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Research Article

Effects of *Hygrophila auriculata* on mitochondrial TCA cycle enzymes in N-Nitrosodiethylamine induced Hepatocellular Carcinoma in Rats.

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Abstract

This study was designed to investigate the Modulatory effect of *Hygrophila auriculata* on mitochondrial TCA cycle enzymes in N-nitrosodiethylamine induced hepatocellular carcinoma in rats. Experimental rats were divided into different groups: normal, N-nitrosodiethylamine induced hepatocellular carcinoma (HCC) bearing rats, *Hygrophila auriculata*(*H. auriculata*)treated hepatocellular carcinoma bearing rats, (200mg/kg body weight doses for 28 days), animals treated with plant extract alone for 28 days. After the treatment period, on 28th day the level of mitochondrial TCA cycle enzymes were assayed and compared with control. These parameters were altered significantly in hepatocellular carcinoma bearing rats. The methanolic extract of *H. auriculata* (200 mg/kg) significantly reverted these altered mitochondrial TCA cycle enzymes level to near normal in *H.auriculata* treated group III carcinoma bearing rats at the end of the treatment period (28days).However, the changes in the above parameters were comparable with control. Thus, methanolic extract of *H. auriculata* reverted the altered level of mitochondrial TCA cycle enzymes which is regulated differently during tumour growth and associated with development of hepatomas to near normal in HCC bearing rats due to the presence of polyphenols and flavonoids in the plant extract..

Keywords: Hepatocellular carcinoma , oxidative stress, *Hygrophila auriculata* , TCA cycle enzymes.

Introduction

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, immunity, nutrient supply and energy provision [1]. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. The importance of a disease for mankind is measured by the number of fatalities it causes. In this aspect cancer is considered to be more important than those most feared infectious diseases. Among various cancers reported, hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Certain specific substances or the conditions can convert a normal body cell into a mutant cancerous type by many factors [2] .Functional and metabolic status of a presumable cell to become a cancerous one. Source of such carcinogens include some fungi and plants[3]and combustion products[4.] N-nitrosodiethylamine (DEN) is another such widely occurring nitrosamine which is present in combustion products, tobacco and various processed food [5]. It is one of the important environmental carcinogens which primarily induces tumor of liver [6] an uncompromised free radical generation in the liver overwhelms the antioxidants status and ultimately proceeds to oxidative stress paving way to carcinogenesis[7]. In order to aim at specific cancer therapy to patients, without deleterious side effects, there is a need for new prototypes and new templates for use in the design of potential chemo preventive agents.

Interestingly natural products are providing the remedy for the search. A number of non-nutrient chemicals from plants and fruits have been reported to possess anticancer activity[8]. Hence, the present investigation was focused to search the anticancer

agent from plant source rich in polyphenols and flavonoids,well known antioxidants. One such interesting plant is *H.auriculata* which contains polyphenols and flavonoids [9]. Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions[10]. though various uses of *H.auriculata* has been evaluated ,there is scarceness of information regarding the modulatory effect of methanolic extract of the whole plant of *H.auriculata* on mitochondrial TCA cycle enzymes in DEN induced HCC bearing rats. The development of different types of tumors is accompanied by characteristic alterations in mitochondrial TCA cycle enzymes. Therefore, *H.auriculata* was selected as plant source to evaluate its effects on mitochondrial TCA cycle enzymes in N-nitrosodiethylamine hepatocellular carcinoma in rats.

Materials and Methods

Healthy albino rats (wistar strain) weighing 140 ±20g of either sex were used for this study. The animals were housed in polypropylene cages at controlled temperature, well ventilated with a 12-12 h light dark cycle.The rats were fed with standard laboratory diet and water was provided *ad libitum.* The animals were maintained as per the CPCSEA guidelines and regulations and the study was approved by the institutional animals ethics committee at Dr. ALM Post graduate institute of basic medical sciences, University of Madras,Taramani, chennai-600 113,India.

