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POTENTIAL EFFECT OF BIS-[(HYDROXYL-NAPHTHALEN-2-YL)-PHENYL-METHYL]-UREA (1,3 BPMU) ON DIETHYL NITROSAMINE (DEN) INDUCED HEPATOCARCINOMA IN WISTAR ALBINO RATS.

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ABSTRACT

Cancer is a leading lethal disease. Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide,Which caused by various factors such as environmental pollutions, Chemical usages, alcohol and Hepatitis B virus (HBV).Now a day researchers involved to develop drug fro HCC.In our present study1,3 –Bis-[(hydroxyl-naphthalen-2-yl)-phenyl-methyl]-urea is a newly synthesized mannich base has showed a wide variety of biological activities for the treatment of oxidative mediated diseases. The present study is aimed to evaluate its receptor binding score value of insilico docking, therapeutic potential of 1, 3BPMU on DEN induced rats were estimated by various biochemical parameters such as total albumin, globulin and bilurubin urea, creatinine enzymatic and non enzymatic antioxidant status in control and experimental rats. 1, 3BPMU treatment significantly normalized the altered biochemical levels and antioxidant activity in DEN induced rats. Further, SEM analysis revealed that the 1, 3BPMU protects the membrane changes induced by the DEN in rats. Therefore, we suggest that 1, 3BPMU could be useful for drug development against Hepatocellular carcinoma.

KEY WORDS: **HCC, 1, 3BPMU, DEN, Insilico docking, antioxidants**

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1. INTRODUCTION

Cancer is a leading lethal disease, among various diseases attributed to mortality in humans all over the world. Liver cancer is a major health problem worldwide. It is the fifth most common neoplasm in the world, and the third most common cause of cancer-related death. Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide with an estimated 50000 to 1 million new cases per year [1]. Many kinds of chemical hepatocarcinogens triggered malignance in different places of the liver, which is lead to different patterns of cellular proliferation preceding development of hepatocellular carcinomas [2] It is based on the activation of different carcinogens in different places of liver lobules. DEN is one of the common environmental chemical carcinogen [3]. It is found in Tobacco, smoke, meat, whiskey cosmetics, gasoline and also found in many processed foods includes milk, meat products, steamed and fried fish, and alcoholic beverages[4]. It has extensive use as a carcinogen in experimental animal model. Di Ethyl Nitrosamine (DEN) is metabolized in pericentral of the liver lobule, but more actively in the periportal zone of the liver and oxidative stress caused by DEN can contribute to hepatocarcinogenesis [5,6] To rectify this problem from various factors, now days researchers are focusing to synthesis a new drug. Synthetic 6,7-substituted 2-phenyl-4-quinolone, was identified as a potent and selective antitumor agent in human hepatocellular carcinoma.[7] Mannich bases possess potent antiviral, antibacterial, antifungal and antioxidant activity in in-vitro model[8] Hence the newly synthesized mannich derived organic compound namely1,3-Bis-[(3-hydroxy-naphthalen-2-yl)-phenyl-methyl)-urea (1,3BPMU) was considered to investigate the protective effect against DEN induced liver cancer in the rat as a model system.

2. MATERIALS & METHODS

DEN and Phenobarbital were purchased from Sigma Aldrich, USA. 1,3BPMU were synthesized at the Department of chemistry, National Institute of Technology (NIT), Tiruchirappalli, Tamilnadu, India, and other chemicals used were of analytical grade.

2.1. Computational Molecular Docking studies

Crystallographic structure of respective protein were retrieved from the PDB (PDBid: 1DV8) computational analysis was done to compute ligand protein binding affinity of the compound, all the 1, 3-bis-[(3-hydroxy-naphthalen-2-yl)-phenyl-methyl)]-urea , molecule is modelled using 3D expansions of real orthogonal spherical polar basis functions to encode both surface shape and electrostatic charge and potential distribution. Hex tools are working on the Fast Fourier transform (FFT) docking methods known for very fast than other traditional docking tools[9].

2.2.Animals

Male *Wister albino* rats of the same age group with body weight of 150- 170 gm were selected for all the experiments. The animals were housed in polypropylene cages at an ambient temperature 25 ^{0}C and 40-55 % relative humidity with 12 hrs each of dark and light cycle. Rats were fed with pellet diet and water ad libitum. All the animal experiments were duly approved by the Institutional Animal Ethics Committee [743/03/abc/ CPCSEA dt 3.3.03] Guidelines.

