Regulation of ROS defense system by *Hybanthus enneaspermus* in CCl_4 induce cardiac damage

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Abstract: The purpose of this study is to regulate the ROS (Reactive oxygen species) defense system in cardiac damage induced by CCl_4 (Carbon tetra chloride) in rats using *Hybanthus enneaspermus.* Administration of CCl4 induces damage in the heart of rats as proved by estimation of antioxidant enzymes such as, SOD (Superoxide dismutase), CAT (catalase), GPx (Glutathione Peroxdiase), GR (Glutathione Reductase), GST (Glutathione-S-Transferaes). However, treatment with HEE in CCl_4 intoxicated rats was found to be protected the heart, as indicated by the enzyme level in serum. A significant increment of serum enzymes level such as SOD, CAT, GPx, GR and GST were observed following HEE treatment during CCl_4 intoxication whereas MDA (Malondialdehyde) level in HEE treated rats were decreased. The results of our experiment suggest that the treatment of HEE may be the critical remedy for the adverse effect of CCl_4 in heart function.

Keywords: *Hybanthus enneaspermus*, oxidative stress, antioxidant enzymes, carbon tetrachloride.

INTRODUCTION

Recent experimental studies suggest that the myocardial failure enhances the oxidative stress. During heart failure, oxygen radicals are increased whereas in normal condition, the antioxidant defense system is preserved. The inability of antioxidants to overcome the tissue oxidants leads to oxidative stress. Mitochondrial electron transport system, cytochrome P450 metabolism, peroxisomes and inflammatory cell activation are the place from which the oxygen radicals are generated and these can be therapeutic target against oxidant induce damage in heart failure (Valko *et al.,* 2006; Halliwell, 1999). Generally ROS is having two faced character both beneficial and non-beneficial and it acts as a secondary messengers in intracellular signaling cascades. The ROS low concentration gives protection against infectious agents and induces the mutagenic response whereas high concentration induce damages in the cell structure (Poli *et al.,* 2004; Halliwell, 1996). The plant compounds give protection to the endogenous antioxidants against the ROS devastation. The prevention actions of plant's isolates from diseases are getting the great importance. Due to continues consumption of oxygen by the cells, oxidative damage which accumulates in lipids, proteins and nucleic acids develops the age related diseases such as cancer, arthritis, arteriosclerosis and neurodegenerative disorders (Yu, 1994; Ray and Husain, 2002). The derived chemical compounds of plants are potent suppressor which nullify the actions of free radicals. To generate energy in the form of adenosine triphosphate (ATP) by electron transport chain (ETC) of mitochondrial membrane needs much oxygen which acts as terminal electron acceptor within ETC. During exercise, sleeping and disease conditions, the cells are utilizing oxygen highly and lead to induce the highly damaging superoxide radical via ETC.

Since ancient times, the plants have been producing better beneficial effect treating the diseases such as cancer, diabetic and kidney related diseases because of bioactive compounds present in plants (Scartezzini and Speroni, 2000; Chandra Mohan *et al.,* 2012; Anand *et al.,* 2012). The therapeutic value of the *hybanthus enneaspermus* has been experimented extensively (Hemalatha *et al.,* 2003; Anand and Gokulakrishnan, 2012; Tripathy *et al.,* 2009). However, its therapeutic efficacy in the state of oxidative stress in myocardial damage has not been evaluated. In this study, made an attempt with the experimental model to induce stress and find out the medicinal efficacy of HEE.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid, glutathione and Carbon tetrachloride were obtained from sigma chemical, Mumbai. All chemicals which were used in this study were being highly purity.

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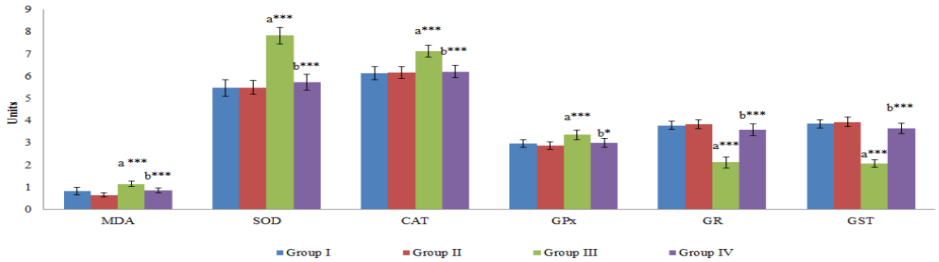

Fig. 1: Effect of *hybanthus enneaspermus* on heart MDA, SOD, CAT, GPx, GR and GST in control and experimental rats.

