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Pharmacological Evaluation of Gatifloxacin in Chemically Induced Hepatocarcinogenesis: A New Tool for Hepatocellularcarcinoma Treatment

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Abstract

Introduction: Hepatocellular carcinoma (HCC) is a major cause of cancer death worldwide. In this study Gatifloxacin an antimicrobial drug which has been banned due to some serious adverse effects evaluated for the other pharmacological activities. Data from the present investigation suggest that Gatifloxacin suppresses the tumors and decrease the biochemical markers which are elevated in HCC.

Objective: This study is an attempt to evaluate the potential chemopreventive influence of Gatifloxacin in hepatocarcinogenic rats. Hepatocarcinogeneis was induced by a single intraperitoneal injection of diethylnitrosamine (DENA) in phosphate buffer (200 mg/kg).

Results and conclusion: Experimental animals exposed to DENA caused a significant alteration in serum indices of liver enzymes glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), acid phophatase (AP), total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL), total proteins (TPR) and blood glucose level in tested animals. Results also revealed severe histological and immunohistochemical changes in hepatic tissues. These included disorganized hepatic parenchyma, appearance of pseudoacinar and trabecular arrays of hepatocytes and alterations in alpha fetoprotein (AFP) levels. Administration of Gatifloxacin relatively improved the biochemical parameters to values approximating those of the normal controls. SGOT, SGPT, ALP, level was significantly decreased ($p<0.001$, $p<0.01$, $p<0.001$ respectively) in therapeutic control while TPR, ALB level was significantly increased ($p<0.001$, $p<0.001$ respectively) and delayed the initiation of carcinogenesis. Rats treated with Gatifloxacin only showed altered levels of liver enzymes, arrhythmic symptoms and abdominal fat deposition. In conclusion, DENA significantly changes the biological enzymatic activities in serum and the integrity of hepatic tissues. Hence, Gatifloxacin proved to possess the potential for the treatment of hepatocellularcarcinomas in rats exposed to DENA.

Keywords: Gatifloxacin; DENA; Hepatocellular carcinoma; Histology; Carcinogen

Introduction

Primary human liver cancer, of which hepatocellular carcinoma (HCC) is the predominant type, is a major cause of cancer death worldwide [1], accounts for about 90% of all cases of liver cancer and is the fourth most common cause of cancer mortality [2]. In 2009 liver cancer incidence in lower middle income countries accounts 10.2% of the total health care cost while in upper middle countries liver cancer resulting 1.9% and in high income countries the number of liver incidence which is accounting 2.7% of economic burden [3].

Nitrosamines are a source of considerable concern due to their potential mutagenesis, carcinogenic and teratogenic influences. Primary sources of human exposure to nitrosamines are agricultural, pharmaceutical and tobacco products, cosmetics and food preservatives [4]. Diethylnitrosamine (DENA) is one of the most frequently used chemical to induce hepatic-carcinogenesis in animals [5], possibly by inducing burst release of reactive oxygen species and cellular injury with the enhanced formation of detrimental free radicals [6]. DENA is metabolized to its active ethyl radical, which interacts with DNA causing mutation and subsequent oncogenesis [7].

Gatifloxacin is a broad-spectrum fluoroquinolone designed for both oral and intravenous administration. Fluoroquinolones act by inhibiting the activity of both the DNA gyrase and the topoisomerase IV enzymes. For most gram negative bacteria, DNA gyrase is the primary fluoroquinolone target. With some exceptions, topoisomerase IV is the primary target of fluoroquinolone action in most of the gram positive bacteria such as Staphylococci and Streptococci, with DNA gyrase being a secondary target. Some serious side effects of Gatifloxacin like diabetes have been reported since 2006. Some other side effects reported are hallucination, liver damage, purpura, to name a few. A relationship between prescriptions of Tegaserod and increased risks of heart attack or stroke has been established in recent past Union Health and Family Welfare Ministry of India on 18 March 2011 banned the manufacture, sale and distribution of Gatifloxacin as it caused certain adverse side effects [8]. Various researchers have explored the uses of Gatifloxacin

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by undergoing various studies on it, and trying to bring Gatifloxacin back to the market by reducing its side effects and exploring new uses of Gatifloxacin which may reduce economic burden of it as well.

