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Protective impact of Chelerythrine and DADS against chemically induced hepatocarcinoma

KEYWORDS: Chelerythrine; DADS; Induced hepatocarcinoma.

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ABSTRACT *Study was performed to explore the efficacy of chelerythrine and DADS in combination against P-DAB and PB induced hepatocellular carcinoma in mice. Five week aged 100 mice were used for the experimental purpose. Animals were divided into five different groups either of sex and each set contained 10 mice. P-DAB in combination with PB were administered to develop hepatocarcinoma. 5mg/kg body weight Chelerythrine and 100mg/kg body weight DADS were used as drug in single and as well as in combination to find out their efficacy in the protection of liver tissue against carcinogenic exposure. After 120 days of consecutive experiment different biochemical parameters such as ALT, AST, ALKP, GGT and histopathological tissue analysis were performed. Toxicity analysis was conducted by determining the TBARS content in control animal after drug treatment with stipulated dose and time. Our obsevation revealed that chelerythrine and DADS in combination successfully restored the activities of enzymatic biomarkers and histopathological liver tissue structure similar to control mice.*

Introduction-

Hepatocellular carcinoma is the 5th most common cancer in the world and causes death of the common people at an interval (Sengupta.D et.al.2014).Majority of primary liver carcinoma arises in hepatocytes (Fielding.L2005). Feeding of carcinogenic azo dye such as paradimethylaminoazobenzene (P-DAB) produces liver damage and neoplastic characteristics (Biswas.SJ et al,2005).Phenobarbital (PB) in association with P-DAB demonstrates carcinogenic impacts in mice(Biswas et al 2005).The conventional therapy of hepatocellular carcinoma including chemotherapy, radiotherapy, resection and radiofrequency ablation (RFA), liver transplantation gives little hope for amelioration of hepatocarcinoma. But they are costly and have many side effects (Zhang CL. et al 2012). Therefore introduction of anticancer agents with less side effects are required.

Chelerythrine is an active component of *chelidonium majus* has anti-viral, anti-inflammatory, anti-tumour and anti-microbial activities in mice (Biswas.S.J 2002). Similarly DADS is an active component of garlic also exhibits anti-microbial, anti-oxidant, immunomodulatory activities (Zhang CL. et al 2012). Study was conducted to determine the combinatorial effects of chelerythrine and DADS against PB and P-DAB induced hepatocellular carcinoma in swiss albino mice.

Material and Methods

Animals

In this experimental work a group of total 100 healthy swiss albino mice (50 male and 50 female), weight of 20-25gms were selected as a model. A group of 10 animals either of sex were set in five groups starting from control, PB+P-DAB, PB+P-DAB+CHEL, PB+P-DAB+DADS and PB+P-DAB+CHEL+DADS respectively. During this experimental work 100mg/kg body weight of DADS and 5mg/kg body weight of chelerythrine were used individually and in combinatorial form against PB and P-DAB induced hepatocellular carcinoma in swiss slbino mice. Experiment was continued for 120 days.

Study on animals was conducted in a cross-ventilated room.at 27 ± 2°C, 44–56% relative humidity and 12 h light/darkness cycle with free access of food and water. Experiments were performed following the ethical guidelines of animal ethics committee of Vidyasagar College, University of Calcutta ,India.

Instruments and Reagents

Phenobarbital and paradimethyalaminoazobenzene, eosin, haematoxyline and all other fine chemicals were purchased from Sigma-Aldrich Corporation,St.Louis, MO,USA. ALT, AST, ALKP, GGT measurement kit were obtained from TECO Diagnostics,CA,USA. A number of apparatus were used to perform experimental work such as cooling centrifuge (Remi C24BL, Remi, Goregaon (East),Mumbai,India); UV-VIS Spectrophotometer (UV mini1240, Shimadzu Corp. Kyoto Japan); Spinwin (Tarsons Pvt.Ltd. NewDelhi India); Microscope (Olympus, Tokyo, Japan).

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Analysis of Biochemical Parameters
After 120 days of experiments different biochemical parameters such as ALT, AST, ALKP and GGT were measured following the kit protocol ((TECO Diagnostics, CA, USA) to analyze their enzymatic activities. In GGT assay absorbance was measured at 405nm for 60sec intervals. For ALKP assay the absorbance was measured at 405nm for 30sec intervals. During ALT and AST the absorbance was measured at 320nm for 30 sec intervals upto 2 min.

