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AMELIORATIVE EFFECT OF *HYGROPHILA AURICULATA* (K. SCHUM) HEINE ON LIPID PEROXIDATION IN A RAT MODEL OF N-NITROSODIETHYLAMINE INDUCED HEPATOCELLULAR CARCINOMA

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ABSTRACT

This study was designed to investigate the ameliorative effect effect of *Hygrophila auriculata* (*H. auriculata*) on lipid peroxidation (LPO) in N-nitrosodiethylamine induced Hepatocellular Carcinoma (HCC) in rats. Experimental rats were divided into different groups: normal, N-Nitrosodiethylamine induced hepatocellular Carcinoma bearing rats, *H. auriculata* treated hepatocellular carcinoma bearing rats, (200 mg/kg body weight, doses for 28 days), Animals treated with plant extract alone for 28 days. After the treatment period of 28th day , effect of *H.auriculata* on LPO in serum , liver and kidney of control and experimental animals were assayed and compared. Under basal and in the presence of inducers (H_2O_2), the levels of LPO were increased significantly ($p<0.001$) in group II cancer bearing animals where as the levels of LPO in the presence of ascorbic acid and $FeSO_4$, were not statistically different. In group III plant extract treated animals, significant decrease on LPO was observed. However, the changes in the above parameters were comparable with control.Thus, methanolic extract of *H. auriculata* ameliorated the LPO which is associated with the development of HCC to near normal in HCC bearing rats which could be attributed to the presence of polyphenols and flavonoids in the plant extract. Antioxidants act as radical scavenger, inhibit LPO and other free radical-mediated processes, thereby protecting the human body from various diseases.

INTRODUCTION: Epidemiological and experimental evidence clearly show that exposure of certain chemicals produce cancers. Certain chemicals particularly lead to Hepatocellular carcinoma(HCC). N-Nitrosodiethylamine (DEN) is another such widely occurring nitrosamine which is present in combustion products, tobacco and various processed food [2]. It is one of the important environmental carcinogens which primarily induces tumor of liver [3]. DEN confers its carcinogenicity through the metabolic activation in the liver microsomes causing the release of alkylating agents that bind to the DNA forming adducts [4, 5] and generation of superoxide radicals [6], paralleled by lipid peroxidation reactions. Lipid peroxidation (LPO) is regarded as one of the basic mechanism of cellular damage caused by free radicals.

Free radicals react with lipid causing peroxidation resulting in release of products such as malandioldehyde (MDA), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH). ROS including oxygen, hydrogen peroxide and hydroxyl radicals (OH) play an important role in carcinogenesis [7]. Free radicals are involved in a wide variety of diseases especiallycancer. The oxidative stress induced breast cancer in experimental rats have been reported [8].

Neoplastic cells may sequester essential antioxidants from circulation to supply the demands of growing tumour. Excess generation of oxygen free radicals can cause oxidative damage to biomolecules resulting in LPO, mutagenesis and carcinogenesis. Oxygen free radicals induced LPO has been implicated in neoplastic transformation [9]. Generally the medicinal plants are considered as potent antioxidants [10]. One such interesting plant is *Hygrophila auriculata* which contains polyphenols and flavonoids [11]. The plant has got potent free radical scavenging and lipid peroxidation inhibitory activity [12].

Therefore, *H. auriculata* was selected as plant source to evaluate its ameliorative effect on LPO against DEN induced HCC.

MATERIALS AND METHODS:

Animals: Healthy wistar albino rats (140 ± 20 g) used in the present study were obtained from Central animal house, Dr. ALM Post Graduate Institute of Basic Medical Sciences, Taramani, campus, University of Madras, Chennai, Tamilnadu, India. The animals were fed with standard rat feed and water ad libitum. The animal house was well ventilated and the animals had 12±1 h day and night rhythm throughout the experimental period. The animals were utilized for the experiment according to the guidelines given by Institutional Animal ethics committee (IAEC approval No.07/060/06).

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 by vacuum 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 [13].

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 topromote the cancer.

