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Pharmacological and Toxicological Evaluation of Methotrexate and Naproxen in Treating Hepatocellular Carcinoma in Wistar Rat

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Abstract

The liver is one of the heaviest organs in the body (1.2–1.5 kg) and serve the most important function of maintaining the body's internal environment. The anatomical location of the liver is key to fulfill this role, as almost all absorption of unknown material into the body takes place in the gut and the portal blood draining the gut flows to the liver, which later controls the release of absorbed nutrients into the systemic circulation. In adding together to its function in metabolizing nutrients, the liver is capable to store and liberate a variety of substrates, vitamins and plays an essential role in drug and bilirubin metabolism. Liver cancer is most of the deadliest disease in the whole world which has been increasing day by day. The present study was aimed to investigate the activity of Methotrexate (MTX) and Naproxen (Np) to reduce the risk of liver cancer induced by Diethylnitrosamine (DENA) on rats. After examination, it was observed that pharmacological assessment of MTX and Np shows positive reactions toward treating DENA-induced Hepatocellular Carcinoma in animals.

Keywords: Portal blood, Bilirubin, Hepatocellular Carcinoma, Methotrexate, Naproxen, Diethylnitrosamine

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Introduction

Liver Cancer

Liver malignant growth, otherwise called hepatic cancer, is a disease that begins in the liver, instead of moving to the liver from another organ or part of the body. In different words, it is an essential liver malignant growth. Malignant growth that begins somewhere else and ultimately arrives at the liver is known as liver metastasis or secondary liver tumors, and are most normally from disease of the gastrointestinal tract, cellular breakdown in the lungs, renal malignancy.[1,2]

Quick Facts on Liver Cancer

Some central issues about liver malignancy.

- The liver is an enormous and fundamental organ on the whole warm-blooded creature.
- Hepatocellular carcinoma (HCC) is the most widely recognized kind of liver malignancy.
- Liver malignant growth influences around 30 individuals for every 100,000.[3]
- One of the significant danger factors is abundance liquor admission.
- Symptoms for the most part don't show up until the malignant growth progressed.
- Diabetes and hepatitis are hazard factors for liver malignancy.
- Diagnosis can be made in various manners, including biopsy and blood tests.
- Treatment choices for liver malignant growth incorporate a medical procedure and liver transfer.
- Cutting down liquor admission can help diminish the odds of liver malignant growth. [4]
- Liver disease comprises harmful hepatic tumors (developments) in or on the liver. The most well-known sort of liver malignancy is HCC (or HCC), and it will in general influence guys more than females.[4,5]

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HCC

- HCC (liver malignancy) is a disease emerging from the liver. It is otherwise called essential liver malignant growth or hepatoma. The liver is comprised various cell types (for instance, bile channels, veins, and fat-putting away cells). Nonetheless, liver cells (hepatocytes) make up 80% of the liver tissue. Subsequently, most of the essential liver tumors (more than 90–95%) emerge from liver cells and are called hepatocellular malignancy or carcinoma.[6] The liver is known as an ideal site for harmful seed second just to the skin, probably as a result of its rich blood supply, liver is the basic site for the spread of dangerous illness. Essential threatening hepatic tumors may emerge from any constituent cells of the liver; however, the solitary two regular liver cell malignant growths are HCC and carcinoma of the biliary epithelium (cholangiocarcinoma). Other uncommon tumors viz., Fibrolamellar carcinoma, squamous carcinoma, epithelial hemangioendothelioma,

Angiosarcoma, Kaposi's sarcoma, and hepatocellular adenoma may likewise emerge from the liver.[7,8]

