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Evaluation of Anti Diabetic Drug Alogliptin for the Treatment of Cancer in Rats

Mohd. Fasih Ahmad[1*] and Dr. D. J. Mani Babu[2]

[1]Sunrise University, Bagad Rajput, Alwar - 301030 (Rajasthan)
Contact No. +91-7838824636 E-mail: fasih435@gmail.com
[2]Hindu College of Pharmacy, Guntur, AP-522002

ABSTRACT

Alogliptin in which variety of pharmacological features are abundant. However, to date anti-cancer activities of this Drug have not been reported. The properties of dipeptidyl peptidase 4 inhibitors (DPP-4) reported by the researchers to opt for the assessment of anti-cancer activities in various experimental animal models. In the present Evaluation of dipeptidyl peptidase 4 inhibitors (DPP-4) opt for the assessment of anti-cancer activities in experimental animal models. Effect of *Alogliptin* on serum biochemical parameters with the Treatment of *Alogliptin* (low, medium and high dose) exhibited a significant anti cancer activity by reducing the level in ALP, AST, ALT, AFP and GLU in DENA induced rats. Liver specimen histology from *Alogliptin* low dose showed minor disorganization and sinusoidal congestions, whereas liver from rat of *Alogliptin* medium and high dose displayed very less damage of hepatocellular organization and low index of necrosis damage. The *Alogliptin* treatment could change those altered parameters to near normal.

KEY WORDS: *Alogliptin*, Anti-Cancer, Anti-diabetics, DPP-4, Hepatocellular carcinoma, Diethylnitrosamine (DENA)

***Corresponding author**

Mohd Fasih Ahmad

Department of Pharmacy

Sunrise University, Bagad Rajput, Alwar - 301030 (Rajasthan), India

Mob No. +91-7838824636 Email: fasih435@gmail.com

INTRODUCTION

In the present Evaluation, we have selected a *Alogliptin* (Anti diabetic drug) in which variety of pharmacological features are abundant. However, to date anti-cancer activities of this Drug have not been reported. Its medicinal properties of dipeptidyl peptidase 4 inhibitors (DPP-4) reported by the researchers to opt for the assessment of anti-cancer activities in experimental animal models.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are novel oral antihyperglycemic agents for treating type 2 diabetes mellitus patients. Recent studies suggest that several DPP-4 inhibitors exert suppressing inflammatory reactions. However, whether or not DPP-4 inhibitors used as anti-cancer drug. *Alogliptin* (2-({6-[(3R)-3-aminopiperidinyl-1-yl]-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)yl} methyl) benzonitrile monobenzoate) (AGP) is a selective DPP-4 inhibitor that has improves glycemic control. However, it remains unknown whether AGP has anti-cancer. DPP4 was first discovered by Hopsu-Havu and Glenner in 1966[1-8].

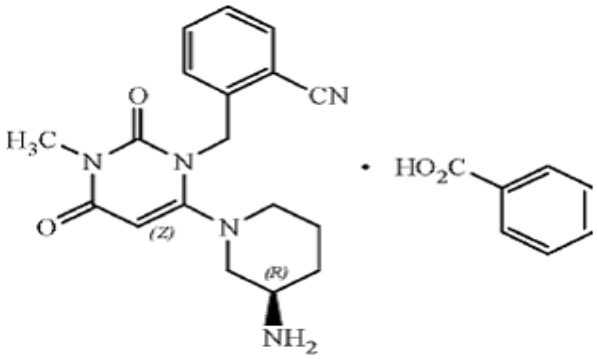
Alogliptin is an oral drug that reduces blood sugar (glucose) levels in patients with type 2 diabetes. *Alogliptin* increased concentrations of the incretin hormones such as glucagon-likepeptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released into the bloodstream from the small intestine in response to meals. These hormones cause insulin release from the pancreatic beta cells in a glucose-dependent manner but are inactivated by the DPP-4 enzyme within minutes. GLP-1 also lowers glucagon secretion from pancreatic alpha cells, reducing hepatic glucose production. In patients with type 2 diabetes, concentrations of GLP-1 are reduced but the insulin response to GLP-1 is preserved. *Alogliptin* is a DPP-4 inhibitor that slows the inactivation of the incretin hormones, thereby increasing their bloodstream concentrations and reducing fasting and postprandial glucose concentrations in a glucose-dependent manner in patients with type 2 diabetes mellitus. *Alogliptin* selectively binds to and inhibits DPP-4 but not DPP-8 or DPP-9 activity *in vitro* at concentrations approximating therapeutic exposures.[9-12]

