Benha Veterinary Medical Journal 40 (2021) 94-98

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Newcastle disease virus and Doxorubicin inhibited the progression of diethyl- nitrosoamine induced hepatocellular carcinoma

Afaf Desoky[1], Kamel Zyan[2], Mahmoud A. Elashrey[1*]

[1]*Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2]*Department of rabbits and birds diseases, Faculty of Veterinary Medicine, Benha University, Egypt*

ARTICLE INFO

Keywords

AFP

Diethylnitrosamine

Doxorubicin

Hepatocellular Carcinoma

LDH

NDV.

Received 03/02/2021
Accepted 25/02/2021
Available On-Line 01/04/2021

ABSTRACT

Doxorubicin is a broad-spectrum antitumor antibiotic. Newcastle disease virus (NDV) is one such virus with an inherent oncolytic property. This study was done to investigate the antitumor effects of Doxorubicin and NDV on Diethylnitrosamine (DEN) induced Hepatocellular carcinoma in rats. Seventy-five male albino rats were divided into five groups. Group (1): rats administered distilled water only. Group (2): rats received diethylnitrosoamine (200 mg/kg b. wt./ i. p), three times at an interval of 15 day at experimental weeks 2, 4 and 6. Group (3): rats received DEN then treated with Doxorubicin at experimental week 10 at a dose level (2 mg/kg b. wt./ i. p). Group (4): rats received DEN then treated with Hitchiner B1 at experimental week 10 at a dose levels (10^7 PFU/rat/ i.p). Group (5): rats received DEN then treated with Doxorubicin at experimental week 10 at a dose level (2 mg/kg b. wt /i. p) and with Hitchiner B1 at experimental week 10 at a dose levels (10^7 PFU/rat/ i.p). All animals were sacrificed after the end of experiment. DEN induced HCC showed elevation in serum AFP, LDH, Creatinine and Urea compared with control group. Treatment with doxorubicin or/ and NDV showed that a significant reduction in serum AFP, LDH, Creatinine and Urea compared with DEN non-treated group. The obtained results confirmed doxorubicin and NDV can inhibit the proliferation of HCC cells through improving hepatocytes.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the most common type of liver cancer that representing 83% of all cases; it represents the third cause of cancer related death and the first cause of death amongst cirrhotic patients. Hepatitis viral infection, food additives, alcohol, fungal toxins (aflatoxins), toxic industrial chemicals, air and water pollutants are the major risk factors of liver cancer (Farazi and DePinho, 2006).

Diethylnitrosamine (DEN) is a potent hepatocarcinogenic nitrosamine, present in cheddar cheese, cured and fried meals, alcoholic beverages, cosmetics, agricultural chemicals and pharmaceutical agents, ground water having high level of nitrate (Mahmoud and Abdul-Hamid, 2012). DEN causes a wide range of tumors in all animal species and considered to be one of compounds which are hazardous to human health (Balamurugan and Karthikeyan, 2012).

Doxorubicin acts on cancer cells through intercalation into DNA resulting in the inhibition of DNA synthesis and function. It inhibits transcription through inhibition of DNA-dependent RNA polymerase (Panno, 2005). Doxorubicin is a DNA topoisomerase II inhibitor, DNA intercalator leading to DNA strand breaks and formation of Reactive Oxygen Species (ROS) in cells (Yurtcu *et al.*, 2015).

Newcastle disease virus (NDV) is one such virus with an inherent oncolytic property (Omar *et al.*, 2003). The cells of various human tumors, such as liver cancer, have been shown to be sensitive to NDV (An *et al.*, 2016). The oncolytic mechanism of NDV mainly include the following aspects. First, the virus selectively infects and replicates in tumor cells. Second, indirect effects of the innate and adaptive immune responses of the host immune system act against the virus, involving natural killer (NK) cells and cytotoxic T lymphocytes targeting the antigen. Third, the envelope protein also participates in the oncolytic effect. Fourth, the apoptotic pathway promotes the oncolytic effect. Fifth, the virus induces immunogenic death, necrosis, and autophagy (Chaurasiya et al., 2018; Ricca et al., 2018)

In this study, the curative and treatment effects of Newcastle disease virus (NDV) were studied during and after hepatic carcinoma induction by DEN.