2.3. Experimental Design

The rats were divided into four groups; each group consists of six animals analyzed for a total experimental period of 16 weeks as follows;Group 1: Control rats fed with standard diet with *ad-libitum* of water. Group 2: 1,3 BPMU alone was orally given to rats (50 mg / kg bwt.) by aqueous form for 16

weeks once in a day. (The dose of 1, 3 BPMU fixed by effective dosage fixation study). Group 3: Rats were induced HCC with DEN by ip injection (0.02%) after two weeks cancer promoted by the administration of Phenobarbital (oral /250mg/kg bwt.) for 6 weeks.[10] Group 4 : Rats were induced HCC with DEN by ip injection (0.02%) after two weeks cancer promoted by the administration of Phenobarbital (oral /250mg/kg bwt.) for 6 weeks then rats were treated with 1,3BPMU (1,3BPMU) oral 50mg / kg/bwt.) for the next 8 Weeks[11].

2.4.PreparationofLiverTissueHomogenate

The liver tissues were excised and rinsed in ice-cold saline. Known amount of the tissue were homogenized in 0.1 M Tris–HCl buffer, pH 7.4 at 4^0C, in a Potter– Elvehjem homogenizer with a Teflon pestle at 600 rpm for 3 minutes. The homogenate was centrifuged at 3000 µg for 10 minutes. The supernatant was collected as tissue homogenate, which was used to assay various parameters.

2.5.EstimationofBiochemical Parameters

From liver homogenate the total protein was estimated by the method of Lowry[12]. Total Bilurubin, Total Albumin also estimated.The activity of Catalase (CAT) superoxide dismutase (SOD) was determined in the tissue homogenate by the method of Sinha[13].

2.6 .Non-Enzymic Antioxidants

GSH,Vitamin C (Ascorbic acid) was measured according to the method of Omaye[14] Vitamin E in tissues was estimated by the method of Varley [15].

2.7. Estimation of Urea and creatinine

Urea was estimated by the method of Natelson [16]. Serum creatinine was carried out by alkaline picrate method [17].

2.8.Histopathological investigation

Liver slices were immersed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 for 8 h, then post fixation was carried out in 1% osmium tetroxide in the same cacodylate buffer for 2 h. Liver sections of 1 µm semi thin sections were cut, picked up on glass slides and stained with toluidine blue for light microscopic examination prior to the final examination Ultrathin sections were cut and picked up on (a formvar coated) 200 mesh copper grids. The ultrathin sections were stained with uranyl acetate for 30 min followed by lead for 15 min. The ultrathin sections were examined under a VEGA 3 Scanning electron microscope (JAPAN) at 10KV accelerating voltage [18].

2.9. Statistical Analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) SPPS-16 version.

RESULTS AND DISCUSSION

The structure of the ligand was drawn using tool Chembiodraw 11.0. in Structure data format and also converted into PDB format using Molecular conversion tool VCC lab online server all ligand was docked with ASGP-R receptor and images shown in Fig.1,interaction with pocket and hydrogen bonding shown in fig.2 and3.