Alogliptin benzoate is a white to off-white, crystalline powder containing one asymmetric carbon in the aminopiperidine moiety. It is soluble in dimethylsulfoxide, sparingly soluble in water and methanol, slightly soluble in ethanol, and very slightly soluble in octanol and isopropyl acetate.[13]

Cancer is a disease of misguided cells which have high potential of excess proliferation without apparent relation to the physiological demand of the process. It is the second largest single cause of death in both men and women, claiming over six million lives each year worldwide. In

modern medicine, chemotherapy, radiotherapy and surgery are the three major existing modes of treatment[14].

Structural formula:



Liver cancer is the fifth most frequently occurring cancer worldwide and the third most common cause of cancer mortality. Established risk factors for liver cancer include infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), excessive alcohol consumption, aflatoxin consumption, obesity, and diabetes. Type II diabetes has been reported to confer a two- to four-fold risk of liver cancer, increasing with diabetes severity and duration. Potential mechanisms for this increase include insulin resistance, compensatory hyperinsulinaemia, and increased growth factor production. In addition, insulin and its precursors may interact with liver cells to stimulate carcinogenesis[15-16].

Primary human liver cancer, of which hepatocellular carcinoma (HCC) is the predominant type, is a major cause of cancer death worldwide, accounts for about 90% of all cases of liver cancer and is the fourth most common cause of cancer mortality. 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.[17-19]

Hepato-carcinogenesis is a multistep process involving different genetic alterations that ultimately lead to malignant transformation of the hepatocytes. Several rodent models have been used indefining the pathogenesis of HCC and have contributed to the current knowledge of HCC. Because of the physiologic and genetic similarities between rodents and humans, the short lifespan and thebreeding capacity, rodents are often used for cancer research. Many chemically induced experimentshave been conducted on rats, but mice are also a favourite modelfor cancer because of the availability of gene targeting methods and the possibility of xenograft implantation. Woodchuck or groundhog (Marmotamonax) is often used for studies concerninghepatitis B infection (HBV)

induced HCC. Thewoodchuck hepatitis causes liver inflammation, injury and repair process similar to those in HBVpatients.[20-23]

The models for HCC research have a broad range of (i) chemically induced models (ii) xenograft models (iii) genetically modified models, in Rats. The experiment had done with the chemically induced models (DENA) have experimented.

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 Diethylnitrosamine (DENA) is one of the most frequently used chemical to induce hepatic-carcinogenesis in animals, possibly by inducing burst release of reactive oxygen species and cellular injury with the enhanced formation of detrimental free radicals. DENA is metabolized to its active ethyl radical, which interacts with DNA causing mutation and subsequent oncogenesis.[24-33]

MATERIALS AND METHODS

Animals:

Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice 16-25 g were procured from National Centre for Laboratory Animal Sciences, C/0 Sri. Venkateswara Enterprises, Bangalore for experimental purpose. Then the animals were acclimatized for 7 days under standard husbandry condition[34].i.e.

Room temperature	-	26 ± 2^0C
Relative humidity	-	45 - 55%
Light/dark cycle	-	12 : 12h

The animals were fed with a synthetic standard diet from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli, Maharastra. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed in accordance to Guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) of Innovative College of Pharmacy, Greater Noida, U.P. CPCSEA registration number was 1346/PO/Re/S/10/CPCSEA and all the procedures were followed as per rules and regulations.

Chemicals:

All chemicals used were of analytical grade. Diethyl nitrosamine (Sigma Aldrich, Bangalore, India) was used for hepatocellular carcinoma induction. Test compound (*Alogliptin*) was provided by Taj Pharma, Mumbai, India.

Grouping of animals:

The animals are divided into 5 groups of six rats in each group and the treatment given once.

