ISSN 2582 – 6050

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2020; 11(4):204-208

RESEARCH ARTICLE

Biochemical and Toxicological Investigations of 5-Fluorouracil, Nimesulide, and Ascorbic Acid in Hepatocellular Carcinoma

Mohd Asif[1], Nazim Hussain[2]*, Mokinur Rahman[3], Shubham J. Khairnar[4], Mithun Rudrapal[4]

[1]Department of Pharmacology, Siddhartha Institute of Pharmacy, Dehradun, Uttarakhand, India, [2]Department of Pharmacognosy, Kingston Imperial Institute of Technology and Sciences, Dehradun, Uttarakhand, India, [3]Department of Pharmaceutical Sciences, North East Frontier Technical University, Along, Arunachal Pradesh, India, [4]Department of Pharmacology and Pharmaceutical Chemistry, Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, Maharashtra, India

Received: 30 October 2020; Revised: 01 December 2020; Accepted: 25 December 2020

ABSTRACT

The objective of this study was biochemical and toxicological investigations of 5-fluorouracil (5-FU), nimesulide, and ascorbic acid (Vitamin C) in Wistar rats with hepatocellular carcinoma in. Results showed that DENA increased the level of alpha fetoprotein (AFP), alkaline phosphatase (ALP), serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), and total bilirubin which was decreased by the various combinations of 5-FU to normal. On the other hand, DENA resulted in decrease of blood glucose level, DFN decreased more than DF, and DFC showed results similar to DFN, while DFNC led to increased AFP, ALP, SGOT, SGPT, and total bilirubin levels to normal. Histopathological evaluations showed normal architecture of tissues of rat liver in normal group. Lesser damage of hepatocytes and low index of necrosis were in pre- and post-treated group of 5-FU+DF, DFN+DFC groups. DFNC treated group exhibited histological features resembling normal control animals.

Keywords: 5-Flurouracil, Ascorbic acid, DENA, Hepatocellular carcinoma, Hepatotoxicity, Nimesulide

INTRODUCTION

The liver is one of the biggest organs in the body. It has an open array of functions, with detoxifications, protein synthesis, and manufacture of biochemical is required for digestion. In humans, this is placed into right upper quadrant of the abdomen, under the diaphragm. Its extra roles into metabolism involve the regulation of glycogen storage, decomposition of red blood cells and the making of hormones.[1] Hepatocellular carcinoma (HCC) (liver cancer) is a cancer arising from the liver. It is also known as primary liver cancer or HCC. The liver is made up of different cell types (e.g., bile ducts, blood vessels, and fat-storing cells). However, liver cells (hepatocytes) make up 80% of the liver tissue. Thus, the majority of primary liver cancers (over 90–95%) arises from liver cells and is called hepatocellular cancer or carcinoma.[2] The liver is known as a favorable site for malignant seed second only to the skin, presumably because of its rich blood supply, liver is common site for the spread of malignant disease. Primary malignant hepatic tumors may arise from any constituent cells of the liver, but the only two common liver cell cancers are HCC and carcinoma of the biliary epithelium (Cholangiocarcinomas). Other rare tumors, namely, fibro lamellar carcinoma, squalors carcinoma, epithelial hemangio endothelium, Angiosarcoma, and Kaposi's sarcoma and his pat cellular adenoma may also arise from the liver.[3,4] The classification present is based on

***Corresponding Author:**
Nazim Hussain,
E-mail: nhussain116@gmail.com

the definitions and nomenclature recommended by the World Health Organization (WHO) (HCC Gastroenterology 2004) The HCC is single of the most general malignancies worldwide.[5-8] The annual global incidence is approximately 1 million cases, with a male to female ratio of approximately 4:1 (1:1 without cirrhosis to 9:1 in many high-incidence countries). According to the National Health Services UK, approximately 18000 people in the US die from HCC each year. The WHO estimates that liver cancer's prevalence is around 30 cases per 100000 people worldwide with rates in parts of Africa and Eastern Asia being particularly high. Expert says that common causes of HCC are regular high alcohol consumption, having unprotected sex; obesity associated liver disease (non-alcoholic steatohepatitis and injected drugs with shared needles).[8]

MATERIALS AND METHODS

Animals

Adult, healthy, and male Wistar rats weighing 100–125 g were procured from the animal house facility of Siddhartha Institute of Pharmacy, Dehradun, for the present protocol. Animals were housed separately in groups of 6 per cage (Perpex) under laboratory conditions recommended by CPSCEA in controlled central animal house facility with light and dark cycle of 12 h each. The animals were acclimatized for 5 days before behavioral studies. All experiments were carried out during day time between 09:00 and 16:00 h. All animals had proper access to standard food and water. Study protocol was approved by the Institutional Animal Ethics Committee Siddhartha Institute of Pharmacy, Dehradun. Normal and healthy animals weighing between 150 and 250 g for rats were included in the study. The Wistar rats which do not fall the above mentioned weights were excluded from the study.