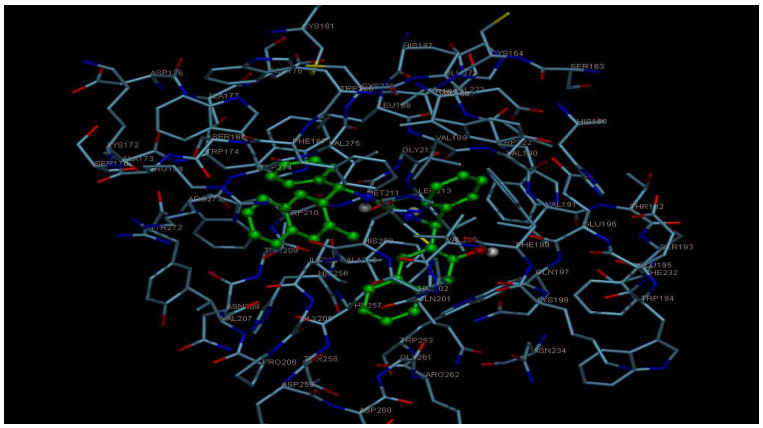


Figure-1
ASGP-R docked with 1, 3BPMU

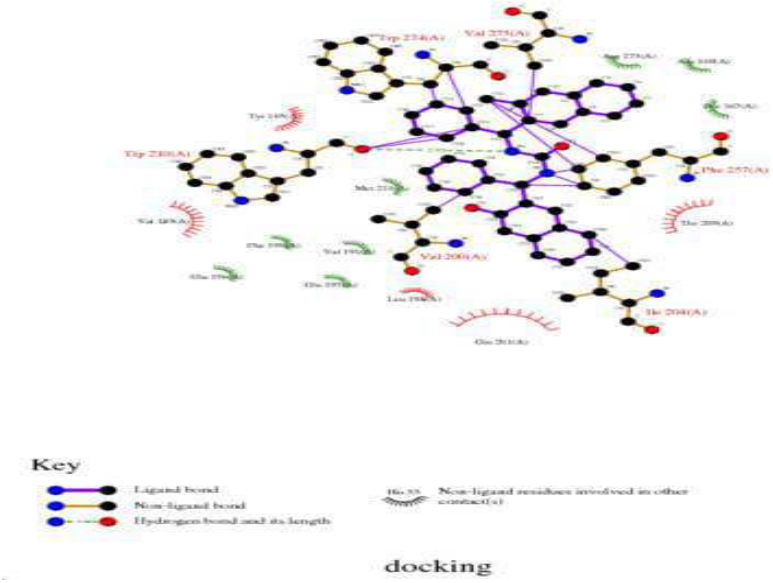


Figure 2
Interaction with pockets

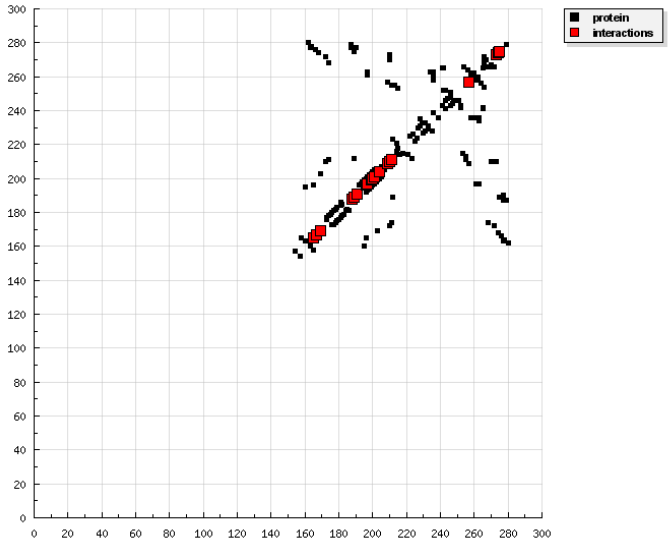


Figure 3
Hydrogen bonding plot

Table 1
Score value of 1,3 BPMU with Asialoglycoprotein (ASGP-R).

Ligand	Protein	Binding energy score
1,3BPMU	(ASGP-R)	+2.50kCal/Mol

The effect of 1, 3 BPMU on the contents of liver total bilurubin in control and experimental rats were presented in Table 2. The level of total bilurubin was found to be elevated in DEN induced rats. In the group IV rats administered with 1,3BPMU, the levels of total bilurubin were reduced when compared with DEN induced rats. There no significant changes were observed in 1,3BPMU alone treated rats when compared to control rats. The levels of liver protein, albumin, globulin ratio in the control and experimental rats were presented in Table 2. The liver total protein, albumin, globulin ratio was decreased in DEN induced rats. The altered levels of the protein, A/G ratio reversed to nearly normal level in rats treated with1,3 BPMU. However, no significant statistical changes were observed in rats treated with 1,3BPMUalone when compared to control rats. The total protein level, including albumin and globulin levels has been reported to decrease in hepatotoxic conditions due to defective protein biosynthesis in liver [19]. The DEN intoxication causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reduces the biosynthesis of protein. The treatment of 1,3BPMU well restored the protein synthesis by protecting the polyribosomes.

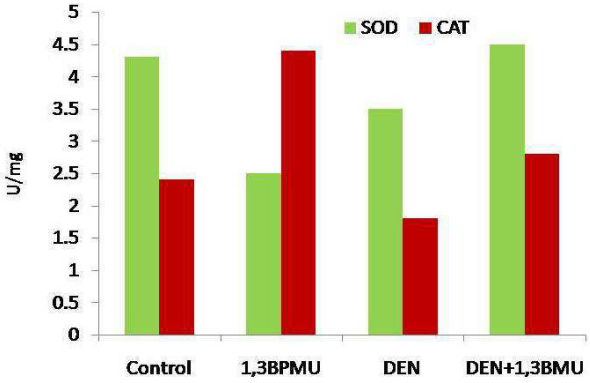
Table 2
Determination Of Liver Protein, Total Bilirubin Albumin,Globulin And Total Bilurubin Of control And Experimental Rats.