2. MATERIAL AND METHODS

2.1. Experimental animals:

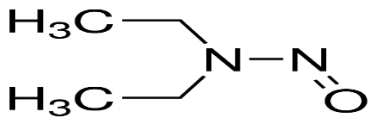
Seventy-five albino rats, 5-6 weeks old and average body weight 80-120 g were used in the experimental investigation of this study. Rats were obtained from the production center for natural toxins and raw plasma at the Helwan farm of the Egyptian Company for vaccines, vaccines and Medicines. Animals were housed in separate metal cages. Pure drinking water was supplied ad-libitum through specific nipple. Rats were kept at constant environmental and nutritional conditions throughout the

* Corresponding author: Mahmoudazmy980@gmail.com

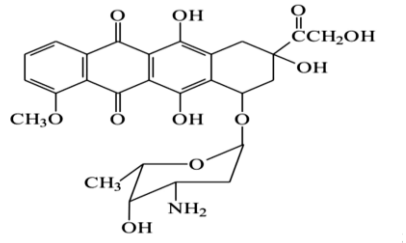
period of experiment. The animals were left 7 days before the beginning of the experiment for acclimatization.

Ration and additives:
The animals were fed on constant ration through the course of the experiment in the form of concentrated diet composed of concentrated mixture 10%, yellow corn 60.91 %, soya bean meal 22.3 %, fat 4.74 %, di calcium phosphate 0.83 %, ground limestone 0.67 %, methionine supplement 0.05 %, mineral and vitamin mixture 0.5%. Concentrate mixture composed of corn gluten meal 60 %, sunflower meal 44%, fish meal 45%, meat and bone meal 50%, di calcium phosphate, common salt limestone, vitamin and mineral, premix, L. lysine and methionine according to (NRC, 1995).

2.2. Chemicals:
The chemicals and natural agents used in the present study were:
Diethylnitrosoamine (DEN):
Common name: N-Nitrosodiethylamine ISOPAC®
Synonym: DEN; DENA; Diethylnitrosamine, N-Nitroso-N, N diethyleamine; NDEA.
Chemical structure



Induction of Hepatocarcinogenesis:
HCC was induced in rats by I.P injection of DEN in normal saline (200 mg/kg b. wt.), three times at an interval of 15 days (Khan *et al.,* 2011).
Doxorubicin:
Chemical anti-cancer drug, It is used at a dose level (2 mg/kg b. wt./ i. p.) (Karuppayil *et al.,* 2018).
Chemical structure



1

Hitchiner B1:
Purchased from Egypt Masters Co. for veterinary products.
Live Newcastle vaccine (Hitchiner B1), It is used at a dose level (10^7 PFU/mouse/i.p.) (Sharma *et al.,* 2017).

2. 3. Experimental design:
Rats were divided into five main equal groups each one contains 15 rats as follow:
Group 1: Control Normal group:
Consisted of 15 male rats, rats fed with ordinary diet only without any treatment during the entire experimental period (13 weeks).
Group 2: DEN- induced hepatocarcinogenesis group: Rats considered as the carcinogen control injected with DEN at a dose of (200 mg/kg body weight i. p.) three times at an interval of 15 day at experimental weeks 2, 4 and 6.
Group 3: DEN- induced hepatocarcinogenesis+ Doxorubicin treated group: Rats considered as the carcinogen control injected with DEN at a dose of (200 mg/kg body weight i. p.) three times at an interval of 15 day at experimental weeks 2, 4 and 6 then treated with Doxorubicin at experimental week 10 at a dose level (2 mg/kg b. wt./ i. p.) until the end of experiment.
Group 4: DEN- induced hepatocarcinogenesis + Hitchiner B1 treated group: Rats considered as the carcinogen control injected with DEN at a dose of (200 mg/kg body weight i. p.) three times at an interval of 15 day at experimental weeks 2, 4 and 6 then treated with Hitchiner B1 at experimental week 10 at a dose levels (10^7 PFU/rat/i. p.) until the end of experiment.
Group 5: DEN- induced hepatocarcinogenesis + Doxorubicin + Hitchiner B1 treated group: Rats considered as the carcinogen control injected with DEN at a dose of (200 mg/kg body weight i. p.) three times at an interval of 15 day at experimental weeks 2, 4 and 6 then treated with Doxorubicin at experimental week 10 at a dose level (2 mg/kg b. wt./i. p.) and with Hitchiner B1 at experimental week 10 at a dose levels (10^7 PFU/rat/ i. p.) until the end of experiment.

