IOSR Journal Of Pharmacy And Biological Sciences (IOSRJPBS)
ISSN : 2278-3008 Volume 3, Issue 5 (Sep.-Oct. 2012), PP 19-22
Www.Iosrjournals.Org

Modulatory Effects Of *Hygrophila auriculata* on Total Proteins And Nucleic Acids In N-Nitrosodiethylamine Induced Hepatocellular Carcinoma In Rats.

Dr.S.Premkumari[1],Dr. M.P. Balasubramanian[2]
[1]*Professsor and HOD,Department of Pharmacology,Sri Venkateswara Dental College, Thazhambur, Chennai-603 103. India.*
[2]*Department of Pharmacology and Environmental Toxicology, Dr. ALM P G IBMS University of Madras, Taramani, Chennai-600 113. India.*

***Abstract*:** *This study was designed to investigate the Modulatory effect of Hygrophila auriculata on Total Proteins and Nucleic acids 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, (200 mg/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 Total Proteins and Nucleic acids was 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 Total Proteins and Nucleic acids 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 Total Proteins and Nucleic acids 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 ,Total Proteins and Nucleic acids.*

I. 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]. The injury to liver caused by virus, chemicals and drugs will lead to the disruption of the important physiological process .Hepatotoxic chemicals may be divided into genotoxic and non-genotoxic. Genotoxic chemicals directly interact with DNA, forming covalent adducts and induces genetic changes upon cell replication. Non- genotoxic chemicals stimulate tumor formation by altering kinetics of cell proliferation, cell death and cell differentiation through a variety of epigenetic pathways [2]. Such cancer producing chemicals are called as carcinogens.

N-Nitrosodiethylamine (DEN) is another such widely occurring nitrosamine which is present in combustion products, tobacco and various processed food [3]. It is one of the important environmental carcinogens which primarily induces tumor of liver [4]. 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 [5] ,[6] and generation of superoxide radicals [7] paralleled by lipid peroxidation reactions. 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 *Hygrophila 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 paucity of information regarding the modulatory effect of methanolic extract of the whole plant of *H.auriculata* on Total Proteins and Nucleic acids in DEN induced HCC bearing rats. The development of different types of tumors is accompanied by characteristic alterations in Total Proteins and Nucleic acids. Therefore, *H.auriculata* was selected as plant source to evaluate its effects on Total Proteins and Nucleic acids in N-nitrosodiethylamine induced hepatocellular carcinoma in rats.

II. 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-darkcycle. 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 *Hygrophila 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[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 *Hygrophila 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.

Estimation of Protein and Nucleic acids:

Protein was estimated by the method of Lowry *et al*. [12]. Deoxyribonucleic acid (DNA) was estimated by the method of Burton (1956) [13].Ribonucleic acid (RNA) was estimated by the method of Rawal *et al*. (1977). [14].

Statistical analysis:

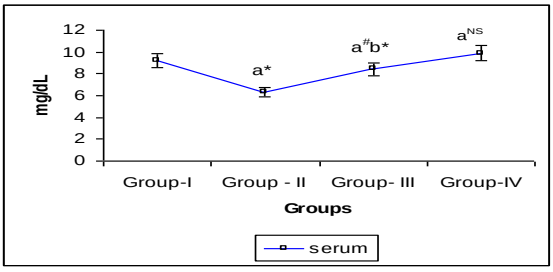
Data are presented as the mean± standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups of by using SPSS 7.5 student versions. Comparisons were made between group II and IV with group I and group II with group III for animal studies.

III. Results

Fig.1 shows the total protein levels in serum, liver and kidney of control and experimental animals. Significant decrease in total protein levels in serum, liver and kidney were observed in group II cancer bearing animals when compared with control (serum, liver $p<0.001$; kidney $p<0.01$). On the other hand, the level of total protein was significantly increased ($p<0.001$) in serum and liver of group III plant extract treated animals. However, there was no statistical significance in kidney of plant extract treated animals. There were no significant changes in group IV animals when compared with control animals.

Fig .2 represents the levels of nucleic acids (DNA and RNA) in liver and kidney of control and experimental animals. In group II animals, the levels of nucleic acids were significantly elevated ($p < 0.001$). These were slightly decreased in plant extract treated animals. However, no significant changes were observed in group IV animals when compared to the control animals.