Group	Liver protein	Albumin	Globulin	Total Billurubin
Control	162.79 ±4.21	22.76 ±2.27	16.12±3.10	1.65±0.21
1,3BPMU	165.86 ± 5.08	24.24±2.23	19.64±2.92	1.78±0.03
DEN	142.39±2.81[a]	17.08±3.14[a]	14.12±4.31[a]	4.62±0.74[a]
DEN+ 1,3BPMU	161.76±4.58[a]	21.16±2.16[a]	18.64±2.13[a]	1.94±0.42[a]

Results are expressed as mean ± S.D for six rats in each group. Statistical significance at p <0.05. Activity is expressed as, mg/g wet tissue for liver and mg/ dl . [a]Comparisons are made with group1 (control). [b]Comparisons are made with group3 (DEN-induced group).

Figure 4

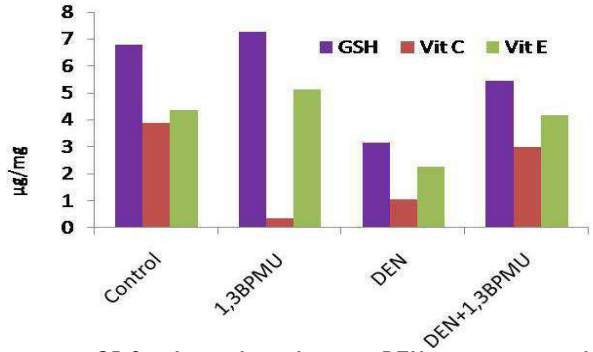
shows the effect of 1,3 BPMU on antioxidant enzymes activities. In the presestudy,changes in the activities of superoxide dismutase (SOD), Catalase (CAT), levels were investigated. DEN treatment reduced the superoxide dismutase and catalase activities as compared with control rats. However, 1,3 BPMU treatment significantly retain the antioxidant activities as compared with DEN induced rats. DEN alone treatment did not change the any antioxidant activities as compared with control rats.



*Each value is expressed as mean ± SD for six rats in each group. DEN induced group compared with control group and DEN+ 1,3BPMU compared with DEN group *p0<.05, SOD-50% of NBT reduction/min/mg protein in tissues; Catalase – µmole of H_2O_2 consumed/min/mg protein in tissues.*

Figure 5

shows the levels of liver non-enzymatic antioxidants. The content of the liver non-enzymatic antioxidants were analysed in control and experimental rats, it shows decreased level of GSH , Vit-C and Vit-E in DEN treated rats as compared with control rats. However, it was nearly normalized by the rats treated with1,3 BPMU as comapared with DEN treated rats. DEN alone treated rats did not shows significant changes in antioxidant level as compared with control rats.



Each value is expressed as mean ± SD for six rats in each group. DEN group compared with control group, DEN+1,3BPMU compared with DEN alone group p<0.001.Vitamin- C, Vitamin - E is formed µg/mg protein in tissues; GST- µmoles CDNB njugated/min/mg protein in tissues.

Urea and cretinine of control and experimental group of rats levels showed in Table 3 shows the urea and cretinine level in control and experimental group of rats. DENtreatment increased the urea and creatinine level as compared with control rats. However, 1,3 BPMU treatment significantly reduced the urea and creatinine level as compared with cancerous rats.

Table -3

Determination of urea and creatinine in control and experimental rats

Parameters	Control	1,3 BPMU	DEN	DEN+1,3 BPMU
Urea	30.42±2.13	44.57±3.30 [a]	33.28±2.33 [b]	34.71±2.42 [b]
Creatinine	0.69±0.04	0.98±0.06 [a]	0.68±0.03 [b]	0.73±0.05 [b]

*Each value is expressed as mean ± SD for six rats in each group. compared with group I, [b]As compared with group III. *p<0.01.*

The ultrastructural changes in hepatocytes of control and experimental rats are shown in Fig.6. A-D gives the clear view about changes in liver membranes by Scaning Electron Microscope (SEM) analysis. The results revelaed the liver membranes of control and 1,3 BPMU alone groups (Fig.6A and 6B) shows similar kind of architecture. However, damage and membrane deformation were found in DEN induced rats (Fig.6C). Conversely, 1,3 BPMU treatment significantly maintain the normal architecture of liver membrane as compared with DEN induced rats. (Fig.6D).