2.4. Sampling:
2.4.1. Blood samples:
Blood samples were collected by ocular vein puncture from all animal groups after overnight fasting in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 rpm for 15 minutes. The serum was taken by automatic pipette and received in dry sterile tubes, then kept in deep freeze at -20 ℃ until use for subsequent biochemical analysis. All sera were analyzed for determination of the following parameters: (AFP (Engall, 1980), LDH (Dito, 1979), Creatinine (Henry, 1974), Blood Urea (Tietz, 1990)

2.5. Statistical analysis:
The results were expressed as mean ± SE using SPSS software program version 16 (SPSS© Inc., USA). The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparison among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p<0.05$.

3. RESULTS

The obtained results presented in Table (1) revealed that, DEN induced HCC (group2) showed elevation in serum AFP, LDH, creatinine and urea compared with control group. Treatment with doxorubicin or/ and NDV (group 3,4 and 5) showed that a significant reduction in serum AFP, LDH, creatinine and urea when compared with DEN group.

Table 1 Effect of Newcastle disease virus on serum AFP, LDH, Creatinine and Urea of DEN induced HCC in rats.

Exp. Groups	AFP (ng/ml)	LDH(U/L).	Creatinine (mg/dl)	Urea (mg/dl)
Group 1	0.36 ± 0.04 ^{c}	178.80 ± 8.62 ^{c}	0.69 ± 0.02 ^{c}	47.60 ± 1.07 ^{b}
Group 2	5.02 ± 0.40 ^{a}	350.10 ± 19.83 ^{a}	1.04 ± 0.04 ^{a}	90.33 ± 6.74 ^{a}
Group 3	1.08 ± 0.10 ^{b}	224.67 ± 12.17 ^{b}	0.78 ± 0.02 ^{b}	49.50 ± 1.70 ^{b}
Group 4:	0.66 ± 0.13 ^{b,c}	182.43 ± 5.05 ^{c}	0.78 ± 0.02 ^{b}	47.67 ± 2.72 ^{b}
Group 5	0.90 ± 0.06 ^{b,c}	208.97 ± 10.66 ^{b,c}	0.77 ± 0.02 ^{b}	47.40 ± 1.80 ^{b}

Data are presented as (Mean ± S.E). Mean values with different superscript letters in the same column are significantly different at ($P<0.05$).

4. DISCUSSION

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis (Alison, 2005). *N*-Nitrosodiethylamine (DEN) is a potent hepatic carcinogen agent (Mahmoud and Abdul-Hamid, 2012). The putative mechanism of DEN-DNA adduct formation. The first bioactivation step is P450- mediated α-hydroxylation, producing an α-hydroxylnitrosamine. Diethylnitrosoamine is hydroxylated principally by the ethanol inducible CYP2El in liver, but other P450 isozymes have been found to be bioactivating as well. DNA-adduct formation proceeds through an ethyldiazonium ion intermediate and evolution of N2. Since this is a relatively simple and rapid alkylation mechanism, DEN may produce DNA adducts in any tissue with appropriate activating P450 isozymes. Nitrosamine alkylation in each organ reflects the local level of bioactivation capacity, since the diazonium ion is too reactive to be transported to other organs in significant amounts. Additionally, P450 isozymes are responsible for detoxification of NDEA, thereby adding to the complexity of the biotransformation. There is a great deal of information concerning the roles of P450 isozymes in the bioactivation of various nitrosamines which is similar to DEN in structure and reactivity, are evaluated together (Talib, 2012).

The obtained results revealed significant elevation in serum AFP, LDH, creatinine and urea with DEN injection to rats. These results came in accordance with the recorded data of (Borai *et al.,* 2017; Salama *et al.,* 2017) who recognized that, AFP concentration was significantly higher in the DEN-treated group as compared to control group. Moreover, (Zaazaa *et al.,* 2018) observed that, increase in serum AFP in DEN-induced HCC in rats compared to control group. Additionally, Alpha fetoprotein (AFP) is the most commonly used tumor markers for the diagnosis of hepatocellular carcinoma (HCC) which is a unique immunomodulatory glycoprotein, which is normally made by the immature hepatocytes in the fetus(oncofetal) (Saffroy *et al.,*2007). Detection of AFP during monitoring of liver cancer treatment is well accepted in patients with increased AFP levels before therapy. It has been recognized that exposure of animals with DEN increases the circulating AFP concentration (Sadik *et al.,* 2008).