Experimental design

In the present study, six groups of animals were prepared and in five groups there were six animals and in one group there were ten animals. All groups of animals will receive treatment for 16 weeks. Forty animals were divided into six groups. Each group had siz animals but Group DC had ten animals.

Group I

Negative control (NC) rats will be administered with normal diet/normal saline and were not given any treatment during research.

Group II

(Positive Control) rats will be exposed to a single dose of DENA (200 mg/kg i.p). Four animals from this group will be sacrifice at termination of the initiation phase, that is, in the 4th week (to identify the histological alteration in hepatic architecture).

Group III

DF rats will be exposed to a single dose of DENA (200 mg/kg i.p) and then from the 4th week they will be treated with 5-fluorouracil (5-FU) (100 mg/kg b.wt i.p) every 3rd day.

Group IV

DFC rats will be exposed to a single dose of DENA (200 mg/kg i.p) and then from the 4th week they will be treated with the combination of 5-FU (100 mg/kg b.wt i.p) every 3rd day and Vitamin C (500 mg/kg per oral) every day.

Group V

DFN rats will be exposed to a single dose of DENA (200 mg/kg i.p) and then from the 4th week they will be treated with combination of 5-FU (100 mg/kg b.wt i.p.) every 3rd day and nimesulide (75 mg/kg b.wt/oral) every day.

Group VI

DFNC rats will be exposed to a single dose of DENA (200 mg/kg i.p.) and then from the 4th week they will be treated with combination of 5-FU (100 mg/kg b.wt, i.p.) every 3rd day,

nimesulide (75 mg/kg b.wt, per oral) and Vitamin C (500 mg/kg, per oral) every day.

Biochemical estimations

The body weight (g) will be recorded on day 1 and then on alternate days in each group. The following serum parameters were examined. Serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), high-density lipoprotein, total cholesterol, triglycerides, uric acid, urea, bilirubin, total bilirubin, direct bilirubin, and alpha fetoprotein (AFP).[9] The following hematological parameters are examined: Hemoglobin, total leukocyte count (TLC), differential leukocyte count (DLC), and erythrocyte sedimentation rate, whole blood will be collected from the animals into ethylenediaminetetraacetic acid bottles and assayed for TLC and DLC using standard laboratory technique.[10]

Histopathology

After sacrifice, livers of different group animals will be collected and preserved in phosphate buffered 10% formalin, embedded in paraffin and were used for histopathological examination. Sections will be cut (5 μm thick), deparaffinized, hydrated, and stained with hematoxylin and eosin. The renal sections will be examined blindly for tubular cell swelling, interstitial edema, tubular dilatation, and to determine moderate to severe necrosis in all treatments.[11]

Statistical analysis

All values were expressed as Mean ± standard error of the mean (SEM) from six animals in five group and ten animals were in last six group. Results were subjected to statistical analysis using one-way analysis of variance (ANOVA), followed by Newman-Keuls Multiple Comparison test set at a significant level of $P \leq 0.05$ to compare the mean values of the different groups to identify the effect of the treatment on the biochemical markers, and comparison between treatment groups, respectively. $P < 0.05$ will be considered as statistically significant. Experimental data were calculated from three independent triplicate experiments. Results presents as the mean values of the chosen triplicate groups. The experimental data were showed as the mean with standard deviations. Results were expressed as mean ± SEM.

RESULTS AND DISCUSSION

HCC, one of the most lethal cancers, results in >1 million deaths worldwide per year.[8] DENA induced hepatocellular damage is clearly evidenced by the marked elevation in serum SGPT, SGOT, ALP, and decrease level of glucose in the liver tissue, these biochemical marker enzymes are indicators of tumor response. SGPT, SGOT, ALP, and decrease level of glucose serves as a marker of liver damage and liver is associated with changes in lipid parameters and oxidative status. Alterations in lipid profiles in malignant tissue are of importance due to the effect on membrane integrity, fluidity, and regulation of cellular processes related to growth and cell survival.[9,10] AFP is a serum protein has higher specificity for hepatocarcinoma and detected in elevated concentration in HCC. AFP is a serum protein similar in size, structure, and amino acid composition to serum albumin, but it is detectable only in minute amounts in the serum of normal adults. Elevated serum concentrations of this protein can be achieved in the adult by exposure to hepatocarcinogenic agents. Its serum concentration confirms hepatocarcinoma and for the diagnosis of tumor response to therapy. The normal organization of the hepatic lobules found in the livers from rats in Group 1 NC and Group 6 (DFNC control). Liver sections from the DENA-treated rats in Groups 2 (DENA only) and 3, 4, 5 (prophylactic groups) exhibited morphological characteristics of HCC these included disorganized hepatic parenchyma represented by thick cords (trabeculae) of polyhedral cells bordering wide sinusoids together with some pseudoacini. In contrast, sections from rats in Group 4 (therapeutic group) did not show such disorganization, despite showing some degenerative changes. Our findings are similar to those obtained from other studies in animal models with hepatocarcinogenicity