Group III- After the induction period group III animals were treated orally with methanolic extract of Hygrophila auriculata at a concentration of 200mg/kg body weight for 28 days.

Group IV- Animals treated with plant extract alone for 28 days.

Collection of Samples: 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.

Estimation of Lipid peroxidation: Lipid peroxidation was estimated by the method of [14]. 1.5 ml of TBA, 0.2 ml of sodium dodecyl sulphate and 1.5 ml of glacial acetic acid were added to test tubes containing 0.1 ml of samples. The test tubes were heated in water bath for 1h. The test tubes were then cooled and 1 ml of distilled water was added. The optical density was determined at 532 nm using a reagent blank. Standard malondialdehyde was also processed in a similar fashion. The results were expressed as nmoles of malondialdehyde liberated /mg protein. LPO products, measured as thiobarbituric acid (TBA) reactive substances (TBARS) [15].

RESULTS: Free radical species are involved in carcinogenesis and chemical induction of liver carcinoma is associated with changes in the oxygen radical metabolism in liver. The changes in hepatic oxygen radical metabolites were demonstrated by measurement of the antioxidant enzymes [16]. The effect of *H. auriculata* on LPO in serum and liver of control and experimental animals are presented in **Fig. 1 and Table 1**. The levels of LPO were found to be significantly increased in group II cancer bearing animals when compared with control animals under basal conditions and also in the presence of inducers ($p<0.001$).

Conversely, the administration of *H.auriculata* reduced the peroxidation reaction in group III plant extract treated animals ($p<0.001$). **Table 2** shows the levels of LPO in kidney of control and experimental animals. Under basal and in the presence of inducers (H_2O_2), the levels of LPO were increased significantly ($p<0.001$) in group II cancer bearing animals where as the levels of LPO in the presence of ascorbic acid and $FeSO_4$, were not statistically different. In group III plant extract treated animals, significant decrease on LPO was observed.

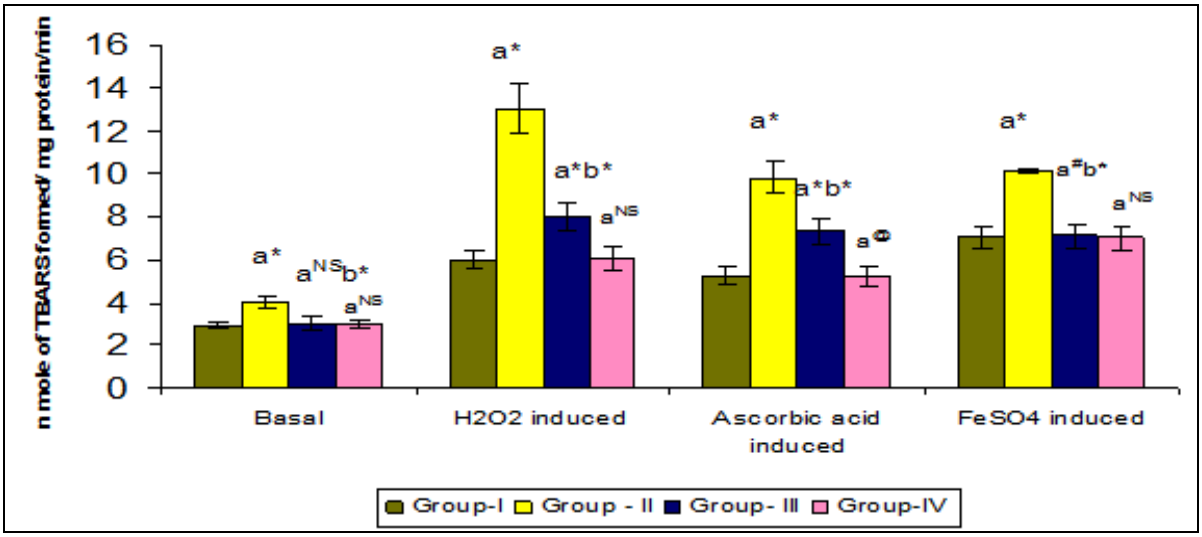

FIG. 1: THE LEVELS OF LIPID PEROXIDATION IN SERUM OF CONTROL AND EXPERIMENTAL ANIMALS

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: THE LEVELS OF LIPID PEROXIDATION IN BASAL AND IN THE PRESENCE OF INDUCERS IN LIVER OF CONTROL AND EXPERIMENTAL ANIMALS