Study on histological structure
Liver was dissected out 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.

Measurement of TBA reactive substances:
For the measurement of TBA reactive substances (TBARS) 1ml of 10mM Tris–HCl buffer (pH 7.4) was added to 0.4mg of membrane protein (in 0.9% saline, pH 7.4) Then 2ml TBA–TCA reagent (15% TCA and 0.4% TBA) was added and the reaction mixture was boiled for 15min. After centrifugation the clear supernatant was measured at 532nm. Quantification was based upon the molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ and was expressed in nmoles/mg protein.

Statistical analysis
Sample mean and SEM were calculated in Microsoft excel. Significance of the treatment groups with the carcinogen exposed group were determined by the Student's t-test in the GraphPad Instat software (Graph Pad, La Jolla, CA, USA). Differences were regarded as statistically significant when $p<0.05$.

Result
[Table-1 about here]
Analysis of Biochemical parameters in hepatocellular carcinoma after DADS and chelerythrine treatment in PB+P-DAB exposed mice
Activities of liver specific enzymatic biomarkers of male and female mice were represented in Table-1.Function of AST and ALT were enhanced after introduction of PB+P-DAB as a corrosive carcinogenic agent. But the level was decreased a little bit after individual chelerythrine and DADS co-treatment in their respective groups of mice. Although combinatorial effect of chelerythrine and DADS were successfully able to restore the carcinogenic activity both in male and female groups of mice.

Table-1: Biochemical Parametric analysis and TBARS content in Swiss Albino Mice.

Male					Female			
Groups	ALT Activity (U/L)	AST Activity (U/L)	ALKP Activity (U/L)	GGT Activity (U/L)	ALT Activity (U/L)	AST Activity (U/L)	ALKP Activity(U/L)	GGT Activity (U/L)
Control	21±2.3	25±2.5	45±2.9	27±2.7	17.5±2.4	19.5±2.8	35±2.6	31.3±2.2
PB+P-DAB	69±10.1	88±10.5	96±10.6	75±11.1	39±9.6	50±10.2	65±10.9	60±10.4
PB+P-DAB+CHEL	40±6.8	60±6.4	76±6.6	54±6.9	25±6.2	35±6.5	58±6.7	53±6.3
PB+P-DAB+DADS	38±5.6	50±5.5	71±5.8	49±5.9	28±5.7	30±5.2	52±5.4	49±5.3
PB+P-DAB+CHEL+DADS	25±3.9	30±3.4	51±3.6	33±3.8	19±3.5	23±3.7	38±3.2	34.7±3.3

TBARS Content		
Groups	Male (n mol/mg Protein)	Female (n mol/mg Protein)
Control	2.53±0.41	2.34±0.37
Control+CHEL	2.538±1.2	2.348±1.1
Control + DADS	2.54±0.9	2.345±0.7
Control +CHEL+DADS	2.536±0.89	2.341±0.68

Determination of toxicity level for both chelerythrine and DADS
Thiobarbituric acid reactive substances are formed as a by-product of lipid peroxidation. TBARS level was estimated after 120 days of consecutive treatment with 100mg/kg body weight of DADS and 5 mg/kg body weight of chelerythrine in the control animals. Values did not display any significant change in the TBARS content both in male and female group of mice (Table-1) after individual as well as combined chelerythrine and DADS co-treatment.

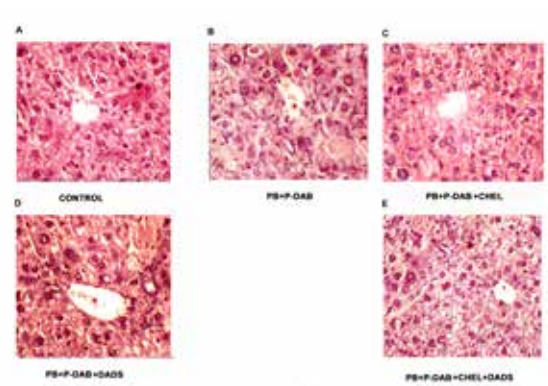

Figure Legend of Figure-1

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Histological study of the liver tissue (40X) obtained from DADS and chelerythrine co-treated (individual and combined) and untreated group of PB+P-DAB exposed male mice. Dysplasia, lipid inclusion and overall distortion of cellular arrangement were observed in PB+P-DAB exposed group (B).Individual chelerythrine co-treatment reduced lipid inclusion(C) while co-treatment with individual DADS reduced the distortion of the tissue (D).DADS and chelerythrine combined co-treatment (E) effectively restored tissues structures more or less similar to control (A).Results were representative of ten independent observations.