In many tumor types, serum LDH levels are indirect marker of tumor hypoxia, neo-angiogenesis, and worse prognosis (Scartozzi *et al.,* 2012). Lactic dehydrogenase, which is a glycolytic enzyme, and exists in various types of human tissue and neoplasms, has also been reported to demonstrate a high level in the serum HCC patients. Serum LDH level of patients with HCC is more closely correlated with the clinical course than the AFP level. The levels of the serum LDH and AFP one week after the right hepatic lobectomy both decreased. Also, HCC with a high serum level of LDH appears to show both a rapid growth and highly malignant tumors (Fujiwara *et al.,* 1997). From another hand, LDH seemed able to predict clinical outcome for HCC patients undergoing trans-arterial chemoembolization (TACE). With respect to the correlation between LDH levels and tumor angiogenesis, it can speculate that patients with high LDH pretreatment levels may be optimal candidates for clinical trial exploring a multimodality treatment approach with TACE in order to improve the overall survival (Scartozzi *et al.,* 2012). Similar results were obtained by (Jagan *et al.,* 2008; Jayakumar *et al.,* 2012) who reported that, DEN administration resulted in a significant increase in serum Lactate dehydrogenase activities in male wistar albino rats.

Serum urea levels were elevated in HCC compared with other chronic liver disease (Badr *et al.,* 2014). The liver is the largest complex organ in the abdominal cavity. Parenchymal cells or hepatocytes comprise the bulk of the organ and carry out complex metabolic processes. Hepatocytes are responsible for the liver's central role in metabolism. These cells are responsible for the many functions such as formation and excretion of bile; regulation of carbohydrate homeostasis; lipid synthesis and secretion of plasma lipoproteins; control of cholesterol metabolism; and formation of urea (Larson and Hauswald, 2014). Thus, liver cell impairment may increase the circulating level of urea.

Similar elevation of serum circulating urea level were obtained after rats' treatment with DEN by (Althaf and Sudaroli, 2012).

Treatment with doxorubicin or Newcastle disease virus (NDV) to DEN induced HCC in rats caused a significant decrease in serum AFP, LDH, creatinine and urea when compared with DEN non-treated group.

Solomon *et al.* (1999) concluded that, among 13 evaluable patients with initially elevated AFP level, 70% had a partial biologic response and survival after hepatic chemoembolization with cisplatin, doxorubicin, mitomycin-C, Ethiodol, and polyvinyl alcohol in a U.S. population of patients with hepatocellular carcinoma, (>50% decrease in AFP), 15% had a minor response (25-50% decrease), and the remaining 15% remained stable. Among 25 patients evaluable for morphologic response, 36% had a partial response, 32% had a minor response, and 32% remained stable. No patients had progression of disease while receiving therapy. The cumulative survival was 60% at 1 year, 41% at 2 years, and 16% at 3 years. Two patients developed progressive hepatic failure. Thirty-day mortality was 3% (one patient). Also, Pritchard *et al.* (2000) reported that, one hundred fifty-four patients were registered in the study, and 138 received preoperative chemotherapy. One hundred thirteen (82%) showed a partial response with tumor shrinkage and serial decrease of serum alpha-fetoprotein levels.

Zhao *et al.* (2015) concluded that, DOX/Cur-NPs and observed their enhanced anti-tumor activity in DNE-induced HCC in mice, which might be regulated through increased apoptosis, and inhibited proliferation and angiogenesis. Cell analysis indicates that modulation on MDR- and hypoxia-related proteins may contribute together to the enhanced activity of DOX/Cur-NPs. Furthermore, In DEN-induced HCC in mice treated with DOX-NPs and DOX/ Cur-NPs, (Zhao *et al.,* 2015) observed that, decrease in liver damage, which is in line with the reported increase in DOX sensitivity loaded in nano-particle- cells for HCC (Barraud *et al.,* 2005).