Materials and Methods

Drugs and chemicals

Gatifloxacin was provided as a gift sample from Elder Pharmaceuticals Pvt Ltd, Dehradun, Diethylnitrosamine (DENA) was procured from Sigma-Aldrich Chemicals Co. St. Louis, USA and Chloroform and Diethyl ether from S.D. Fine Chem. Ltd. Mumbai. All the chemicals were of analytical grade.

Animals

Adult, healthy, experimental Wistar albino rats weighing 100-125 g was procured from the animal house facility of Siddhartha Institute of Pharmacy for these experiments. The rats were housed in groups in polypropylene cages under controlled conditions of temperature (22°C+3°C) and light (14:10h light and dark cycle) and provided balanced pallet diet and water ad libitum. The Protocol was approved by the Institutional Animal Ethical Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA); Ministry of Social Justice and Empowerment, Government of India and taken for conducting research studies.

Induction of hepatocarcinoma

Liver cancer was induced by a carcinogenic dose of 200 mg/kg body weight, I.P. Diethyl nitrosamine (DENA) when associated with fasting/refeeding [9,10].

Experimental Design

Rats were divided into five groups (n=6), Group-I rats served as normal control and were treated with vehicle orally. Group-II rats were administered a single dose of DENA (200 mg/kg, i.p.) Group-III rats were administered DENA (200 mg/kg i.p.) on day 7 followed by Gatifloxacin (80 mg/kg) from day 1. Group-IV rats were treated with Gatifloxacin (80 mg/kg) alone from day 7 given DENA dose. Group-V rats were treated with Gatifloxacin (80 mg/kg) as control Gatifloxacin. The dose of Gatifloxacin was selected by performing an effective dose fixation study. At the end of the experimental period, animals were subjected to ether anesthesia, blood was collected from retro orbital plexus and serum was separated by centrifugation.

Estimation of biochemical parameters

Blood samples were collected on the termination day of the experiment from the retro-orbital plexus under light ether anesthesia and allowed to stand for 30 minutes at room temperature, centrifuged at 2500 rpm for 10 minutes to separate the serum. The serum obtained was kept at 2-4°C for further use. The serum blood glucose, SGOT, SGPT, ALP, GGTP, TC, TG, HDL, TPR, TBR and ALB were performed using a standard kit (Nicholas India Pvt. Ltd.) with semi-auto analyzer (photometer 5010, Nicholas India Pvt. Ltd). Serum α-feto protein (AFP) was also estimated [11].

Histopathological examination

The livers were preserved in phosphate-buffered 10% formalin, embedded in paraffin and used for histopathological examination. Then, 5 µm-thick sections were cut, deparaffinized, hydrated and stained with haematoxylin and eosin. The sections were examined blindly for structural alterations like tubular cell swelling, interstitial edema, tubular dilatation, and moderate to severe necrosis in all treatments.

Statistical analysis

The results were expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparison test or an unpaired two-tailed student's t-test as appropriate using computer based fitness program (Prism, Graphpad). Differences were considered to be statistically significant ($p<0.05$).

Results

Animal weight

In the disease group body weight was reduced significantly as compared to a normal control group. In prophylactic group, the body weight decreased when compared to disease control group. While in the Gatifloxacin control group there were no significant alterations in the body weight when compared to normal controls (Table 1).

Total protein (TPR)

DENA control animals exhibited significantly decreased ($p<0.001$) TPR levels as compared to a normal control group. Prophylactic group slightly while therapeutic group significantly increased ($p<0.001$) TPR levels when compared to the DENA control group. Gatifloxacin control group exhibited significantly decreased ($p<0.001$) TPR levels as compared to a normal control group (Table 1).

Blood glucose

Acute effect (hyperglycemia) and chronic effect (hypoglycemia): The DENA control animals showed significantly decreased ($p<0.001$) glucose levels than normal controls. The blood glucose level in the prophylactic and therapeutic group was significantly altered ($p<0.001$) when compared to the disease control group. No significant decrease ($p<0.001$) in glucose level of Gatifloxacin treated groups (both prophylactic and therapeutic was observed when compared to disease controls. Gatifloxacin control group had also exhibited the significantly decreased ($p<0.01$) blood glucose level as compared to a normal control group (Table 1).

Serum α-feto protein (AFP)

The level of AFP in serum was significantly ($p<0.01$) increased in DENA treated rats when compared to normal control, whereas their levels remained near the control in animals treated with Gatifloxacin (Table 1). Treatment of Gatifloxacin restored the level of AFP better in therapeutic group as compared to disease controls.