Induction of hepatocellular carcinoma[35]*:*

Diethylnitrosamine (200 mg/kg) (DENA) was administered in phosphate buffer solution intra-peritoneally to induce hepatocellular carcinoma, as single dose, after animals were fasted overnight. Induction of hepatocellular carcinoma was confirmed after 7 days of administration of DENA by measuring serum alpha fetoprotein (AFP) in different group ani-mals. Animals were grouped as follows: group I was kept normal control and administered saline, group II was given DENA only. Groups III, IV, and V rats were treated with *Alogliptin* (1, 2, and 3 mg/kg, p.o.) once daily for 16 weeks, respectively.

Estimation of anti-hepatocellular carcinoma activity:

Blood was collected from all groups directly from retro-orbital plexus after anesthetized by a mixture of chloroform–ether (2:3) at the end of protocol period and animals were sacrificed followed by liver speci-men collection. Serum was separated after coagulating blood for 30 min and centrifuged at 1,500 rpm for 20 min; serum was then separated and was used for estimation of biochemical parameters.

Biochemical estimation:

The animals were subjected to ether anesthesia, blood was collected from retro-orbital plexus, and serum was separated by centrifugation after 16 weeks. Anti-hepatocellular carcinoma activity of the drug was determined by measuring serum levels of enzymes (ALT, AST, ALP, AFP and Glucose).

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:

All results will be expressed as mean ± SEM from 6 animals. Statistical difference in mean will be analyzed using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's't' test). $P< 0.05^{*}$, 0.01^{**} and 0.001^{***} will be considered as statistically significant

RESULTS:

Biochemical parameters

After the end of protocol period of 16 weeks, animals were sacrificed and blood was collected for estimation of serum levels. The level of serum ALT in group II which was given DENA only is 76.25 ± 1.99 which is significantly higher than the group I which was kept as normal control (36.53 ± 1.18) ($p<0.001$). The groups receiving *Alogliptin* 1 mg/kg (group III), 2 mg/kg (group IV), and 3 mg/kg (group V) have ALT levels 70.07 ± 1.94, 63.75 ± 2.11 and 57.41 ± 1.72 respectively which is significantly below ($p<0.001$) than the level of that of group II (Table 1 and Fig.1).

Serum AST in group II which was given DENA only is 151.90 ± 1.34 and this level is significantly higher than the group I which was kept as normal control 24.78 ± 0.99) ($p<0.001$). The groups receiving *Alogliptin* 1 mg/kg (group III), 2 mg/kg (group IV), and 3 mg/kg (group V) have AST levels 72.65 ± 0.99, 63.66 ± 1.87 and 52.85 ± 1.88 respectively which is significantly below than the levels of that of group II ($p<0.001$) (Table 1 and Fig. 2).

Enzyme ALP level in group II which was given DENA only is 135.09 ± 2.98 which is significantly higher ($p < 0.001$) than the group I which was kept as normal control 73.45 ± 0.58. The groups receiving *Alogliptin* 1 mg/kg (group III), 2 mg/kg (group IV), and 3 mg/kg (group V) have ALP levels 112.23 ± 2.74, 103.36 ± 2.25 and 96.20 ± 1.56 respectively which is significantly below than the levels of that of group II ($p<0.001$) (Table 1 and Fig. 3).

The level of serum AFP in group II which was given DENA only is 74.11 ± 0.69 which is significantly higher than the group I ($p<0.001$)which was kept as normal control 2.00 ± 0.31. The groups receiving *Alogliptin* 1 mg/kg (group III), 2 mg/kg (group IV), and 3 mg/kg (group V) have AFP levels 27.39 ± 1.87, 14.95 ± 1.60 and 7.01 ± 0.94 respectively which is significantly below than the levels of that of group II ($p<0.001$) (Table 1 and Fig. 4).

The level of serum GLU in group II which was given DENA only is 77.77 ± 1.21 which is significantly higher than the group I ($p<0.001$)which was kept as normal control 56.02 ± 1.70. The groups receiving *Alogliptin* 1 mg/kg (group III), 2 mg/kg (group IV), and 3 mg/kg (group V) have GLU levels 47.54 ± 1.97, 40.69 ± 1.65 and 36.78 ± 1.56 respectively which is significantly below than the levels of that of group II ($p<0.001$) (Table 1 and Fig. 5).

