for seven weeks. The activity of Cytochrome P-450 and, were significantly decreased but the activity of glutathione -S- transferase were increased in the PCC administered rats rather than in control rats. Significant decrease in the activities of Cytochrome P-450 and Met hemoglobin and increase in the level of glutathione -S- transferase , indicate the defensive cytoprotective effects of Poorna Chandrothaya Chendooram .

Keywords: Cytochrome P-450, Met hemoglobin and Glutathione -S- transferase

Introduction

Liver is the largest and almost complex organ in the body. It plays an important role in the maintenance of internal environment through its multiple and diverse function. It plays a central role in detoxification and excretion of many exogenous compounds (Ames and Gold .,1993) A group of enzymes located in the endoplasmic reticulum, known as cytochrome P-450. It is the most important family of metabolizing enzymes in the liver. Cytochrome P-450 is the terminal oxidase component of an electron transport chain (Rendic and Di Carlo, 1997) It consists of a large family of GSH-utilizing enzymes that play an important role in the detoxification of xenobiotics in mammalian systems (Sohini and Rana, 2007; Tabrez , 2009). GSTs to have an important protective function (Polit *et al.,* 2009) Oxidative stress, there is a considerable rise in the level of methemoglobin, which is known to be incapable of reversible oxygen binding. Human erythrocytes normally contain 3% met hemoglobin. For converting methemoglobin into oxyhemoglobin, there are two enzymatic systems in erythrocytes, one of which is related to glycolysis and the other is associated with the pentose phosphate pathway (Masana *et al*., 1990; Yoneyama, *et al*., 1980)

Poorna chandrodayam chendooram is a well known ,mercurial preparation with gold and sulphur (Thiagarajan, 1992) widely used for many ailments like tuberculosis ,jaundice ,fever ,rat bite ,cancerous ulcer ,sprue and male sterility (Muthaliar and Uttamarayan.,1987). Hibiscus and Aloe juice is added for titration (Mahdihassan ,1985). These drugs are mostly a mixture of compounds and because of its synergistic action and purification process (Austin et al .,2002) toxicity is being diminished (Hardy et al .,1995)thereby increasing bioavailability through the cells of our body (Sudha.et al .,2009) These drugs are known to be effective even in low concentration (Kumar et al .,2006). The aim of the present study was to investigate and evaluate the Cytochrome P450, GST and blood Met Hb activity of PCC treated rats.

MATERIALS AND METHODS

Selection of animal

Healthy and pure strain Male Wistar rats, *Rattus norvegicus,* ranging from the body weight of 120-150 g were procured from the Venkateshwara Enterprises, Bangalore and maintained in the Central Animal House, Department of Siddha Medicine, Tamil University, and Thanjavur. Experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Tamil University, Thanjavur. The animals were maintained on standard diet (Kamadhenu Agencies, Bangalore) and water was given ad libitum.

Drug Preparation

The (Poorna Chandrodayam Chendooram drug obtained from the SKM Siddha and Ayurvedic Medicine's India Private Limited, Saminathapuram, mudakurichi, Erode-638104. Tamilnadu, India. The drug (Poorna Chandrodayam Chendooram) is not soluble in water therefore a suspension of gum acacia is made for oral administration. The 10 gm. of gum acacia dissolved in 100 ml of distilled water by gradual trituration in a mortar. Then well prepared solution was taken and added Poorna chandrodayam chendooram at the dose of 3 mg/ml/100 gm.

Experimental design

After acclimatization, the rats were divided into 2 groups, each having 8 rats. **Group I :** Untreated control were received water only . **Group II :** Young rats were treated with Poorna chandrodaya chenduram (3.0 mg / kg body wt. calculated from human dose) with honey for 7 weeks (orally administered) (p.o.).

Collection of samples

On completion of the experimental period, animals were sacrificedwith ether anaesthesia.Liver tissue were excised immediately andimmersed in physiological saline and blotted with filter paper and used for 10% homogenate preparation using Tris-Hcl buffer (pH 7.5).

Estimation of Cytochrome P450

Cytochrome P450 was estimated by the method of Omura and Sato (1964).The post mitochondrial supernatant was diluted with phosphate buffer and they were reduced by a few milligram of solid Sodium dithionate. The baseline was adjusted to zero at 450nm. The contents of the sample cuvette were gassed gently for about one minute with carbon monoxide (the CO was generated by the reaction of concentrated H2SO4 with sodium formate). This leads to the formation of Cyt P450 that has an absorbance at 450nm. The results were expressed in terms of nmols/mg proteins

Assay of glutathione –S- transferase (EC.2.5.2.18)

GST was assayed by the method of Habig *et al* (1974).The reaction mixture contained 1.0 ml of phosphate buffer, 0.01ml of CDNB, 0.1ml of sample and 0.7 ml of distilled water. This mixture was pre incubated at 37º C for 5 minutes, and then the reaction was started by the addition of 0.1ml of GSH. The change in the absorbance was read at 340 nm for 5 minutes, the reaction mixture without the enzyme used as the blank. The enzyme activity was expressed as µmol CDNB – GSH Conjugates/ min/mg protein.