Abdullahi et al. (2018) concluded that, although lactate dehydrogenase (LDH) assays seemed to demonstrate a slightly reduced level of cytotoxicity at early time points after infection with rVSV-NDV compared to the parental viruses, we believe this to be an artifact of the assay, rather than a reflection of a delayed response to the virus. Cytotoxicity data, as determined by LDH assay, similarly revealed that rVSV-F caused slightly faster and more potent tumor cell killing than rVSV, although these results were not statistically significant. Furthermore, Keshavarz *et al.* (2019) investigated that, Antitumor activities of oncolytic NDV were assessed by cell proliferation (MTT) and lactate dehydrogenase (LDH) release analysis. In addition, molecular changes of early stage of apoptosis and

the role of reactive oxygen species (ROS) were analyzed by flow cytometry and Western Blot in NDV-treated TC-1 cells. Chan *et al.* (1996) Concluded that administration of carotenoids (CARs) following DOX treatment decreased levels of LDH in a dose dependent manner. Furthermore, An *et al. (*2016) investigated that, in order to assess potential toxicities associated with recombinant virus *in vivo*, a toxicity study was performed. AST and ALT are the important indexes in evaluating liver function, the blood urea nitrogen and creatinine are the important indexes in evaluating renal function. We have detected the BUN, creatinine, AST, and ALT. Results showed that these parameters were in the normal range, indicating that rNDV-P53 is relatively safe for cancer therapy. Also, Shinozaki *et al.* (2004) reported that, there were also no elevations of blood urea nitrogen (BUN) or creatinine at all time points, indicating a lack of nephrotoxicity at day 1 after hepatic arterial administration of (vesicular stomatitis virus (VSV)) rVSV-h-gal, although the levels rapidly returned to baseline at day 3).

5. CONCULOSIONS

The present findings showed that doxorubicin or Newcastle virus improve liver cells damage and ~~can~~ inhibit the proliferation of HCC cells which decreased the serum AFP, LDH, Creatinine and urea concentrations that increased by DEN induction. It could be concluded that, the chemo preventive effect of doxorubicin or Newcastle as powerful agents may be useful in hepatocellular carcinoma treatment.