Histology:

The histological sections of livers from animals of normal control group (Fig. 6) show the normal organization of hepatic lobules consisting of one to two cell thickness of hepatic cords radiating from a central vein towards the lobular periphery. In contrast to it, the sections of animal livers from DENA group (Fig. 7) of cancer control group show disorganized hepatic parenchyma with trabeculae of polyhedral cells bordering wide sinusoids which represent cellular necrosis and hepatocellular carcinoma development by diethylnitrosamine treatment. Liver specimen histology from *Alogliptin* low dose (Fig. 8) showed minor disorganization and sinusoidal congestions, whereas liver from rat of *Alogliptin* medium and high dose (Fig. 9 and 10) displayed very less damage of hepatocellular organization and low index of necrosis damage.

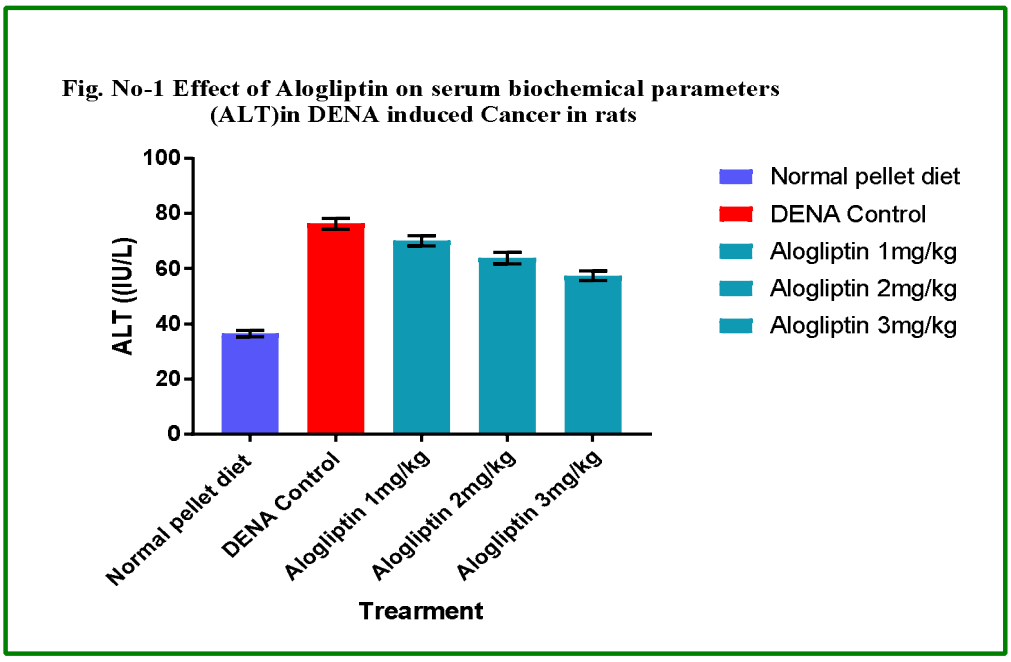


Figure 1 Effectof alogliptin

Fig. No-2 Effect of Alogliptin on serum biochemical parameters (AST) in DENA induced Cancer in rats