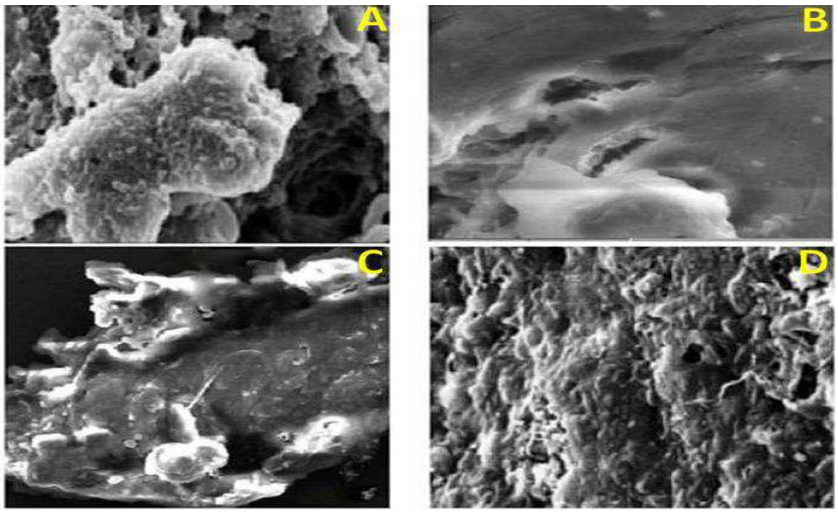


Figure 6

Scanning electron microscope examined liver section showed the morphological feature. Control (A) and 1,3BPMU treated liver (B). DEN induced rats - Arrow indicates the morphological changes in liver (C). DEN and 1,3BPMU treated rats (D).

DISCUSSION

Antioxidants are substances that either directly or indirectly protects cells against adverse effects of xenobiotics, drugs, carcinogens and toxic radical reactions [20]SOD is the primary step of defense mechanism in the antioxidant system against the oxidative stress. Deleterious effects of oxidants progression of neoplastic condition are affronted by primary antioxidants such as SOD and CAT . In a variety of malignancies, the elevated LPO is associated with reduced activity of antioxidants. Deficiency of SOD and CAT results in decreased detoxification of oxygen radicals, which leads to attack of ROS on protein and nucleic acids. the present study there was a significant depletion in the activities of enzymic antioxidants in liver of animals treated with DEN when compared to normal animals. The findings of this study signify that 1,3BPMU treatment to DEN induced rats were able to reverse the reduced activities of antioxidant status.Vitamin C, Vitamin E and reduced glutathione are well known non-enzymic antioxidant defense system of cells. These are interrelated with each other in the recycling process. Vitamin C is water soluble antioxidant and can react with radicals to regenerate vitamin E [21]. Vitamin E is chain breaking antioxidant present in the cell membrane. It provides protection against superoxides as well as H_2O_2 [22]GSH is the major cytosolic thiol compound and is required to maintain the normal reduced state of the cells and to counteract ROS, thereby reducing the oxidative stress [23]. GSH also preserves the cellular levels of active forms of vitamin C and vitamin E [24]. The levels of these non-enzymic antioxidants were decreased in hepatoma bearing animals [25]. The results of the present study also correlate with such findings. It might be due to over utilization of these antioxidants to scavenge free radicals. On the other hand, the simultaneous administration of 1,3BPMU reversed the changes induced by DEN

exposure to near normal, and supporting the hypothesis that 1,3BPMU is an effective chemotherapeutic agent.Kidney plays a vital role to excreated the metabolic wastes . Inour study we concentrated on the excretion of urea and creatinine in serum.The level of urea and creatinine is the blood rises if the kidney does not function properly [26]. Anticancer drugs has been altered the renal functions were reported by Wu [27]. In the present study also observed the increased level of urea and creatinine in DEN intoxicated rats. Supplementation of compound restored the increased level of urea and creatinine in DEN induced toxicity.

CONCLUSION

From the analysis of molecular docking, biochemical and histopathological studies on the possible effect of the synthesized mannich based 1, 3BPMU could be considered as a novel therapeutic drug for Hepato cellular carcinoma and development of drug for liver cancer in the future.

Conflict of Interest

Conflict of interest declared none

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