IV. FIGURES



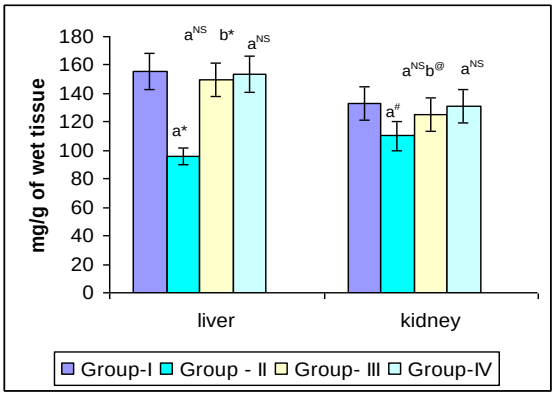


Fig 1: The levels of total protein in serum, liver and kidney of control and experimental animals

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

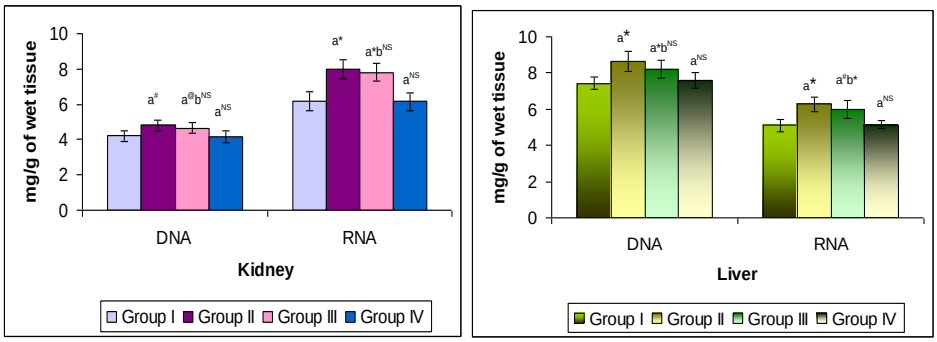


Fig 2: The levels of nucleic acids in liver and kidney of control and experimental animals

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

V. Discussion

Reactive oxygen species (ROS) have great potential to damage cellular proteins, lipids and DNA and have been implicated in various diseases, including atherosclerosis, cancer, arthritis, neurodegenerative disease, pulmonary fibrosis, acute and chronic inflammation etc. Protein metabolic perturbations in the tissue of host may also favor the growth of tumor. It has been reported that tumor growth elicited marked loss of body weight in growing ascitic hepatoma bearing rats [15].This might be due to changes in protein metabolism during tumor formation. In contrast to decrease in body weight, there was an increase in the liver and kidney weight of the tumor bearing animals. The increase in the liver weight might be due to the progression of the tumor growth in the liver. The administration of *H. auriculata* extract decreased both the liver and kidney weight, Body weight gradually increased leading us to understand that the plant extract helps in the restoration of protein metabolism due to the free radical scavenging activity of the plant extract.

Neoplasms are associated with abnormalities in their DNA content, which increase with the degree of malignancy. The determination of DNA content was more meaningful with regard to biological and functional aspects of the tumor. Since it is indicative of proliferative activity in tumor conditions. DNA content is an independent indicator and often correlates with DNA content of tumor [16].

Oxidants can utilize several different avenues to alter cellular function including signal transduction pathways and expression of genes relevant to generation of mitogenic signals to the oxidant resistant carcinogen initiated cells causing tumor promotion, apart from initiating a toxicity signal to normal cells [17],[18]. In this aspect to meet the mitogenic signals the nucleic acids play a vital role. The assessment of DNA content which is an index of proliferative activity in cancer conditions is very essentials. DNA content is found to be an independent indicator of prognosis, since the size of the tumor often correlates well with DNA content of tumor.

In the present study, there was an increase in the amount of DNA content in tumor bearing animals when compared to control animals. DNA synthesis is minimum in normal hepatocytes which have a very slow rate of cell division [19].In contrast the development of HCC is associated with increase DNA synthesis [20].There was an increase in the RNA content of both liver and kidney in tumor bearing animals which could be due to mitosis related increased transcription in neoplastic conditions.The levels of DNA & RNA were reverted to normal in the plant extract treated animals.

Considering the points of attack of free radicals, antioxidants may prevent the initiation of carcinogenesis by protecting DNA from mutagenic change and the promotion and progression by protecting damage to cell membrane. The time internal over which these sequential changes occur seem to provide more opportunity to intervene in the processes and thus, to prevent the development of cancer [21]. This action of *H. auriculata* is due to the oxidant detoxifying capacity of the Plant. This action could be attributed to the presence of polyphenols and flavonoids in the plant extract. The plant have been reported to contain many polyphenolic compounds mainly flavonoids .The antioxidant activity of this plant extract might be due to the presence of polyphenolic constituents [22].Flavonoids are reported to have inhibitory action on various stages of tumor development [23].Thus the present study suggest that the presence of flavonoids and polyphenols in the extract might have facilitated free radical scavenging activity and thus might be useful in inhibition of free radical mediated DNA damage thereby restoring the total protein and nucleic acids in HCC bearing rats.

VI. Conclusions

Antioxidants such as the polyphenols and flavonoids present in the whole plant extract of of *H. auriculata* act as radical scavenger, inhibit LPO and other free radical-mediated processes, thereby protecting the human body from various diseases including cancer. Therefore an extended study on this plant extract might be of immense value as an herbal chemotherapeutic agent for cancer.