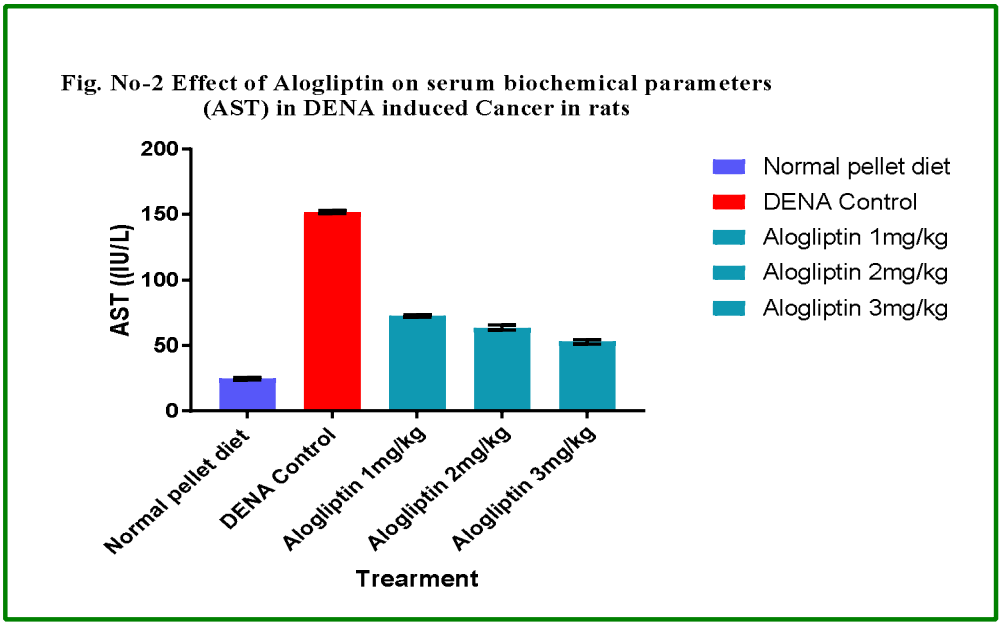


Figure 1 Effectof alogliptin

Fig. No-3 Effect of Alogliptin on serum biochemical parameters (ALP) in DENA induced Cancer in rats

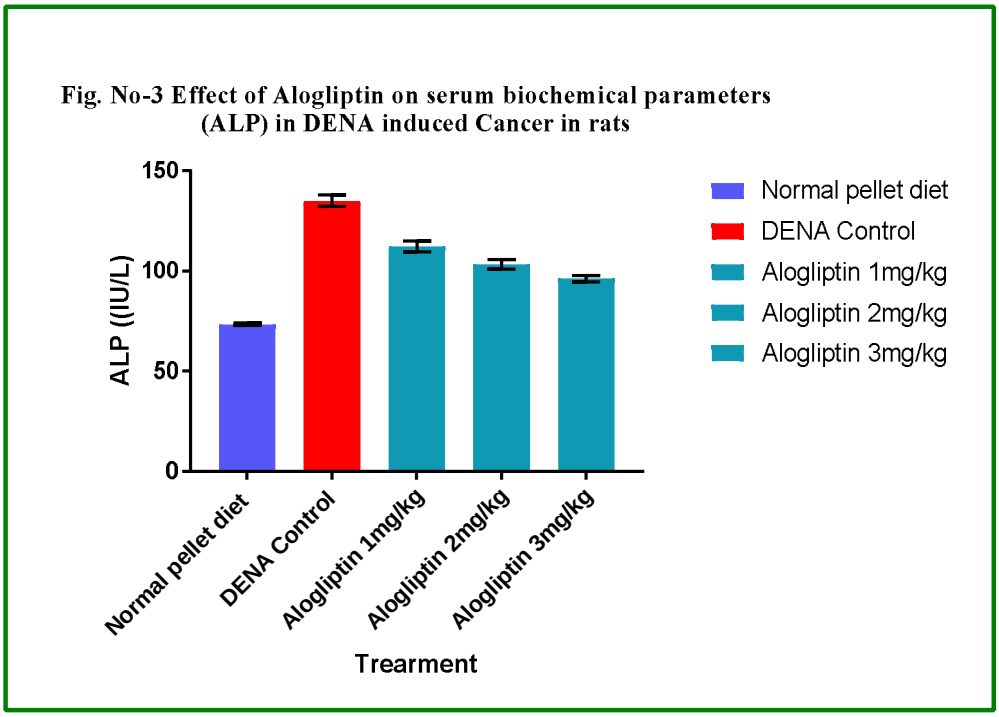


Figure 1 Effectof alogliptin

Fig. No-4 Effect of Alogliptin on serum biochemical parameters (AFP) in DENA induced Cancer in rats

