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Original Research Paper

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AMELIORATING EFFECT OF CHELERYTHRINE AND DADS AGAINST CHEMICALLY INDUCED HEPATOCARCINOMA IN MALE SWISS ALBINO MICE.

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ABSTRACT The main aim of our study is to find out efficacy of combined drug Chelerythrine and DADS against chemically induced hepatocarcinoma in male Swiss albino mice.4-6 weeks aged mice were considered for experimentation.Liver cancer was induced by genotoxic carcinogen para- dimethyal-aminoazobenzene along with nongenotoxic promoter carcinogen phenobarbital exposure.During study animals were co-treated with 100mg/kg body weight DADS,5 mg/kg body weight chelerythrine individually or in combination for 120 days. Morphological and histopathological tissue analysis were performed for the confirmation of our objectives.Lots of nodule formation were observed after carcinogen exposure, that causes morphological changes in liver tissue.Histological analysis of the said tissue also demonstrated the reservation of tissue structures in the treated groups, most significantly in the combined treatment.So in concluding remark we can say combined impact of drugs protect the liver tissue structure from harmful carcinogenic exposure in male Swiss albino mice.

KEYWORDS : Hepatocellular carcinoma;Carcinogen; DADS; Chelerythrine ; Combination therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is a cancer arising from liver. It is known as primary liver carcinoma [Kumar *et al.*, 2003]. Liver is made up of different cell types such as hepatocytes, epithelial cell, bile duct, endothelial cell in blood vessels and fat storing cells amongst them, liver hepatocytes alone make up 80% of liver tissue [Kmieć Z, 2001]. Thus majority of the primary hepatocarcinoma arises from hepatocytes [Fielding L,2006]. Liver cancer often referring to a cancer that has spread to the liver, having originated from other organs as metastatic liver cancer or hepatocellular carcinoma. This is among the fifth most common cancers worldwide [Andrade LJDO *et al.*,2009].The geographic areas at higher risk are located in China and eastern Asia, middle Africa and some countries of western Africa. Lower incidences are encountered in Japan, Europe and America, but this incidence is still rising in part mostly because of the high level of hepatitis C virus infection [El-Serag HB,2012]. HCC is an epithelial tumour and generally develops in the setting of chronic hepatitis or cirrhosis [Gomes MA *et al.*,2013] in which there is a continuous inflammation and regeneration of hepatocytes.

Mechanisms of hepatocarcinogenesis are not completely understood but, like most solid tumours, the development and progression of HCC are believed to be caused by the accumulation of genetic changes resulting in altered expression of cancer-related genes, such as oncogenes or tumour suppressor genes, as well as genes involved in different regulatory pathways [Thorgeirsson SS *et al.*,2002]. To understand the mechanism of liver cancer development a number of models are designed using carcinogenic chemicals, hormones, and viruses [Marszałek M, 2000].This process is almost always a multistep and during the long period of cancer development, discrete cells or cell populations acquire step-by step the various properties that go to make up a cancer [Marszałek M, 2000].The hepatocarcinogeneic models are useful in identification and analysis of the preneoplastic and neoplastic alterations during HCC [Marszałek M,. 2000].The remarkable similarities between many models with different carcinogens in animals and humans suggest the importance of such studies in understanding of molecular basis of liver cancer development.

On the basis of this theoretical foundation we treated Swiss albino mice *Mus musculus* with phenobarbital and paradimethylaminoazobenzene for the study of the mechanisms of hepatocarcinogenesis. Phenobarbital (PB) is mitogenic non-genotoxic carcinogens while para-dimethylaminoazobenzene (P-DAB) is genotoxic carcinogen [Bhattacharya S *et al.*,2004].We have found that PB in combination with P-DAB induces hepatotoxicity and tumor generation in mice. Using this PB+P-DAB treated group, our objective was to identify novel chemo preventive agents and their combined treatment procedure with traditional medicine for the successful ameliorative means during HCC development.

MATERIAL AND METHODS

ANIMALS

A group of total 50 healthy male Swiss Albino mice weight of 20- 25 gms were selected as model for the in vivo studies about the experimental work. A group of 10 animals were considered as control separately for experimental set. An alternative group of 10 animals were set to see the extent of damage at morphological, and histological, level after carcinogenic exposure. To know the efficacy of the treatment a set of groups were co-treated with 100mg/kg body weight DADS, an active organosulpher component of garlic; and another set of groups were co-treated with 5 mg/kg body weight chelerythrine,an active component of Chelidonium majus and performed tissue morphology as well as histological study.Experimental animals were bred in-house and maintained at 27 ± 2°C, 44–56% relative humidity and 12 h light/darkness cycle with free access of food and water in a cross-ventilated room. Experiments were designed following the ethical guidelines of animal ethics committee of Vidyasagar College, University of Calcutta to minimize animal suffering and to use the minimum number required for statistical validity.

Study on morphological tissue structure:

Liver was dissected out from the respective group of male mice and tissue was cleaned. The photographs were taken for further analysis.

Study on histological structure

After dissecting out the liver from the respective group of mice

and the paraffin block was prepared following the standard procedure. Tissue was sectioned and was stretched on the mayers albumin coated slide. Finally sections were stained following the double staining procedure and were observed under microscope.