References

[1] F.M.Ward and M.S.Daly. Hepatic disease. In: Walker R, Edwards C, editors. *Clinical pharmacy and Therapeutics*. (Churchill Livingstone: NewYork, 1999) 195-212.

[2] G.N. Wogan .Impacts of chemicals on liver cancer risk. *Semin. Cancer. Biol*, 2000, 201-210.

[3] G .Lucier and G.E.R Hook .Dietary mutagens special issue. *Environ. Health Perspect*, 1986, 67-72.

[4] S.S. Hecht. Approaches to cancer prevention based on an understanding of N-nitrosamine carcinogenesis. *Proc. Soc. Exp. Biol. Med., 216*, 1997, 181-191.

[5] M.C.Archer. Mechanism of action of N-nitrosocompounds. *Cancer Surv., 8***,** 1989, 241-250.

[6] D .Nakae, Y. Kobayashi, H Akai, N .Andoh, H. Satoh and K .Ohashi .Involvement of 8-hydroxyguanine formation in the intiation of rat liver carcinogenesis by low dose levels of N-nitrosodiethylamine, *Cancer Res. 57***,**1997, 1281-1287.

[7] B .Halliwell and J.M.C. Gutteridge). Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity, in: K.H. Cheeseman, T.F. Slater (Eds), *Free Radicals in Biology and Medicine* (Clarendon Press, Oxford, 1989) 144-147.

[8] L.W .Wattenberg. Chemoprevention of cancer. *Cancer Res., 45*, 1985, 1.

[9] P. Bairaj and S. Nagarajan .Apigenin- 7-0-Glucuronide from the flowers of *Asteracantha longifolia* Nees. India Drugs. 1982, 150-152.

[10] A.K .Tiwari. Antioxidants: New-generation therapeutic base for treatment of polygenic disorders. *Curr Sci,86*, 2004,1092-1100.

[11] M.L .Tan, S.F. Sulaiman, N. Najimuddin, M.R.Samian and T.S. Tengku Muhammad. Methanolic extract of *Pereskia bleo* (Kunth) DC (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. *Journal of Ethnopharmacology***.** *96*, 2005, 287-294.

[12] O.H. Lowry, N.J .Rosebrough, A.L. Farr and R.J. Randall. Protein measurement with the Folin-phenol reagent. *J. Biol. Chem. 193*, 1951, 265-275.

[13] K .Burton .A study of the conditions and mechanisms of diphenylamine reaction for colorimetric estimation of deoxyribonucleic acid. *Biochem. J. 62***,**1956,315-323.

[14] V.M. Rawal, V.S .Patel, G.N. Rao and R.R. Desai. Chemical and biochemical studies on cataractous human lenses-III - Quantitative study of protein, RNA and DNA. *Arogya J. Health Sci. 3*, 1977, 69-75.

[15] L Tessitore, G Bonelli and F.M. Baccino .Early development of protein metabolic perturbations in the liver and skeletal muscle of tumour-bearing rats. *Biochem. J., 241***,** 1987. 153-159.

[16] C.N .Ellis, J.J Burnette, R /Sedlack, C. Dyas and W.S. Blackmore .Prognostic applications of DNA analysis in solid malignant lesions in humans. *Surgery. 173*, 1991, 329-342.

[17] G .Witz .Active oxygen species as factors in multistage carcinogenesis, *Proc.Soc. Exp.Biol.Med. 198***,**1991, 675-682.

[18] K .Frenkel ,Carcinogen mediated oxidant formation and oxidative DNA damage, *Pharmacol. Ther. 13***,** 1992, 127-166.

[19] M .Board, S. Human and E.A Newsholme ,Maximum activities of key enzymes of glycolysis, glutaminolysis, pentose phosphate pathway and carboxylic acid cycle in normal, neoplastic and suppressed cells. *Biochem. J., 265*, 1990, 503-509.

[20] E.B .Pierr, D .Dominque, .J.M Fabre, G. Pageaux, J.P .Daures, D. Larrey, B .Saint Aobert, H. Michel and P. Maurel. Contribution of primary cultures of adult human hepatocytes to the pathophysiology of hepatocellular carcinoma. *J. Hepatol., 25*, 1996,663-669.

[21] I.O.Aruoma. Free radicals and antioxidants strategies in sports, *J.Nutr. Biochem. 5, 1994*, 370-381.

[22] R.P .Rastigo and B.N. Mehrotra, *Compendium of Indian medicinal plants*. (2). (Publications and Information Directorate, New Delhi, India1991).

[23] S .Hollman and O .Touster .Alterations in tissue levels of UDP-glucose dehydrogenase, UDP-glucuronic acid pyrophosphatase and glucuronyl transferase induced by substances influencing the production of ascorbic acid. *Biochem. Biophys. Acta., 62*,1962, 338-352..