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Research Article

# PROTECTIVE ROLE OF ETHANOLIC EXTRACT OF *HYBANTHUS ENNEASPERMUS* ON MITOCHONDRIAL MEMBRANE $Na^+/K^+$ATPase, $Ca^{2+}$ATPase AND $Mg^{2+}$ATPase ACTIVITIES ON CARBON TETRACHLORIDE INDUCED OXIDATIVE STRESS IN RATS

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**ABSTRACT:**
In this present study, we evaluated the liver mitochondrial membrane ATPases activities on carbon tetrachloride induced oxidative stress in rats. Wistar strain male albino rats were divided into four groups. Group I animals were served as normal and group II was administrated with corn oil as vehicle control. Group III was given single dose ($29^{th}$ day) of $CCl_4$ in corn oil (1:1 v/v, 3ml/kg, i.p.). Groups IV was treated with ethanolic extract of *Hybanthus enneaspermus*. (Orally at the dose of 500mg per kg body weight). In $CCl_4$ + *Hybanthus enneaspermus* treated rats, $Na^+/K^+$ATPase, $Ca^{2+}$ATPase and $Mg^{2+}$ATPase activities were attained normally when compared with $CCl_4$ treated rats.
**KEYWORDS:** Oxidative stress, Carbon tetrachloride, *Hybanthus enneaspermus,* $Na^+/K^+$ATPase, $Ca^{2+}$ATPase

## INTRODUCTION:

In biomembranes, Molecular interactions play a bottom-line role to communicate the cells and signal transduction pathways. Membrane receptors serve as the main targets able to recognize specific ligands selectively, which can trigger a cascade of functional cell responses. Biological membranes are the first fence that has to be overcome by toxic compounds targeting the cell. One of the most important membrane proteins is adenosinetriphosphathase (ATPase, EC 3.6.1.3), an integral part of a sodium-potassium pump and the largest protein complex member of P –type family of active cation transport proteins.[1] ATPases play an important role in the maintenance of Ionic gradient by coupling ATP hydrolysis with energy process.[2] ATPases decomposes the adenosine triphosphate into adenosine diphosphate and a free phosphate ion.[3,4] $Na^+/K^+$ATPase is a membrane bound enzyme and inactivation of this enzyme leads to produce oxidative stress. The rabid metabolic nature of ccl4 is highly induces the toxicity when it is administrated into living things.[5-7] $CCl_4$ is bio transformed by the cytochrome $P_{450}$ is a isoenzyme in endoplasmic reticulam to convert $CCl_4$ into trichloromethyl radical ($CCl_3^{\bullet}$) in the liver after the initiation of lipid peroxidation. $CCl_3^{\bullet}$ reacting with oxygen of cellular proteins and lipids to produce a trichloromethyl peroxyl radical which attacks rabidly lipid membrane of endoplasmic reticulam than trichloromethyl free radical. It has been leads to liver cirrhosis, aging, reduced glutathione, accumulation of triacyl glycerol, $Ca^{2+}$ and $Na^{2+}$ influx and finally cell swelling in mitochondria which allows the mitochondrial membrane damage, , reduced carbonylation of protein , loss of enzyme activity and cell death. These result in changes of structure of the endoplasmic reticulum and other membrane, and loss of glucose-6-phosphatase activation, leading to liver damage. The medicinal value of the chosen plant *Hybanthus enneaspermus* has not been extensively worked out. Previously reported that the chosen plant having alkaloids, flavanoids, tannins, cardio glycosides, saponins, and terpenoids like compounds in *Hybanthus enneaspermus*.[8-10] Hence in the present study, an attempt has been made to create an animal model with oxidative stress using $CCl_4$ and the ethanolic extract of *Hybanthus enneaspermus* on liver mitochondrial membrane Na+/K+ATPase, $Ca^{2+}$ATPase and $Mg^{2+}$ATPase activities were evaluated.

## MATERIALS AND METHODS:

### Plant material and preparation of extract

Whole plants of *H. enneaspermus* were collected in the month of November and December from PRIST University Campus, Thanjavur, Tamil Nadu, India. The collected plants were identified and authenticated by a Botanist Dr. John britto, Department of Herbarium, St.Joseph college, Trichy, Tamil Nadu, India. The collected plants were open-air-dried under the shade, pulverized in to a moderately coarse powder (using pestle and mortar). Three-hundred grams (300 g) of the powered plants were extracted with ethanol (70%) using soxhlet apparatus for 48 h. A semi-solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract contains both polar and non-polar phytocomponents

**Animals**: Wistar strain male albino rats, weighing 180-200 g were selected for the study. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (28±2ºC, humidity 60-70%, 12 h light/dark cycle). The animals were allowed a standard feed and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use. All the animal experiments were duly approved by the Institutional Animal Ethics Committee (743/03/abc/CPCSEA dt 3.3.03) Guidelines. (IAEC)

**Induction of oxidative stress:** Oxidative stress through hepatic injury was created by intraperitoneal injection of $CCl_4$ in corn oil (1:1 v/v, 3ml/kg).[11] The control animals received vehicle alone through intraperitoneal injection.