RESULT:

Tumour regression after DADS and chelerythrine treatment in PB+P-DAB exposed mice:

Swiss albino mice were exposed to PB and p-DAB to induce hepatocarcinoma.Liver morphology demonstrated development of liver tumour (Figure 1B marked by white arrows) in male mice. Our data suggested significant enhancement in tumour number tissue surface area and corresponding increase in the size of liver tissue after 120 days of PB+p-DAB exposure. To analyze the efficacy of the tissue protective drug, carcinogen exposed male mice were co-treated with 100 mg/kg body weight of DADS as well as 5 mg/kg body weight of chelerythrine .Result suggested that increase of no of tumour nodules(Figure1B) in the liver tissue were successfully attenuated by combination therapy of Chelerythrine and DADS (Figure1E).

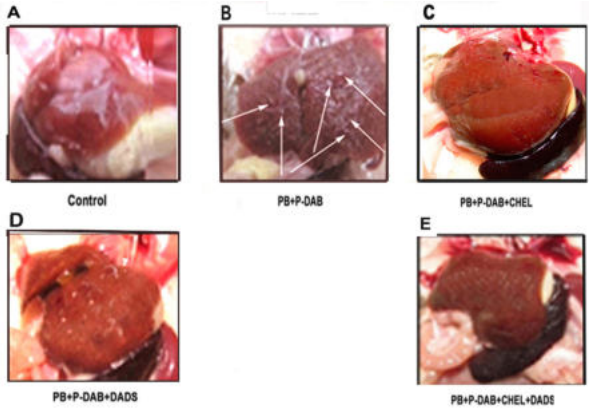

Figure 1:Tumour regression after DADS and chelerythrine treatment in PB+P-DAB exposed mice.

Change in liver histology due to PB+P-DAB exposure and its restoration in the DADS and chelerythrine co-treatment:

Histological analysis suggested distinct amount of lipid inclusion within the hepatocytes, cellular enlargement, dysplasia and overall distortion of cellular arrangement after PB+P-DAB exposure(Figure 2B). Individual chelerythrine co-treatment reduced lipid inclusion to some extent (Figure 2C),while DADS individually restored cell morphology to a certain level as observed by the (Figure 2D).Drugs in combination effectively maintained cellular arrangement with a reduction in lipid accumulation as well as defined central vein like structure (Figure 8E)more or less similar to control animals (Figure 2A).

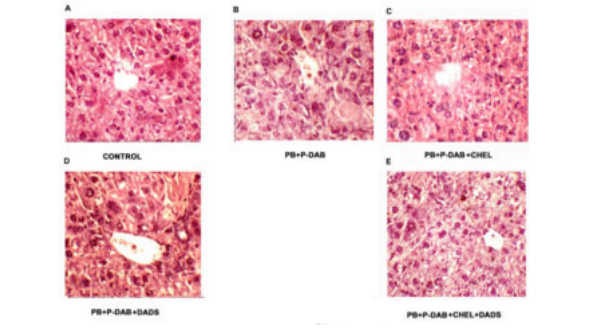

Figure 2:Histological study of the liver tissue obtained from DADS and chelerythrine co-treated (individual and combined) and untreated group of PB+P-DAB exposed male mice.

DISCUSSION

Hepatocellular carcinoma is known as primary liver carcinoma, which is associated with unregulated growth and proliferation of hepatocytes [Kitisin K *et al*.,2007]. It has already been reported that the occurrence of HCC is predominant in males and it is the commonest in subjects over the age of 40, although can be observed in younger people too [Farhi DC *et al*.,1983]. The prognosis is generally poor. Causes of more than 85% of HCC cases are hepatitis B and C infection aflatoxin B1, carcinogenic toxicants and ethanolic exposure, as well as metabolic disorder [Thorgeirsson SS *et al*.,2002].

On the basis of this theoretical foundation we exposed Swiss albino male mice (*Mus musculus)* with Phenobarbital and paradimethylaminoazobenzene for the generation of chemically induced hepatocarcinoma.Our study clearly revealed that cumulative exposure of genotoxic paradimethyl aminoazobenzene (P-DAB) and the promoter phenobarbital (PB) lead to effective generation of hepatocellular cytotoxicity, and liver tumour formation associated with change in the structure of morphology as well as histology.Exposure of P-DAB produces reactive electrolytes [OhnishiS *et al*.,2001] and reactive oxygen species (ROS).ROS accumulation played a significant role in the generation of DNA mutation. This in turn in association with covalent binding of carcinogenic metabolites to the DNA probably lead to generation of liver tumour after PB+P-DAB exposure [Biswas SJ et al.,2002]. Experimentally morphological study clearly revealed the enhancement of tumorigenic growth after PB+P-DAB exposure. More over exposure with carcinogen pointed out a remarkable change in male mice considering the tumour development .Our aim was to determine the efficacy of the combination of tissue protective herbal drugs with traditional medicine in the restoration of normal cell physiology of liver tissue considering PB+P-DAB exposed Swiss albino mice as a model for *in-vivo* studies.Morphological tissue analysis and histological study revealed that chelerythrine and DADS,as combine drug were successfully reduced the carcinogenic effect in male mice.

CONCLUSION:

Our study confirm that chelerythrine and DADS individually can reduce the carcinogenic effect in the tissue but to eradicate the harmful effect,combination therapy (Chlerythrine +DADS) play most significant role.

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