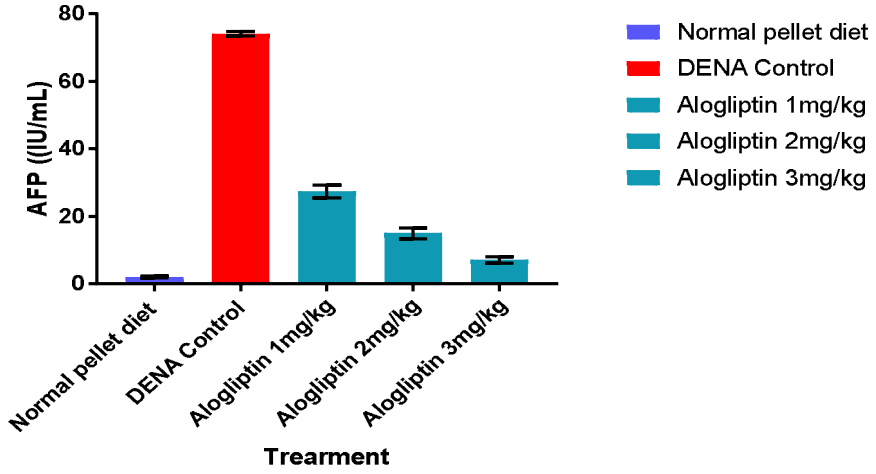


Figure 1 Effectof alogliptin

Fig. No-5 Effect of Alogliptin on serum biochemical parameters (GLU)in DENA induced Cancer in rats

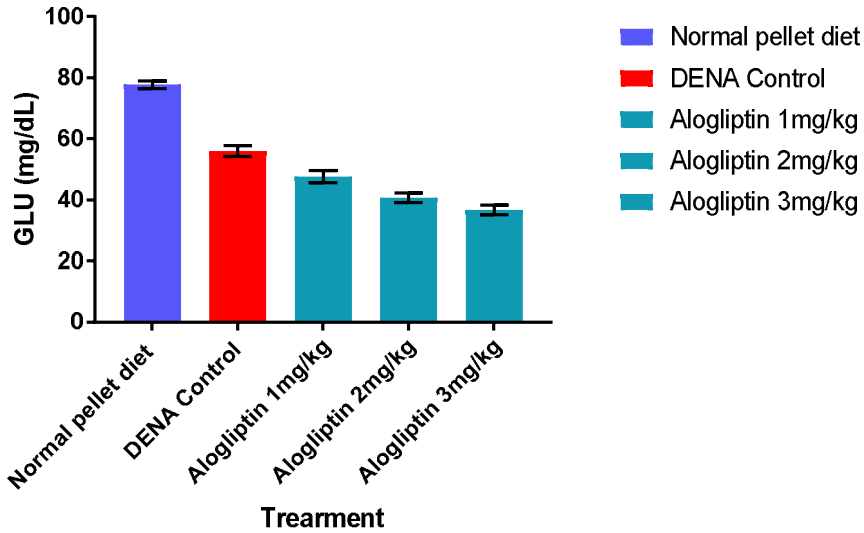


Figure 1 Effectof alogliptin

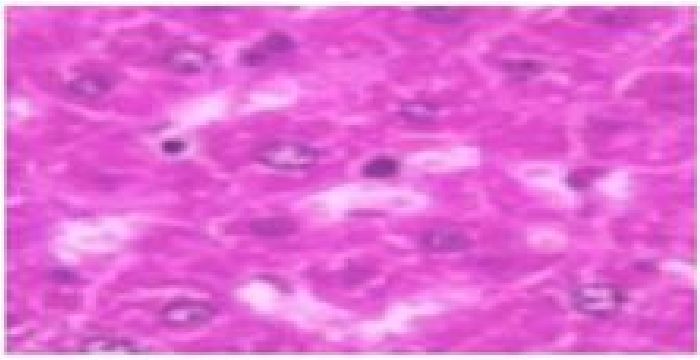


Fig. No 6: Photomicrographs (original magnification 45×) of histopathological studies of livers of normal control.

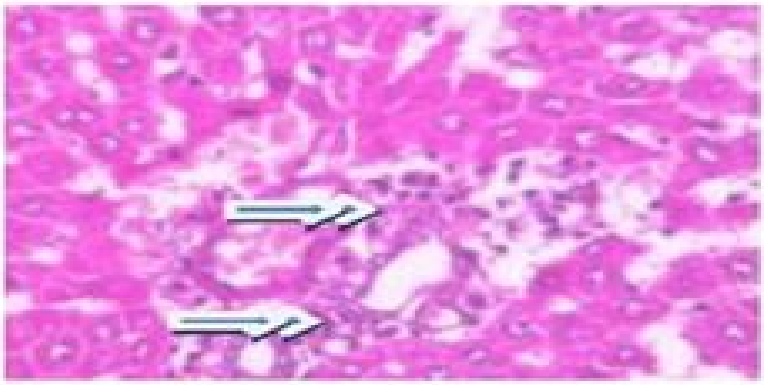


Fig. No 7: Photomicrographs (original magnification 45×) of histopathological studies of livers of DENA control.