**Experimental protocol:** Rats were divided into four groups with six animals in each group. Group I animals were served as normal control. Group II was administered with corn oil (3ml/kg, i.p.) as vehicle control. Group III was given single dose ($29^{th}$ day) of $CCl_4$ in corn oil (1:1 v/v, 3ml/kg, i.p.). Groups IV was treated with(5E, 13E)-5,13-Docosadienoic acid (6 mg/kg body weight) for 28 days and given single dose of ($29^{th}$ day) $CCl_4$ in corn oil (1:1 v/v, 3 ml/kg, i.p.). Six hours after $CCl_4$ intoxication, the experimental animals were sacrificed. The blood was collected with EDTA as

anticoagulant. Serum was separated by centrifugation. Liver was excised immediately and immersed in physiological saline. It was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) and cut into small pieces. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate and serum were used for the estimation of various biochemical parameters.

**BIOCHEMICAL ANALYSIS**

**ATPases:**

The mitochondrial homogenate was used for assaying membrane bound enzymes like $Na^+/K^+$ATPase,[12] $Ca^{2+}$ATPase,[13] and $Mg^{2+}$ATPases[14], activities. The amount of inorganic phosphorus was determined by the method of Fiske and Subbarow.[15] Protein was estimated by the method of Lowry *et al.*[16]

**STATISTICAL ANALYSIS:**

Statistical analysis values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of

variance (ANOVA) followed by the Tukey's test for multiple comparisons.[17] Statistical analysis carried out by Ms-Windows based graph pad Instat software (Graph Pad Software, San Diego, CA, USA) 3 version was used. A value of $p < 0.001$ was considered statistically significant.

**RESULTS AND DISCUSSION:**

The activity levels of mitochondrial $Na^+/K^+$ATPase, $Ca^{2+}$ATPase and $Mg^{2+}$ATPase in liver was studied after inducing oxidative stress by using Ccl4 as a toxic inducer. All three ATPases showed conspicuous inhibition in $CCl_4$ induced oxidative stress rats. In *Hybanthus enneaspermus* treated rats restored the levels of $Na^+/K^+$ATPase, $Ca^{2+}$ATPase and $Mg^{2+}$ATPase in liver (Table 1).

Table 1: Effect of *Hybanthus enneaspermus* on mitochondrial $Na^+/K^+$ATPase, $Ca^{2+}$ATPase and $Mg^{2+}$ATPase in liver of control and experimental rats.

| Parameters | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| $Na^+/K^+$ATPase | 8.03 ± 0.54 | 8.11 ± 0.58 | 6.05 ± 0.57 [a***] | 7.94 ± 0.53 [b***] |
| $Ca^{2+}$ATPase | 14.22 ± 1.27 | 14.31 ± 1.33 | 10.46 ± 1.14 [a***] | 14.14 ± 1.09 [b***] |
| $Mg^{2+}$ATPase | 13.27 ± 0.69 | 13.40 ± 0.82 | 8.42 ± 0.64 [a***] | 13.12 ± 0.79 [b***] |

Values are expressed as mean ± SD for six rats in each group.
[a]As compared with Group I & II rats, [b]As compared with Group III rats. ***$p<0.001$

Oxidative stress is mainly caused by the mitochondrial dysfunction and energy depletion and alteration in the ionic homeostasis leads to loss of cellular integrity and cell death. Oxidative stress can be defined as a disturbance in the prooxidant/antioxidant balance and it is associated with more than hundred diseases such as arthritis, carcinogenesis, and aging and acquired immunodeficiency syndrome.[18] The number of organic compounds and inorganic salts, including cardiovascular and anti-cancer drugs, biologically important elements, heavy metals, organic solvents and some toxic organic compounds, such as pesticides and herbicides, strongly modulate enzyme activity on the concentration dependent manner. Because of its high sensitivity to the broad spectrum of toxic compound, as well as potential cardiotonic and anticancer drugs, Na+,K+-ATPase activity can be taken as meaningful index of cellular activity and forms a useful toxicological tool in medicine, pharmacy and environment. In conclusion, the restored activities of liver mitochondrial $Na^+/K^+$ATPase, $Ca^{2+}$ATPase and $Mg^{2+}$ATPase were observed after treatment with $CCl_4$ and *Hybanthus enneaspermus* rats and thus the results proved that the *Hybanthus enneaspermus* extract have strong membrane stabilizing action.

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