6. REFERENCES

1. Abdullahi, S., Jäkel, M., Behrend, S.J., Steiger, K., Topping, G., Krabbe, T., Colombo, A., Sandig, V., Schiergens, T.S., Thasler, WE., Werner, J., Lichtenthaler, S.F., Schmid, R.M., Ebert, O., Altomonte, J., et al., 2018. A novel chimeric oncolytic virus vector for improved safety and efficacy as a platform for the treatment of hepatocellular carcinoma. J Virol 92 (23): e01386-18.
2. Alison, M.R., 2005. Liver stem cells: implications for hepatocarcinogenesis. Stem Cell Rev. 1(3):253-60.
3. Althaf, F.D., Sudaroli, M., 2012. Influence of Vitex Leucoxylon Linn on Oxidative stress and hepatocarcinogenesis induced by Diethylnitrosamine and Phenobarbital in rats. International Journal of Toxicological and Pharmacological Research; 4:96-107.
4. An, Y., Liu, T., He, J., et al., 2016. Recombinant Newcastle disease virus expressing P53 demonstrates promising antitumor efficiency in hepatoma model. J Biomed Sci. 2016;23(1):55.
5. Badr, E.A.E., Korah, T.E., Abdel Ghani, A., El-Sayed, S., Badr, S., 2014. Role of serum glypican-3 in the diagnosis and differentiation of small hepatocellular carcinoma from hepatitis-C virus cirrhosis. Alexandria Journal of Medicine; 50:221–226.
6. Balamurugan, K., Karthikeyan, J., 2012. Evaluation of Luteolin in the Prevention of N-nitrosodiethylamine-induced Hepatocellular Carcinoma Using Animal Model System. Indian J Clin Biochem.; 27: 157-163.
7. Barraud, L., P. Merle, E. Soma, L. Lefrancois, S. Guerret, M. Chevallier, et al.,2005. Increase of doxorubicin sensitivity by doxorubicin-loading into nanoparticles for hepatocellular carcinoma cells in vitro and in vivo, J. Hepatol. 42 (2005) 736–743.
8. Borai, I.H., Ghanem, H., Mamdouh, M., Abdel-Halim, A.H., Hegazi, A. E.A., Mousa, F., 2017. Chemopreventive Effect of Momordicacharantia Extract Against Chemically-Induced Hepatocellular Carcinoma in Experimental Animals. RJPBCS 8 (2) : 519- 529 ISSN: 0975-8585.
9. Chan, E.M., Thomas, M.J., Bandy, B., Tibbits, G.F., 1996. Effects of doxorubicin, 4′- epirubicin, and antioxidant enzymes on the contractility of isolated cardiomyocytes. Can J Physiol Pharmacol; 74:904-910.
10. Chaurasiya S, Chen NG, Fong Y. (2018). Oncolytic viruses and immunity. Curr Opin Immunol ; 51: 83-90
11. Dito, W.R., 1979. Lactate dehydrogenase A brief review. In: Griffiths JC, ed. Clinical Enzymology. New York :masson publishing USA; 1979:18.
12. Engall, E., 1980. Methods in Enzymology, Volume 70, Van Vunakis, H. and Langone, J. J. (eds.), Academic Press, New York, 419-492(1980).
13. Farazi, P.A., Depinho, R.A., 2006. Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer 6:674–687
14. Fujiwara, Y., Takenaka, K., Kajiyama, K., Maeda, T., Gion, T., Shirabe, K., Shimada, M., Sugimachi, K., et al., 1997. The characteristics of hepatocellular carcinoma with a high level of serum lactic dehydrogenase: a case report. Hepatogastroenterology; 44:820-823.
15. Henry, T.J., 1974. Clinical Chemistry Principles and Techniques. 2nd ed. Harper and Row Publishers; New York:
16. Jagan, S., Ramakrishnan, G., Anandakumar, P., Kamaraj, S., Devaki, T., et al., 2008. Antiproliferative potential of gallic acid against diethylnitrosamine-induced rat hepatocellular carcinoma. Mol Cell Biochem.; 319:51-59.
17. Jayakumar, S., Madankumar, A., Asokkumar, S., Raghunandhakumar, S., Gokula dhas, K., Kamaraj, S., Divya, M.G., Devaki, T., et al., 2012. Potential preventive effect of carvacrol against diethylnitrosamine-induced hepatocellular carcinoma in rats. Mol Cell Biochem.; 360:51-60.
18. Karuppayil, S.M., Wakharde, A.A., Awad, A.H., Bhagat, A., et al., 2018. Synergistic Activation of Doxorubicin against Cancer: A Review. American journal of clinical microbiology. ,Antimicrobials Volume 1 | Issue 2 | Article 1009
19. Keshavarz, M., M. Nejad , A. Sasan, E. Maryam, B. Farah, D. Hassan, Keyvani, H., Ghaemi, A., et al., 2019. Oncolytic Newcastle disease virus reduce growth of cervical cancer cell by inducing apoptosis. Saudi Journal of Biological Sciences. 27. 10.1016/j.sjbs.2019.04.015.
20. Khan, M.S., Devaraj, H., Devaraj, N., et al., 2011. Chrysin abrogates early hepatocarcinogenesis and induces apoptosis in N-nitrosodiethylamineinduced preneoplastic nodules in rats. Toxicol Appl Pharmacol.; 251: 85-94.
21. Larson, A.M., Hauswald, M., 2014. Normal Functional Biology of the Liver. Diseases of the Liver in Children: Evaluation and Management. Edition: 2014. Springer. pp. 23-51.
22. Mahmoud, M.S., Abdul-Hamid, M., 2012. Green tea extract ameliorates Liver and pituitary gland toxicity induced by diethylnitrosamine in male rats. JAS, 8(3): 58-71.
23. NRC (National Research Council). (1995). Nutrient Requirement of Laboratory animals 4th edition, Washington National Academy Press.
24. Omar, A., Ideris, A., Ali, A., Othman, F., Yusoff, K., Abdullah, J., Wali, S., Zawawi, M., Meyyappan, N., et al., 2003. An Overview on the Development of Newcastle Disease Virus as an Anti-Cancer Therapy. The Malaysian journal of medical sciences: MJMS. 10. 4-12.
25. Panno J., 2005. Cancer: the role of genes, lifestyle, and environment. Infobase Publishing. New York, USA.
26. Pritchard, J., Brown, J., Shafford, E., et al., 2000. Cisplatin, doxorubicin, and delayed surgery for childhood hepatoblastoma: a successful approach--results of the first prospective study of the International Society of Pediatric Oncology. J Clin Oncol. 2000;18(22):3819-3828.
27. Ricca JM, Oseledchyk A, Walther T, Liu C, Mangarin L, Merghoub T, Wolchok JD, Zamarin D., (2018) Preexisting Immunity to Oncolytic Virus Potentiates Its Immunotherapeutic Efficacy. *Mol Ther*; 26: 1008-1019 [PMID: 29478729 DOI: 10.1016/j.ymthe.2018.01.019].
28. Sadik, N.A.H., EL-Maraghy, S.A., Ismail, M. F., 2008. Diethylnitrosamine-induced hepatocarcinogenesis in rats: possible chemoprevention by blueberries: Afr. J. Biochem. Res. Vol.2 (3), pp. 081-087.
29. Saffroy, R., Pham, P., Reffas, M., Takka, M., Lemoine, A., Debuire, B., et al., 2007. New perspectives and strategy research biomarkers for hepatocellularcarcinoma. ClinChem Lab Med.45:1169–1179.