Parameters (n moles of TBARS formed/mg protein/min)	Group I (Control)	Group II (DEN)	Group III (DEN + *H. auriculata*)	Group IV (*H. auriculata*)
Basal	2.05±0.11	3.19±0.16a*	2.12±0.12a^{NS}b*	2.03±0.11a^{NS}
H_2O_2 induced	3.17±0.25	6.14±0.26a*	4.58±0.35a*b*	3.08±0.29a^{NS}
Ascorbic acid induced	2.27±0.20	5.09±0.22a*	3.01±0.21 a$^{\#}$b*	3.43±0.24a^{NS}
$FeSO_4$ induced	2.55±0.23	5.38±0.25a*	3.16±0.23 a$^{@}$b*	2.47±0.21a^{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.

TABLE 2: THE LEVELS OF LIPID PEROXIDATION IN BASAL AND IN THE PRESENCE OF INDUCERS IN KIDNEY OF CONTROL AND EXPERIMENTAL ANIMALS

Parameters (n moles of TBARs formed/mg protein/min)	Group I (Control)	Group II (DEN)	Group III (DEN+ *H. auriculata*)	Group IV (*H. auriculata*)
Basal	1.64±0.11	2.15±0.12a*	02.01±0.11a$^{@}$ b$^{\#}$	1.52±0.14 a^{NS}
H_2O_2 induced	5.39±0.26	8.79±0.42 a*	5.89±0.29 a* b*	5.29±0.19 a^{NS}
Ascorbic acid induced	3.38±0.29	4.01±0.31 a$^{\#}$	3.58±0.29 a^{NS}b$^{@}$	3.26±0.29 a^{NS}
$FeSO_4$ induced	5.77±0.32	5.98±0.41a^{NS}	5.85±0.48a^{NS}b^{NS}	5.56±0.38 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; Control animals.

DISCUSSION: Clinical, epidemiological and experimental studies provide evidence implicating the role of free radicals on the etiology of cancer [16]. Recently considerable attention has been focused on the inter relationship of lipid peroxidation process, free radical generation and the development of various diseases, especially cancer. LPO induced by oxidative stress is found to be the key process in many pathological events. Free radicals react with lipid causing peroxidation resulting in release of products such us MDA, H_2O_2, OH radicals. ROS including O^{2-} H_2O_2 and OH radicals play an important role in carcinogenesis [17]. It has been reported that serum LPO products are increased in patients with liver disease [18]. An increase in circulating LPO products (determined as the TBARS), as it is observed in different human hepatic diseases [15].

There are evidences to support that oxidative stress is an obligatory component of carcinogenesis and clear examples of the participation of ROS in hepatocarcinogenesis in rats [19]. It has been showed that initiation with low doses of N-diethyl nitrosamine (DEN) induced liver DNA-8-hydroxydeoxy-guanosine adducts and suggested that oxidative stress participates in hepatocarcinogenesis [20] . There was an abnormal increase of LPO in the present study in the cancer bearing rats when compared to control. This might be due to oxidative stress induced free radical mediated changes by DEN. The decreased level of LPO in plant extract treated animals could be attributed to free radical quenching effect of *H. auriculata*.

There is a natural dynamic balance between the output of free radicals generated in the body and the antioxidant defense system that quenches or scavenges them and thereby protecting the body against pathogenesis [21].

CONCLUSION: From the above results, it may be concluded that, the methanolic extract of *H.auriculata* has ameliorated the lipid peroxidation induced by DEN .The present findings shows that extract of *H.auriculata* by inhibiting LPO has suppressed the vigour of development of HCC which is attributed to the flavonoids and polyphenols present in the *H.auriculata* extract.

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