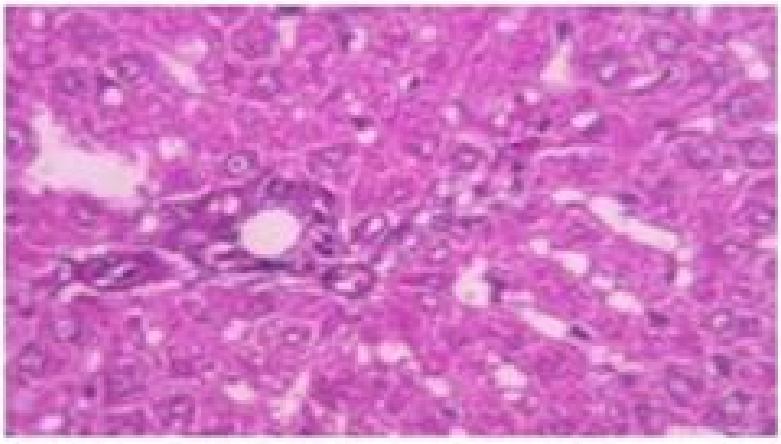


Fig. No 8: Photomicrographs (original magnification 45×) of histopathological studies of livers of *Alogliptin* 1 mg/kg.

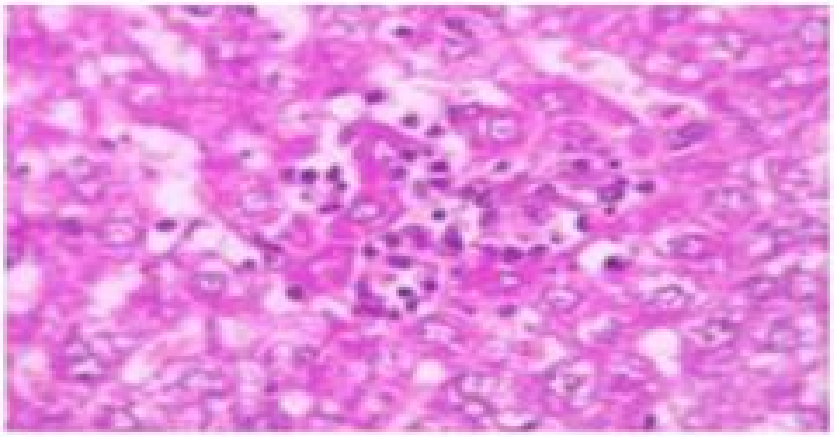


Fig. No 9: Photomicrographs (original magnification 45×) of histopathological studies of livers of *Alogliptin* 2 mg/kg.

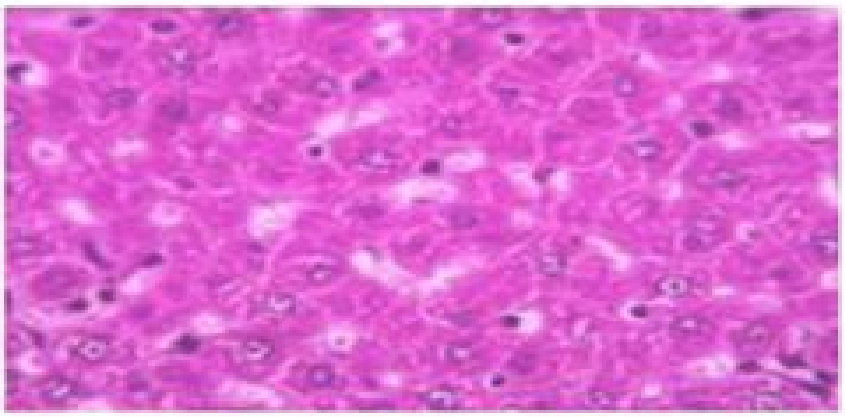


Fig. No 10: Photomicrographs (original magnification 45×) of histopathological studies of livers of *Alogliptin* 3 mg/kg.