induced by DENA. Results showed that DENA increased the level of AFP, ALP, SGOT, SGPT, and total bilirubin which was decreased by the various combinations of 5-FU to normal. On the other hand, DENA resulted in decrease of blood glucose level, DFN decreases more than DF, and DFC showed results similar to DFN, while DFNC led to increased AFP, ALP, SGOT, SGPT, and total bilirubin levels to normal [Figure 1].

In the histopathological study, liver sections of the normal control group showed normal liver histology with unremarkable central veins, no evidence of hepatocytes injury or fibrosis or dysplasia or malignancy noticed1.[11,12] Disease control animals

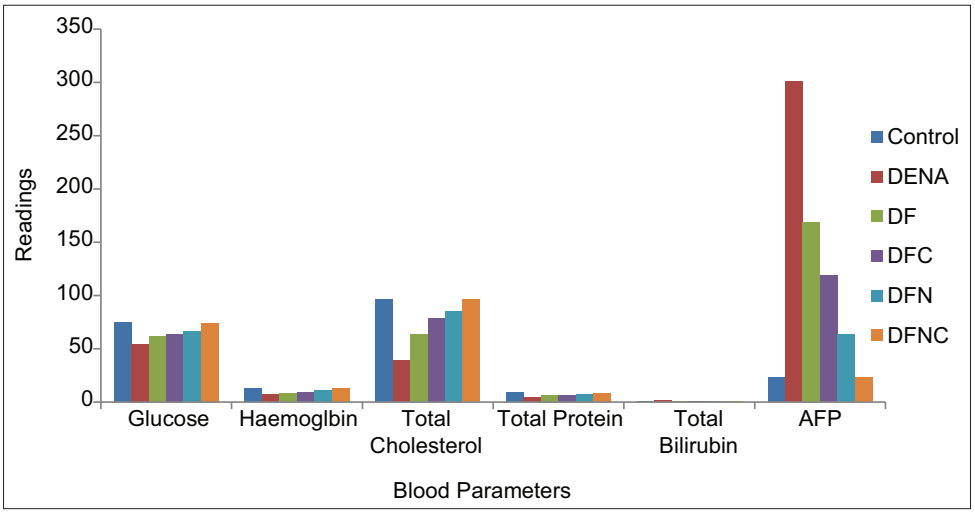

Figure 1: Blood parameters with DENA profile and its treatments

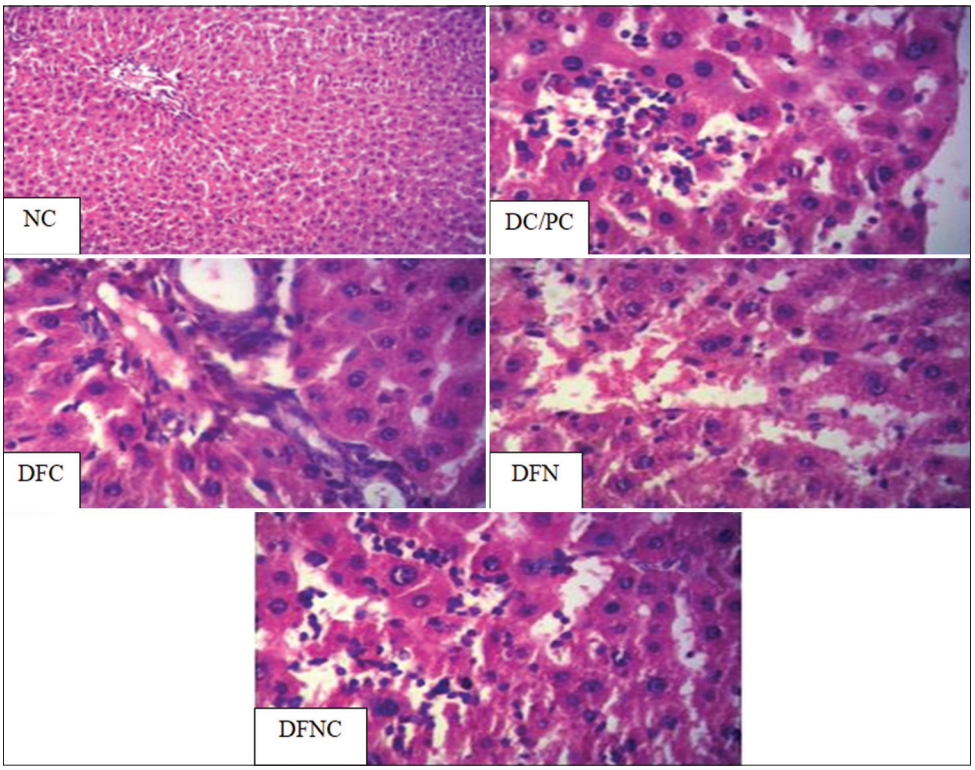

Figure 2: Photomicrographs (original magnification 45×) of Histopathological studies of livers of various groups. NC group showed rat liver hepatocytes and positive control showed necrosis and hepatocellular degeneration in positive control (DC) group. Treated groups (5-FU+DFN+DFC+DFNC) showed lesser damage of hepatocyte and low index of necrosis as compare to positive control group

showed central veins surrounded by extensive necrosis and inflammatory infiltrate, clusters of hepatocytes necrosis and the portal tract with bile duct proliferation and marked atypical. The tumor cells resembling hepatocytes show pleomorphism and were seen 2–8 cell, wide trabeculae which are separated by endothelium lined sinusoidal spaces. The prophylactic group showed that periportal inflammation with conspicuously dilated blood vessels and ballooning degeneration mononuclear infiltrates associated with regenerative cellular changes of the adjacent hepatocytes, mild bile duct proliferation, and intra-acinar inflammatory cell infiltrates was observed. Liver section from DFNC group shows the normal architecture of the liver, no necrosis was observed. Lesser damage of hepatocytes and low index of necrosis pre- and post-treated group of 5-FU+DF, DFN+DFC group's result like normal control in DFNC treated group [Figure 2].[13]

CONCLUSION