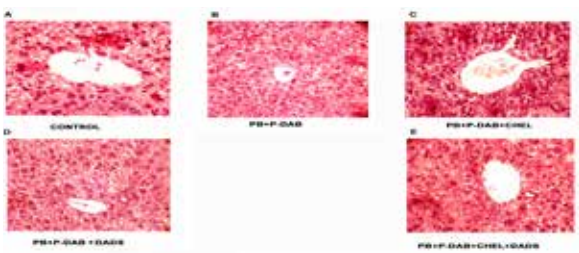

Figure Legend of Figure-2

Study on histological structure (40X) of liver tissue isolated from drug (individual DADS and chelerythrine as well as in combination) co-treated and untreated group of PB+P-DAB exposed female mice. Distinct distortion on cellular arrangement was observed after PB+P-DAB exposure (B). Co-treatment with individual chelerythrine restrained cellular distortion to some extent (C) and individual treatment with DADS effectively maintained central vein and associated tissue structure (D).Co-treatment with combined drug (E) efficiently protected tissue from any toxic effect of PB+P-DAB exposure and restored tissue structure similar to control (A). Results were representative of ten independent observations

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 1B). Individual chelerythrine co-treatment reduced lipid inclusion to some extent (Figure 1C), while DADS individually restored cell morphology to a certain level as observed by the Figure 1D. Drugs in combination effectively maintained cellular arrangement with a reduction in lipid accumulation as well as defined central vein like structure (Figure 1E) more or less similar to control animals (Figure 1A).

Considering the female perspectives result depicted significant modification in cellular morphology and its associated tissue structure after PB+P-DAB exposure (Figure 2B).Chelerythrine effectively maintained cellular structure (Figure 2C) and a reservation of tissue morphology with definite central vein was observed after DADS (Figure 2D) individual co-treatment. Although combined treatment of DADS and chelerythrine (Figure 2E) significantly conserved the tissue structure even after PB+P-DAB exposure as depicted by the overall similarities in the histological analysis with control mice (Figure 2A).

Discussion

Hepatocellular carcinoma is the second most frequent cause of death in 2010 worldwide (Jemal A et al 2011).In our experimental work we have used P-DAB and PB for the generation of liver cancer mice. Experimental observation revealed that P-DAB in association with PB can generate reactive electrolyte (Ohnishi S, et al 2001) and reactive oxygen species leading to destruction of liver physiology .Our aim was to explore the efficacy of combinatorial effect of chelerythrine and DADS in restoration of liver tissue during PB+P-DAB exposure.

Enhanced activity of ALT, AST and ALKP in serum are usually recognized as an indicator of liver tissue injury (Ha W.S et al 2001).While the serum GGT is an established biomarker for preneoplastic lesion (Zhang CL. et al 2012). This is an established fact that after the damahe in liver cells excess amount of enzymes usually release from cytoplasm to blood circulation (Shaarawy S.M et al 2009).This in turn the possible reason for the eventual enhancement in the enzymatic activities during PB+P-DAB exposure. In our study chelerythrine and DADS were able to reduce the carcinogenic effect of PB+P-DAB by improving the histopathological damage and biochemical indices in the liver tissue individually and most significantly in a combination form (Figure-1, Figure-2 and Table-1).

Experimental analysis also suggested that 5 mg/kg body weight of chelerythrine and 100 mg/kg body weight of DADS individually or in a combination did not introduce any toxic effect after a consecutive treatment of 120 days in control animal. Data further portrayed the restoration of tissue structure to some extent in individual chelerythrine and DADS co-treatment. Combination of this two drugs displayed more or similar histological structure of liver tissue with respect to control animal both in male and female group of mice.

Conclusion

Data analysis indicated the effective co-treatment of DADS and chelerythrine against PB+P-DAB induced hepatocercinoma.Experiment also suggested the consideration of DADS and chelerythrine in the therapeutics against chemically induced hepatoceracinoma.

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