Determination of Methaemoglobin

Methaemoglobin was estimated by the method of Evelyn and Malloy (1938). Dilute 6.5 ml of blood sample were diluted to 25ml with phosphate buffer. Initital reading was measure at 630 n.m. with dilute buffer as blank (R1). To another portion, 1 drop of cyanide solution added and read again at 630 nm (R2). Then a few drop of ferric cyanide solution was added to another portion and the above procedure repeated before (R3) and after (R4) adding cyanide.

Statistical Analysis

Values are expressed as mean ± SD for 8 rats in each group and significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey‟s test for posthoc multiple comparison tests. GRAPHPAD INSTAT Statistical Software package was used for analysis and $P < 0.05$ was considered to be significant (Motulsky, 1990-98).

RESULTS AND DISCUSSION

Table- 1 demonstrates the activity of Cyt- P450, GST enzymes and blood Met hemoglobin level in PCC treated and untreated liver tissue of rats. The activity of Cyt. P450 and blood Methemoglobin level were significantly decreased in PCC treated rats than untreated young control rats of liver tissues. The decrease being 18 % for Cyt P450 and 30 % for blood Methemoglobin of PCC treated rats. But GST level was significantly increased in PCC treated rats than untreated young rats. The increase was 16% in PCC treated rats.

Detoxification of trace metals involves a combination of displacement of non-toxic metals already bound to protein by toxic one and also denovo synthesis of protein. Earlier reports suggest that flavonoid inhibit Cyt-P450 (Dai *et al*., 1998). The presence of phytocompound in herbomineral drug might also evidence the reduction in Cytochrome- P450. Reports also evidenced that decease liver Cytochrome P450 is related to increasing life span (Ames *et al*., 1993). The reduced level of Cyt-P450 also evidence non availability of toxic metals during the treatment of PCC.Our results is also in supportive of previous report which evidences inhibition of human Cyt-P450 by *G.biloba* extract (Budzinski *et al.,* 2000). Non-glycosylated flavonoids

were also known to inhibit human P450 enzymes (Lautraite *et al*., 2002). Thus our finding suggests the possibility of PCC the herbo-mineral drug available as non toxic organometallic complexation. Decreased Cyt-P450 activity observed during PCC treatment may suggest the possibility of PCC as a non toxic herbomineral drug.

Glutathione S-transferases are a group of enzymes that catalyse the conjugation of GSH to a variety of electrophiles, thereby decreasing its reactivity with cellular components (Bickers and Athar, 2006). The ethanolic extract of *herbal drug* increased the activity of hepatic GST in sodium arsenate-induced oxidative stress in rats (Usoh *et al*., 2005).

The role of GST in catalyzing the reaction of a wide variety of electrophiles (reactive carcinogenic form of chemical carcinogen) to GSH (one of the endogeneous antioxidant) has been well established and formation of GSH conjugated of xenobiotics has been associated with the cellular detoxification system (Hartman *et al.,* 1990). The present study suggests that PCC may be an effective chemopreventive agent in organ sites wherein Phase II detoxification enzymes are GST levels are induced. It is evidenced coordinately regulated detoxification enzyme system such as GST and Cyt P450 mixed function oxidases can also confer resistant to drug which can be related to effective anticancer drug (Schuetz *et al.,* 1990).

Generally it is reported that heavy metals cause damage to the hemoglobin and convert the active hemoglobin to toxic met-heamoglobin. Determination of met haemoglobin will implicates the chemical reaction of the compounds / or drugs that could reveal the toxicity of the chemicals. In our present study, administration of PCC to normal young rats had shown decrease in the methaemoglobin to a maximum of 30 % inhibition than normal young rats. Earlier reports had shown flavonoids against oxidative damage of hemoglobin and red blood cells caused by hydroperoxides (Cesquini *et al.,* 2003; Pereira *et al.,* 2003). This effect seems to be, at least in part, connected with the ability of flavonoids to scavenge ferrylHb which is formed in the reaction of oxyHb with hydrogen peroxide or organic hydroperoxides. FerrylHb reacts with oxyHb to form metHb (Giulivi & Davies, 1990).

The reduced levels of met Hb observed in our present PCC treated rats could reveal the non toxic mercurial compounds with its complexation with herbal juices enriched with flavonoidal compounds that might have limited or excluded the toxic effect of the drug. Met Hb determination is a good tool as a biomarker to justify the non toxic capacity of the PCC drug that are processed with herbal juices which are rich in flavonoids (Grinberg *et al*.,1994). It is suggests the possible non toxic nature of mercurial and gold drug preparation thus could increase the oxygen capacity of RBC resulting in the increased resistance to the health. Our finding with Poorna Chandrodhaya Chenduram is also have the potential as above and thus suggestive of defensive cytoprotective effects.

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Table-1. Effect of PCC on liver Cytochrome P450, Glutathione -S-transferase Activities and Serum methemoglobin Level

Liver	Normal Control	PCC Treated
Cyt P450	1.027 ±0.013	0.842±0.032***
GST	7.29±0.18	8.42±0.28***
Methemoglobin	9.31±0.48	6.50±0.32**

Each value are expressed as mean ± SD for eight rats in each group; Cytochrome P450, nmol/mg protein; GST, Units /mg protein; Methemoglobin, gm % Hb; Superscript letters represent p<0.05 (Turkey-Kramer Multiple comparison Test)
*As compared with Normal Control; * P<0.001 ** P<0.02; *** P<0.5

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