The present study reports that significant hepatoprotective effect of various combinations of 5-FU in DENA-induced hepatotoxicity in HCC. Various combinations of 5-FU such as DFN, DFC, and DFNC can be used as prophylactic and/or therapeutic measure in HCC.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Eder-Czembirek C, Czembirek C, Erovic BM, Selzer E, Turhani D, Vomitting L, *et al*. Combination of betulinic acid with cisplatin--different cytotoxic effects in two head and neck cancer cell lines. Oncol Rep 2005;14:667-71.
2. Cullen KJ, Yang Z, Schumacher L, Guo Z. Mitochondria as a critical target of the chemotheraputic agent cisplatin in head and neck cancer. J Bioenerg Biomembr 2007;39:43-50.
3. Barac A, Mitulovic G, Hallström S, Zehetmayer S, Grasl MC, Erovic BM. Impact of combined treatment with nimesulide and cisplatin on oral carcinoma cells. Onco Targets Ther 2017 10:3607-16.
4. Liver Function Tests. Available from: https://www.vdocuments.site/documents/small-animal-radiology-and-ultrasound-a-diagnostic-atlas-and-text.html. [Last accessed on 2020 Nov 21].
5. Yoneda K, Yamamoto T, Osaki T. p53-and p21-independent apoptosis of squamous cell carcinoma cells induced by 5-fluorouracil and radiation. Oral Oncol 1998;34:529-37.
6. Nimesulide. Available from: https://www.1mg.com/generics/nimesulide-2103. [Last accessed on 2020 Dec 28].
7. Imawari M. liver cancer-prevention and early diagnosis. Jpn Med Assoc J 2002;45:130-3.
8. Acharya SK. Epidemiology of hepatocellular carcinoma in India. J Clin Exp Hepatol 2014;4 Suppl 3:S27-33.
9. European Association for the Study of the Liver, European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: Management of hepatocellular carcinoma. J Hepatol 2012;56:908-43.
10. Bagi CM, Andresen CJ. Models of hepatocellular carcinoma and biomarker strategy. Cancers (Basel) 2010;2:1441-52.
11. Mo Kim B, Won J, Ah Maeng K, Han YS, Yun YS, Hong SH. Nimesulide, a selective COX-2 inhibitor, acts synergistically with ionizing radiation against A549 human lung cancer cells through the activation of caspase-8 and caspase-3. Int J Oncol 2009;34:1467-73.
12. Lv H, Wang C, Fang T, Li T, Lv G, Han Q, *et al*. Vitamin C preferentially kills cancer stem cells in hepatocellular carcinoma via SVCT-2. NPJ Precis Oncol 2018;2:1.
13. Nadia RA, Zeid A. Ameliorative effect of Vitamin C against 5-fuorouracil-induced hepatotoxicity in mice: A light and electron microscope study. J Basic Appl Zool 2014;67:109-18.