Liver profile study

Serum glutamate pyruvate transaminase (SGPT/ALT): The DENA control group exhibited significantly elevated ($p<0.01$) level of SGPT as compared to a normal control group, while prophylactic and therapeutic group showed significantly decreased ($p<0.001$ and $p<0.01$ respectively) SGPT levels as compared to the DENA control group. Gatifloxacin control group showed the significantly decreased ($p<0.01$) in SGPT level as compared to normal controls (Table 2).

Serum glutamate oxaloacetate transaminase (SGOT/AST): SGOT level was significantly increased ($p<0.001$) in DENA control as compared to a normal control group. The prophylactic and therapeutic

group exhibited significantly decreased ($p<0.01$ and $p<0.001$ respectively) SGOT levels when compared to the DENA control group. Gatifloxacin control group exhibited the significantly decreased ($p<0.001$) SGOT level as compared to a normal control group (Table 2).

Serum alkaline phosphatase (SALP): Prophylactic and therapeutic group exhibits a significant decrease in ($p<0.001$) SALP levels as compared to disease controls (Table 2).

Gamma-glutamyltranspeptidase (GGTP)

Disease controls did not show any significant alteration in the GGTP level as compared to normal control. In prophylactic group GGTP level was significantly increased ($p<0.001$) when compared to DENA control animals. There were no significant changes in GGTP level in the Gatifloxacin control group as compared to a normal control group (Table 2).

Total bilirubin (TBR)

There was no significant alteration found in the TBR levels in all groups.

Albumin (ALB)

In DENA control group the ALB level was significantly decreased ($p<0.01$) as compared to normal control where as prophylactic and therapeutic treatment with Gatifloxacin significantly increased ($p<0.001$) ALB levels. In the Gatifloxacin control group the ALB level was significantly decreased ($p<0.001$) as compared to a normal control group (Table 2).

Lipid profile

Significant elevation in the levels of TC and TG and reduction in HDL was observed in the animals exposed to DENA when compared to a normal control group. These parameters were maintained to normal by the treatment of Gatifloxacin in both the prophylactic, therapeutic and Gatifloxacin control groups (Table 3).

Histopathological study

Liver sections of the normal control group showed normal liver histology with unremarkable central veins, no evidence of hepatocyte injury or fibrosis or dysplasia or malignancy noticed. Disease control animals showed central veins surrounded by extensive necrosis and inflammatory infiltrate, clusters of hepatocyte necrosis and the portal tract with bile duct proliferation and marked atypia. 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 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 Gatifloxacin control group shows the normal architecture of the liver, no necrosis was observed (Figure 1).

Discussion

Hepatocellular carcinoma (HCC), one of the most lethal cancers, results in >1 million deaths worldwide per year. DENA is reported to be hepatotoxin and the hepatocarcinogenic agent [12]. In the present study, DENA induced hepatocellular damage is clearly evidenced by the marked elevation in serum SGPT, SGOT, GGTP, ALP and decrease level of glucose in the liver tissue, These biochemical marker enzymes are indicators of tumor response [13]. SGPT, SGOT, GGTP, ALP and decrease level of glucose serves as a marker of liver damage and

S.No.	Groups	Body weight (g)	Total protein (mg/dl)	Blood glucose (mg/dl)	AFP (ng/dl)
1	Vehicle control	135.23 ± 2.52	8.10 ± 0.19	76.14 ± 0.86	26.45 ± 0.89
2	DENA control	121.68 ± 2.65	4.06 ± 0.26***	52.35 ± 1.44***	302.03 ± 12.14**
4	PGTG	126.76 ± 2.12	6.44 ± 0.36**	31.36 ± 0.79***	63.68 ± 1.44***
3	GTG	083.60 ±1.66	6.34 ± 0.29***	33.12 ± 0.42***	41.45 ± 1.04***
5	Gatifloxacin	076.88 ±1.09	6.56 ± 0.45***	48.38 ± 1.67**	20.48 ± 0.17 **

Values are expressed as Mean ± SEM, (N=6); Values are expressed as Mean ± SEM, (N=6); * Significance level: *p<0.05, **p<0.01 and ***p<0.001 as compared to DENA control. PGTG=Prophylectic gatifloxacin treatment group and GTG=Gatifloxacin therapeutic group

Table 1: Effect of gatifloxacin on body weights, total proteins and blood glucose of animals.