Preparation of the extract

The whole plants of *H. auriculata* were shade dried and coarsely powdered and was extracted by using methanol as a solvent in a soxhlet extraction apparatus. The solvent was completely removed byvacuum and semisolid mass was obtained (11% w/w with respect to the powdered material), the extract was dried under reduced pressure using rotary flash evaporator and stored in refrigerator for further studies.methanolic extracts were normally used for anticancer screening because traditional practitioners believed that mostly the polar compounds were responsible for the claimed anticancer properties[11].

Experimental design

The rats were divided into four groups of six animals each. Group I animals received normal saline (control), Group II animals were administered with single i.p injection of DEN at a dose of 200mg/kg body weight in normal saline to induce liver cancer. Two weeks after administration of DEN, Phenobarbital at a concentration of 0.05% was incorporated into rat chow for up to 14 successive weeks to promote the cancer, after the induction period Group III animals were treated orally with methanolic extract of *H. auriculata* at a concentration of 200mg/kg body weight for 28 days.Group IV animals treated with plant extract alone for 28 days.

Biochemical estimations

After the experimental period the animals were sacrificed by cervical decapitation. Blood was collected and the serum was separated by centrifugation. Liver and kidney were immediately excised from the animals and washed in ice cold saline, blotted and then weight was determined. Liver and kidney tissues were homogenized in Tris-HCl buffer (0.1M pH 7.4). The supernatants were used for the assay of biochemical parameters.

Mitochondrial TCA cycle enzymes

Isolation of Mitochondria

Tissues were rinsed with ice-cold saline immediately after removal from the animal. A 20% homogenate was prepared using 0.02 M Tris-HCl buffer containing 0.25 M sucrose. It was centrifuged first at 5000 rpm for 10 minutes to remove the nuclear fraction and the broken cell debris and then centrifuged at 12,000 rpm for 10 minutes to sediment the mitochondrial fraction. The resulting pellets were washed again in 0.25 M sucrose, centrifuged at 12,000 rpm for 10 minutes. The final pellet was suspended in 0.25 M sucrose, gently homogenized and used as the mitochondrial enzyme source.

Isocitrate dehydrogenase [12],Succinate dehydrogenase [13],Malate dehydrogenase[14],α-Ketoglutarate dehydrogenase [15]

Statistical analysis

Data are presented as the mean± standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups of by

using SPSS 7.5 student versions. Comparisons were made between group II and IV with group I and group II with group III for animal studies.

Results

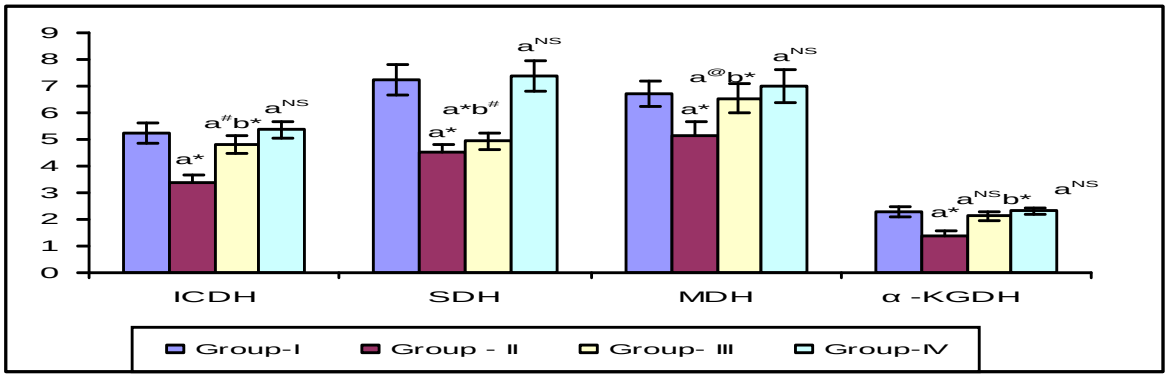
Mitochondrial TCA cycle enzymes

Mitochondria are the major intracellular source during oxidative phosphorylation 16 and are primary target of reactive oxygen species (ROS). It has been established that defects in the respiratory chain leads to enhanced production of ROS and free radicals in mitochondria, resulting in mitochondrial DNA mutations which indirectly impair glucose sensing by reducing intracellular concentrations of ATP.

