

## ***Hybanthus enneaspermus* is a potent regulator for membrane bound enzymes in mitochondria on carbon tetrachloride induced oxidative stress in rats.**

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### **Abstract**

In this study, we created an experimental animal model for evaluating the kidney and heart mitochondrial Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase and Mg<sup>2+</sup>ATPase 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 animals were administrated with corn oil as vehicle control. Group III was given single dose (29<sup>th</sup> day) of CCl<sub>4</sub> 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<sub>4</sub> and *Hybanthus enneaspermus* treated rats, the Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase and Mg<sup>2+</sup>ATPase activities were attained normally when compared with CCl<sub>4</sub> treated rats.

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### **Key words:**

Oxidative stress, Carbon tetrachloride, *Hybanthus enneaspermus*, Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase

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### **Introduction:**

ATPases play an important role in the maintenance of molecular mechanisms of membrane proteins [1]. ATP itself, as a neurotransmitter, neuromodulator, and participate in the synthesis and release of nitric oxide by shear stress in endothelial cells [ 2,3,4 and 5 ]. The inactivation of Na<sup>+</sup>/K<sup>+</sup>ATPase leads to oxidative stress is the condition of unbalanced state between oxidants and antioxidants. The rapid metabolic nature of CCl<sub>4</sub> is highly inactivate the

membrane bound enzymes when it is administrated into living things [ 6,7 and 8 ]. CCl<sub>4</sub> is metabolized by the cytochrome P450 is an isoenzyme which convert the CCl<sub>4</sub> into CCl<sub>3</sub>• and it reacts with oxygen of cellular proteins and lipids to produce a trichloromethyl peroxy radical which attacks rapidly lipid membrane of endoplasmic reticulum than trichloromethyl free radical. It has been leads to liver cirrhosis, aging, Ca<sup>2+</sup> and Na<sup>2+</sup> influx and finally cell swelling in mitochondria which allows the mitochondrial membrane damage, reduced carbonylation of protein, loss of enzyme activity and cell death. 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* [ 9, 10 and 11]. Hence in the present study, an attempt has been made to create an animal model with oxidative stress using CCl<sub>4</sub> and the ethanolic extract of *Hybanthus enneaspermus* on mitochondrial Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase and Mg<sup>2+</sup>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 Prof. Dr. K. Singaravadivel, Department of Microbiology, Indian Institute of Crop Processing Technology, Thanjavur, 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 powdered 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±2°C, 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<sub>4</sub> in corn oil (1:1 v/v, 3ml/kg) [12]. 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<sup>th</sup> day) of CCl<sub>4</sub> in corn oil (1:1 v/v, 3ml/kg, i.p.). Group IV was treated with ethanolic extract of *Hybanthus enneaspermus* (500 mg/kg body weight) for 28 days and given single dose of (29<sup>th</sup> day) CCl<sub>4</sub> in corn oil (1:1 v/v, 3 ml/kg, i.p.). Six hours after CCl<sub>4</sub> intoxication, the experimental animals were sacrificed. The blood was collected with EDTA as anticoagulant. Serum was separated by centrifugation. Kidney and heart were 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:**

The mitochondrial homogenate was used for assaying membrane bound enzymes like Na<sup>+</sup>/K<sup>+</sup>ATPase,<sup>[13]</sup> Ca<sup>2+</sup>ATPase,<sup>[14]</sup> and Mg<sup>2+</sup>ATPases<sup>[15]</sup>, activities. The amount of inorganic phosphorus was determined by the method of Fiske and Subbarow<sup>[16]</sup>. Protein was estimated by the method of Lowry *et al.*<sup>[17]</sup>

**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 [18]. 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 actions of mitochondrial Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase and Mg<sup>2+</sup>ATPase in kidney and heart were studied after inducing oxidative stress by using Ccl<sub>4</sub> as a toxic inducer. All three ATPases showed apparent inhibition in Ccl<sub>4</sub> induced oxidative stress rats. In *Hybanthus enneaspermus* treated rats restored the levels of Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase and Mg<sup>2+</sup>ATPase in kidney and heart ( Table 1 and 2). Oxidative stress is mainly caused by the mitochondrial dysfunction and energy depletion. 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[ 19]. In conclusion, the restored activities of kidney and heart mitochondrial Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase and Mg<sup>2+</sup>ATPase were observed after treatment with CCl<sub>4</sub> and *Hybanthus enneaspermus* rats and thus, the *Hybanthus enneaspermus* extracts were proved as a potent membrane bound enzyme stabilizer.

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**Table 1:** Effect of *Hybanthus enneaspermus* on mitochondrial Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase and Mg<sup>2+</sup>ATPase in kidney of control and experimental rats.

Parameters	Group I	Group II	Group II	Group IV
Na <sup>+</sup> /K <sup>+</sup> ATPase	5.14 ± 0.30	5.22 ± 0.28	3.64 ± 0.33 <sup>a***</sup>	5.09 ± 0.31 <sup>b***</sup>
Ca <sup>2+</sup> ATPase	11.49 ± 0.94	11.56 ± 0.87	8.18 ± 0.91 <sup>a***</sup>	11.35 ± 0.93 <sup>b***</sup>
Mg <sup>2+</sup> ATPase	19.46 ± 1.24	19.54 ± 1.29	14.07 ± 1.16 <sup>a***</sup>	19.32 ± 1.20 <sup>b***</sup>

Values are expressed as mean ± SD for six rats in each group.

<sup>a</sup>As compared with Group I & II rats, <sup>b</sup>As compared with Group III rats. \*\*\**p*<0.001

**Table 2:** Effect of *Hybanthus enneaspermus* on mitochondrial Na<sup>+</sup>/K<sup>+</sup>ATPase, Ca<sup>2+</sup>ATPase and Mg<sup>2+</sup>ATPase in heart of control and experimental rats.

Parameters	Group I	Group I	Group III	Group IV
Na <sup>+</sup> /K <sup>+</sup> ATPase	7.04 ± 0.52	7.09 ± 0.44	4.98 ± 0.53 <sup>a***</sup>	6.95 ± 0.49 <sup>b**</sup>
Ca <sup>2+</sup> ATPase	16.08 ± 1.14	16.17 ± 0.98	11.84 ± 1.03 <sup>a***</sup>	16.02 ± 1.11 <sup>b***</sup>
Mg <sup>2+</sup> ATPase	16.12 ± 1.03	16.43 ± 1.14	11.16 ± 1.08 <sup>a**</sup>	16.07 ± 1.16 <sup>b***</sup>

Values are expressed as mean ± SD for six rats in each group.

<sup>a</sup>As compared with Group I & II rats, <sup>b</sup>As compared with Group III rats. \*\*\**p*<0.001

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