S.No.	Treatment	SGPT (mg/dl)	SGOT (mg/dl)	ALP (mg/dl)	GGPT (mg/dl)	Total bilurubin (mg/dl)	ALB (mg/dl)
1	Vehicle control	49.99 ± 046	55.23 ± 1.04	06.13 ± 0.46	1.75 ± 0.11	0.61 ± 0.06	3.89 ± 0.23
2	DENA control	76.11 ± 1.29**	75.24 ± 1.61***	9.78 ± 0.56***	2.67 ± 0.11 ***	1.49 ± 0.65 **	2.22 ±0.09**
4	PGTG	72.23 ± 0.89 ***	49.36 ± 0.85**	06.13 ± 0.37***	1.59 ± 0.23 **	0.45 ± 0.11***	3.25 ± 0.14***
3	GTG	99.68 ± 1.25**	64.11 ± 1.33***	06.46 ± 0.71***	1.56 ± 0.56**	0.46 ± 0.19 ***	3.21 ± 0.09***
5	Gatifloxacin	40.51 ± 1.45**	39.26 ± 1.19***	03.77 ± 0.64***	0.72 ± 0.07 **	0.25 ± 0.24**	3.71 ± 0.32***

Values are expressed as Mean ± SEM, (N=6); * Significance level: *p<0.05, **p<0.01 and ***p<0.001 as compared to DENA control.

Table 2: Effect of gatifloxacin on serum SGOT, SGPT, ALP, GGTP and TB of animals.

S.No.	Groups	TC	TG	HDL
		(mg/dl)	(mg/dl)	(mg/dl)
1	Vehicle control	098.14 ± 1.35	39.68 ± 0.88	32.62 ± 0.56
2	DENA control	041.11± 0.24**	99.55 ± 1.36***	11.09 ± 0.68 **
3	GTG	109.25 ± 1.37**	58.81 ± 2.42***	04.22 ± 1.08**
4	PGTG	038.38 ± 0.89**	86.01 ± 1.98***	14.05 ± 1.66***
5	Gatifloxacin	035.12 ± 0.63***	94.27 ± 1.09**	20.18 ± 1.92***

Values are expressed as Mean ± SEM, (N=6); * Significance level: *p<0.05, **p<0.01 and ***p<0.001 as compared to DENA control.

Table 3: Effect of gatifloxacin on serum lipid profile.

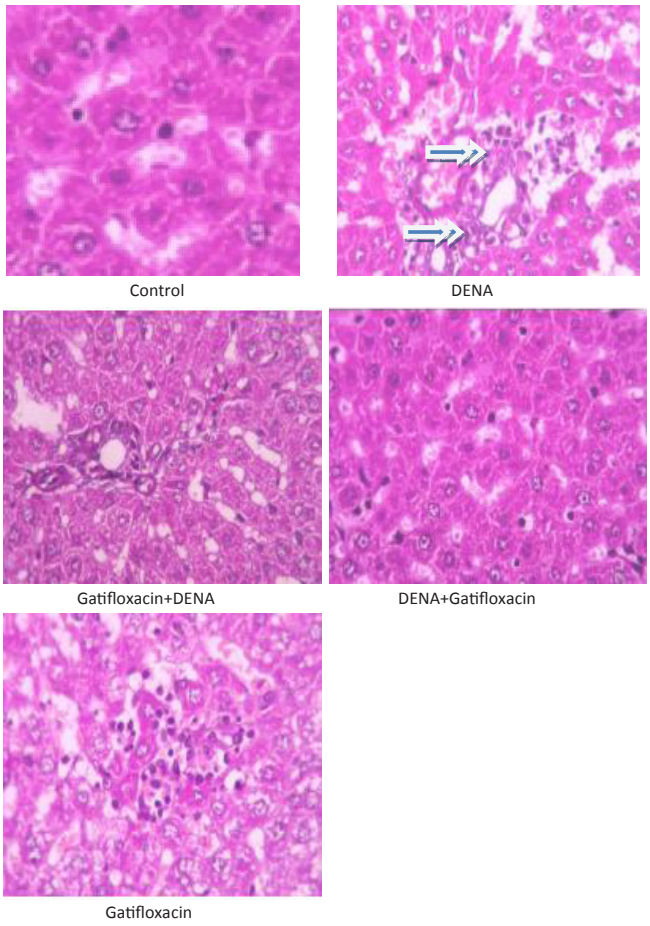

Figure 1: Photomicrographs (original magnification 45×) of histopathological studies of livers of various groups. normal architecture of rat liver (normal group). Necrosis and hepatocellular degeneration of DENA (DENA+Gatifloxacin and Gatifloxacin+DENA). Lesser damage of hepatocytes and low index of necrosis pre and post-treated group control Gatifloxacin (80 mg/kg) treated group.