Animals

Albino rats were preferred for this study and kept separately by using polypropylene cages. The enough water and *ad libitum* were given to the animals. The approval was given by the IAEC to experiment the fed animals.

Preparation of HEE

Hybanthus enneaspermus was gathered and later it was identified by Dr. John peter. The collected plant materials were washed, sliced, and fully dried in a hot-air oven at 37°C. The dried materials were ground into fine powder and used for extraction. Three hundred grams (300g) of the powdered plants were extracted with ethanol (70%) using "Soxhlet apparatus" for 48 h. A semisolid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in the refrigerator until used.

The dose determination of Hybanthus enneaspermus

At the initial stage, the dose determination was experimented with HEE.HEE were given to the rats in the following dosage, (75mg, 150mg, 250mg, 500mg and 700mg/kg body weight). Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were retrieved significant level at the 500mg dose. Hence the 500mg dose was chosen for the study.

Oxidative stress induction

Rats were induced by oxidative stress by receiving a suspension of carbon tetrachloride (CCl4) in corn oil (1:1, v/v, 3 ml of CCl4, i.p) on 29th day (Caroline *et al*., 2008).

Experimental protocol

Each six animals were separated into four groups. Among the four groups, group I was treated as normal, group II was served as vehicle control and the single dose was given to the group III. Group IV was administrated with *Hybanthus enneaspermus* for 28 days and single dose (CCl4) was given at 29^{th} day.

Biochemical analysis

Releasing MDA (Malondialdehyde) from endogenous lipid peroxidation meant indicating the lipid peroxidation estimated by the thiobarbituric acid assay (Beuge and Aust, 1978). Superoxide dismutase (SOD) activity was determined by the procedure of Kakkar *et al*. (1984). The activity of catalase (CAT) was assayed by the method of Beers and Sizer (1952). Glutathione peroxidase (GPx) was assayed by the procedure of Rotruck *et al* (1973). The activity of glutathione reductase was measured by the method of Staal *et al*., (1969). Glutathione S Transferase was assayed by the method of Habig *et al* (1974).

STATISTICAL ANALYSIS

Values were displayed as ±SD to six rats of each group and significant differences were analyzed by ANOVA.

RESULTS

The purpose of the study was to evaluate the ROS defense system in myocardial damage induced by CCl_4 by using *hybanthus enneaspermus* extract. The suggestion of result that the extract regulates the ROS defense system to protect the myocardial damage from oxidative stress. The chosen plant extract was given better protectiveness at the dosage of 500mg/kg-day to CCl_4 intoxicated rats as shown in fig. 1. In the study, the antioxidant and non antioxidants level were decreased significantly in CCl_4 induced rats. It may be the reduced function of SOD in heart tissue in rats, due to the increased level of lipid peroxidation. HEE was able to significantly restore the function of SOD compared to the group which treated CCl_4.MDA is acted as denoter of oxidative stress in lipid peroxidation. MDA concentration was increased significantly in CCl_4 given animals which indicate the heart membrane damage, but free radical scavenging activity of HEE decreased the MDA level. Reversely, the content of the GSH in heart in-group III showed a decline significantly when comparing with control animals. But in the group IV, the level of GSH was obtained near normalcy. The oxidative stress markers level was altered significantly (p<0.001) in-group III comparing to the group I, II and group IV.

DISCUSSION

It is well established that the toxic effect of CCl_4 induce the free radical formation for the large number of diseases and metabolic alterations (Moron *et al.*, 1979). In cytochrome P450, CCl_4 is metabolized into CCl_3O• and/or

$CCl_3OO•$. CCl_4 induces the organ damage through lipid peroxidation and reduces the antioxidant enzymes like SOD, GH and CAT. (Recknagel *et al*., 1989; Aleynik *et al*., 1997). Antioxidant constitutes a team which considered as a mutual support of defense against ROS (Reactive oxygen species) (Leeuwenburgh and Heinecke 2001; Ohta *et al.*, 1997; Ogawa *et al*., 1992).

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