30. Salama, A.F., Abdel-Hamid, N. M., El-sheekh, M., Tosson, E., Gabr,, A. M., 2017. Spirulina Platensis Microalgae Protects against Diethyl Nitrosamine Carcinogenic Effect on Female Albino Rats. AJVS. Vol. 53(1): 167-179. April.
31. Scartozzi, M., Faloppi, L., Bianconi, M., Giampieri, R., Maccaroni, E., Bittoni, A., Del Prete, M., Loretelli, C., Belvederesi, L., Svegliati Baroni, G., Cascinu, S., et al., 2012. The role of LDH serum levels in predicting global outcome in HCC patients undergoing TACE: implications for clinical management. PLoS One; 7: e32653.
32. Sharma, K.K., Kalyani, I.H., Mohapatra, J., Patel, S.D., Patel, D.R., Vihol, P.D., Chatterjee, A., Patel, D.R., Vyas, B., et al., 2017. Evaluation of the oncolytic potential of R2B Mukteshwar vaccine strain of Newcastle disease virus (NDV) in a colon cancer cell line (SW-620). Arch. Virol., 162, 2705–2713.
33. Shinozaki, K., Ebert, O., Kournioti, C., Tai Y.S., Woo, S.L., et al., 2004. Oncolysis of multifocal hepatocellular carcinoma in the rat liver by hepatic artery infusion of vesicular stomatitis virus. Mol Ther. 2004;9(3):368-376.
34. Solomon, B., Soulen, M.C., Baum, R.A., Haskal, Z.J., Shlansky-Goldberg, R.D., Cope, C., et al., 1999. Chemoembolization of hepatocellular carcinoma with cisplatin, doxorubicin, mitomycin-C, ethiodol, and polyvinyl alcohol: prospective evaluation of response and survival in a U.S. population. J Vasc Interv Radiol. 1999;10(6):793-798.
35. Talib, H., 2012. Ph. D Thesis, FOP, IU, Lucknow.
36. Tietz, N.W., 1990. Serum triglyceride determination. In: Clinical Guide to Laboratory Tests, second ed. W.B. Saunders Co, Philadelphia, USA, pp. 554–556.
37. Yurtcu, E., Iseri, O., Sahin, F., et al., 2015. Genotoxic and cytotoxic effects of doxorubicin and silymarin on human hepatocellular carcinoma cells. Hum Exp Toxicol; 33:1269-1276.
38. Zaazaa, A.M., Lokman, M.S., Shalby, A.B., Ahmed, H.H., El-Toumy, S.A., et al., 2018. Ellagic Acid Holds Promise Against Hepatocellular Carcinoma in an Experimental Model: Mechanisms of Action.*APJCP.19.2.387*.
39. Zhao, X., Chen, Q., Li, Y., Tang, H., Liu, W., Yang, X., et al., 2015. Doxorubicin and curcumin co-delivery by lipid nanoparticles for enhanced treatment of diethylnitrosamine-induced hepatocellular carcinoma in mice. Eur J Pharm Biopharm.;93:27-36.