mechanisms of neoplastic process. It has been studied that serum GGTP and ALP levels increases linearly with tumor mass [14]. Serum GGTP levels increased linearly with increases in small tumor mass, ALP levels elevated in association with small tumors and further increases with increasing tumor mass [15]. ALP is used as a specific tumor marker during diagnosis in the early detection of cancer. It is well established that (ALT) level signifies the presence of active disease and increases risk, particularly if the ALT is persistently or intermittently elevated over the years [16].

Gatifloxacin at a dose of 125 mg/kg restored the level of ALP, SGPT, SGOT and GGTP. An increase GGTP activity found in the preneoplastic foci that enhances cell proliferation and increase tumor promotion [17]. The GGTP level elevated significantly in the disease control group as compared to Normal control. These results established the role of Gatifloxacin as a chemopreventive agent in DENA induced HCC.

The development of HCC has also been associated with disorders in plasma lipid and lipoprotein metabolism [18]. Cancer development is associated with alterations in lipid metabolism, affecting cellular function and growth. The development of hepatocyte nodules in rat liver is associated with changes in lipid parameters and oxidative status [19]. 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 [20,21].

The increase in cholesterol level increases the membrane fluidity, regulates membrane permeability and alters internal viscosity and also the internal chemical composition. In the present research it was observed that Gatifloxacin maintained the lipid profile, hence it can be suggested that Gatifloxacin may play the role in inhibition of carcinoma progression.

AFP is a serum protein has higher specificity for hepatocarcinoma and detected in elevated concentration in hepatocellular carcinoma [22,23]. 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 [24]. Its serum concentration confirms hepatocarcinoma and for the diagnosis of tumor response to therapy.

In the present study, serum AFP level of DENA treated rats showed a significant increase compared to that of control group, proving the occurrence of premalignant liver changes in DENA treated rats. The elevation of serum AFP in HCC was well documented [25,26]. Treatment with Gatifloxacin significantly reduced serum AFP.

Phenotypically altered hepatocyte populations including persistent nodules (PNs) were found scattered in the livers of DENA exposed groups (i.e., Groups 2 and 5); but no such alterations were noticeable in untreated normal control (Group 1) or in the Gatifloxacin control group (Group 3).

Unlike the normal organization of the hepatic lobules found in the livers from rats in Group 1 (normal control) and Group 3 (Gatifloxacin control), liver sections from the DENA-treated rats in Groups 2 (DENA only) and 5 (Prophylactic group) 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 hepatocarcinogenity induced by DENA [7].

Chemopreventive activities of Gatifloxacin may be ascribed to its molecular modeling of the complex formed between Gatifloxacin and DNA, presented the full ability of the drug for participating in the formation of a stable intercalation site. The properties of the isolated intercalator and its stacking interactions with the adeninethymine (AT) and guaninecytosine (GC) nucleic acid base pairs, were studied previously using the DFTB method and structure changes in base pairs showed that GTFX is effective on DNA special for GC. Thus, revealing that Gatifloxacin effectively reacts with DNA and more prominently in rapidly dividing cell and hence it could be beneficial for the treatment of HCC [27]. This binding, specifically to the complex of DNA gyrase/Topoisomerase enzyme and DNA appears to stabilize the enzyme-DNA complexes which in turn results in breaks in the DNA that may be fatal to the cancerous cell.

Conclusion

Gatifloxacin an antimicrobial drug which has been banned due to some serious adverse effects globally can be evaluated for the

other pharmacological activities. Data from the present investigation suggest that Gatifloxacin possesses potential chemopreventive action at reduced dose (125 mg/kg) suppress the tumors and decrease the biochemical marker which are elevated in HCC. This will open new perspectives that Gatifloxacin is a chemopreventive compound to prevent, slow or treat the occurrence of liver cancer.

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