Table 1: Effect of *Alogliptin* on serum biochemical parameters in DENA induced Cancer in rats

Group	Treatment	ALT	AST	ALP	AFP	GLU
1	Normal Pellet diet	36.53 ± 1.18	24.78 ± 0.99	73.45 ± 0.58	2.00 ± 0.31	56.02 ± 1.70
2	DENA Control	76.25 ± 1.99	151.90 ± 1.34	135.09 ± 2.98	74.11 ± 0.69	77.77 ± 1.21
3	*Alogliptin* 1mg/kg	70.07 ± 1.94	72.65 ± 0.99	112.23 ± 2.74	27.39 ± 1.87	47.54 ± 1.97
4	*Alogliptin* 2mg/kg	63.75 ± 2.11	63.66 ± 1.87	103.36 ± 2.25	14.95 ± 1.60	40.69 ± 1.65
5	*Alogliptin* 3mg/kg	57.41 ± 1.72	52.85 ± 1.88	96.20 ± 1.56	7.01 ± 0.94	36.78 ± 1.56

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. 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. Diethylnitrosamine (DENA) is one of the most frequently used chemical to induce hepatic-carcinogenesis in animals, possibly by inducing burst release of reactive oxygen species and cellular injury with the enhanced formation of detrimental free radicals. DENA is metabolized to its active ethyl radical, which interacts with DNA causing mutation and subsequent oncogenesis.[20-24]

Alogliptin is a new drug and is only evaluated for antidiabetic activity. Hence, in current scenario, present study designed to evaluate anticancer potential of this new drug.[12-13]

DENA is frequently used to induce hepatocarcinoma in animal models possibly by causing oxidative stress and cellular injury with enhanced formation of detrimental free radicals. DENA has been shown to be metabolized to its active ethyl radical, which can interact with DNA causing mutation and subsequent oncogenesis. It is present in tobacco smoke, water, cheese, cured, and flamed meats.

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 . In the present study, DENA induced hepatocellular damage is clearly evidenced by the marked elevation in serum ALT/SGPT, AST/SGOT, SALP/ALP, AFP and decrease level of glucose in the liver tissue, These biochemical marker enzymes are indicators of tumor response. ALT/SGPT, AST/SGOT, SALP/ALP, AFP and decrease level of glucose serves as a marker of liver damage and mechanisms of neoplastic process. It has been studied that serum GGTP and ALP levels increases linearly with tumor mass. 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. 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.[24-30]

Alogliptin at a dose of 3 mg/kg restored the level of ALP, SGPT, SGOT. An increase GGTP activity found in the preneoplastic foci that enhances cell proliferation and increase tumor promotion. These results established the role of *Alogliptin* as a chemopreventive agent in DENA induced HCC[31].

The development of HCC has also been associated with disorders in plasma lipid and lipoprotein metabolism. 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. 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[32].

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 *Alogliptin* maintained the lipid profile, hence it can be suggested that *Alogliptin* may play the role in inhibition of carcinoma progression[33].

AFP is a serum protein has higher specificity for hepatocarcinoma and detected in elevated concentration in hepatocellular carcinoma. 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.[25-30]

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. Treatment with *Alogliptin* 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); but no such alterations were noticeable in untreated normal control (Group 1) or in the *Alogliptin* control group (Group 3, 4 and 5).

Unlike the normal organization of the hepatic lobules found in the livers from rats in Group 1 (normal control) and Group 5 (*Alogliptin* 3mg/kg), liver sections from the DENA-treated rats in Groups 2 (DENA only) and 4 (*Alogliptin* 2 mg/kg) 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 3 (*Alogliptin* 1 mg/kg) 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[48].

Chemopreventive activities of *Alogliptin* may be ascribed to its molecular modeling of the complex formed between *Alogliptin* 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 *Alogliptin* is effective on DNA special for GC. Thus, revealing that *Alogliptin* 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

CONCLUSIONS:

From our study, it is concluded that the evaluation of *Alogliptin* in dose-dependent manner prevented the carcinogenesis in the liver because it significantly prevent the markedly increase in the levels of serum marker enzymes and also suppressed the free radical processes by scavenging hydroxyl radicals. Hence, the treatment of hepatocarcinogenesis with *Alogliptin* could be a novel approach in the field of chemotherapy. *Alogliptin* an antidiabetic drug which has been evaluated for the other pharmacological activities. Data from the present investigation suggest that *Alogliptin* possesses potential chemopreventive action at reduced dose (3 mg/kg) suppress the tumors and decrease the biochemical marker which are elevated in HCC. The present study will help and assist designing proper medication for patients suffering from hepatic cancer.

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