Fig.1 shows the levels of ICDH, SDH, MDH and α-KGDH in liver of control and experimental animals. A highly significant decrease in the levels of TCA cycle enzymes were observed in group II cancer bearing animals when compared to group I control animals ($p<0.001$). The *H.auriculata* treatment normalized the levels of ICDH, SDH, MDH and α-KGDH in group III animals (ICDH, MDH and αKGDH $p<0.001$; SDH; SDH $p<0.01$). There was no noticeable change observed in group IV animals compared to control.

The activities of ICDH, SDH, MDH and α-KGDH are shown in kidney of control and cancer bearing animals in Table 1. All these enzymes were decreased in group II cancer bearing animals, when compared to group I control animals (ICDH< SDH, MDH $p<0.001$; α-KGDH $p<0.05$). In group III plant extract treated animals these enzyme levels were reverted to near normal (ICDH, SDH $p<0.001$; MDH $p<0.01$). The group IV animals showed no change in these parameters when compared to control.

Fig 1: Activities of mitochondrial TCA cycle enzymes in liver of control and experimental animals



Each value represents mean ± SD , a – Group II, III, IV compared with Group I, b – Group III compared with Group II, $^{*}p<0.001$; $^{\#}p<0.01$; $^{@}p<0.05$; ^{NS} – Not significant

Table 1: Activities of mitochondrial TCA cycle enzymes in the kidney of control and experimental animals

Parameters	Group I (control)	Group II (DEN)	Group III (DEN+ *H. auriculata*)	Group IV (*H. auriculata*)
Isocitrate dehydrogenase (n mole of α-keto glutarate formed/mg protein/min)	5.17±0.18	3.69±0.16 a^{*}	5.08±0.17a^{NS} b^{*}	5.29±0.18 a^{NS}
Succinate dehydrogenase (µ mole of succinate oxidized /mg protein/min)	7.69±0.16	5.32±0.18 a^{*}	7.48±0.17a^{NS} b^{*}	7.56±0.18 a^{NS}
Malate dehydrogenase (n mole of NADH oxidized /mg protein/min)	6.87±0.19	6.32±0.32a^{*}	6.76±0.23a^{NS} $b^{\#}$	6.93±0.16 a^{NS}
α-ketoglutarate dehydrogenase (µ mole of potassium ferrocyanide /mg protein/min)	3.35±0.21	2.97±0.18$a^{@}$	2.88±0.18 $a^{\#}b^{NS}$	3.40±0.19 a^{NS}

Each value represents mean ± SD, a – Group II, III, IV compared with Group I, b – Group III compared with Group II $^{*}p<0.001$; $^{\#}p<0.01$; $^{@}p<0.05$; ^{NS} – Not significant

Discussion

The rate of ROS generation is closely related to oxygen consumption and proportional to the amount of mitochondria in the tissues. Lipid peroxidation has been reported as a major contributor to the loss of cell function under oxidative stress situations. For instance, attack on mitochondrial membranes can alter permeability and induce disruption of cellular energetics [17]. Increased superoxide production results in oxidative damage to mitochondria, thus compromising their ability to meet cellular energy demands [18] which could have reduced the activities of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. The accumulation of pyruvate and α-ketoglutarate results in the lowering of activities of isocitrate dehydrogenase and succinate dehydrogenase [19]. In the present study, isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and α-ketoglutarate dehydrogenase (α-KGDH) were decreased in liver and kidney of cancer bearing animals. This might be due to mitochondrial damage caused by DEN-induced oxidative stress. A number of workers have reported that the mictrochondrial TCA cycle enzymes such as succinate dehydrogenase, malate dehydrogenase and NAD-isocitrate dehydrogenase were decreased in stressed animals. They suggested that the stressed animals are meeting their energy requirements through anaerobic oxidation[20, 21] Researchers also reported a significant decrease in the activity of liver succinate dehydrogenase and suggested that anaerobic metabolism was favoured over aerobic oxidation of glucose through Kreb's cycle in order to mitigate the energy crisis for survival. [22]

Administration of *H. auriculata* extract markedly increased the levels of ICDH, SDH and α-KGDH in plant extract treated animals that might be due to antioxidant nature of plant extract, which nullified the mitochondrial damage due to DEN administration.

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