- HCC is the 6th most regular harm and is the main source of mortality in patients with cirrhosis. An expected half million new cases are determined every year worldwide to have illness trouble most noteworthy in non-industrial nations (85% of all cases). This pattern is particularly found in non-industrial nations and has suggestions for treatment. Paces of HCC are two to multiple times higher in men contrasted with ladies.[9]
- The yearly worldwide frequency is roughly 1,000,000 cases, with a male to female proportion of around 4:1 (1:1 without cirrhosis to 9:1 in some high-rate nations). As per the National Health Services UK, roughly 18,000 individuals in the US bite the dust from HCC every year. The World Health Organization gauges that liver malignancy's pervasiveness is around 30 cases for each 100,000 individuals around the world, with rates in pieces of Africa and Eastern Asia being, especially high. Master says that normal reasons for HCC are customary high liquor utilization, having unprotected sex, heftiness-related liver illness (non-alcoholic steatohepatitis), and infused drugs with shared needles.[2,10,11]

Materials and Methods

Animals

Adult, healthy, male Wistar albino rats weighing 100–125 g were procured from the animal house facility of Siddhartha Institute of Pharmacy for the present protocol. The rats were housed in groups in polypropylene cages under controlled conditions of temperature (22 + 3°C) and light (14:10 h light and dark cycle) and provided with balanced pallet diet and water. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) with approval no SIP/IAEC/PCOL/2017 under 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.

Acclimatization

After procurement, rats are acclimatized for the time of 10 days with the goal that they are acclimating to a changed climate.

Induction of Disease

Animals were subjected to carcinogenic dose of Diethylnitrosamine (DENA) 200 mg/kg body weight, followed by promotion with Phenobarbital (0.05%) in drinking water.

Experimental Design

The rats were acclimatized and randomly divided into five groups each having six rats except Group 2 DENA control (DC) having10 rats for a 16-week study.

- Group I: - Normal control (NC) rats served as vehicle control and were administered with saline orally.
- Group 2: - DC rats were administered with a single dose of DENA (200 mg/kg).
- Group 3: - DENA + Methotrexate (MTX) control (DMC) rats will be administered with DENA (200mg/kg) as a single dose and after 4 weeks treated with MTX (1.5 mg/kg/week).
- Group 4: - DENA + Naproxen (Np) control (DNC) rats were administered with DENA (200 mg/kg) as a single dose and after 4 weeks treated with Np (10 mg/kg/day).
- Group 5: - DENA + MTX + Np control (DMNC) rats were administered with DENA (200mg/kg) as a single dose and after 4 weeks treated with MTX (1.5 mg/kg/week) and Np (10 mg/kg/day).

Estimation of Biochemial Parameters

Blood tests were gathered on the end day of the test from the retro-orbital plexus under light ether sedation with no anticoagulant and were permitted to represent 30 min at room temperature, centrifuged at 2500 rpm for 10 min to isolate the serum. The serum acquired was kept at 2–4°C for additional utilization.

Estimation of serum Serum Glutamic-Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphate (ALP), Alpha-feto Protein (AFP), Hemoglobin (Hb), Blood glucose, Total bilirubin (Tb), and Total Cholesterol (Tc) were performed using standard kits.

- Hb
- AFP
- ALP
- SGOT
- SGPT
- Blood Glucose
- Tb
- Tc.

Statistical Analysis

Statistical analysis was carried out using Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA, USA). The results were expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Tukey's multiple comparison tests. Values of $P < 0.05$ were regarded as significant.

Biochemical Parameter

Hb

Administration of DENA rats, produced a significant decrease in Hb level in rats as compared to normal control. Treatment with Methotrexate Naproxen in rats showed significant increase in Hb level as compared to DENA control group [Table 1] [Figure 1].

AFP

Administration of DENA rats, produced a significant increase in AFP level in rats as compared to normal control. Treatment with Methotrexate +Naproxen in rats showed significant decrease in AFP level as compared to DENA control group [Table 2] [Figure 2].

ALP

Administration of DENA rats, produced a significant increase in ALP level in rats as compared to normal control. Treatment with Methotrexate Naproxen in rats showed significant decrease in ALP level as compared to DENA control group [Table 3] [Figure 3].

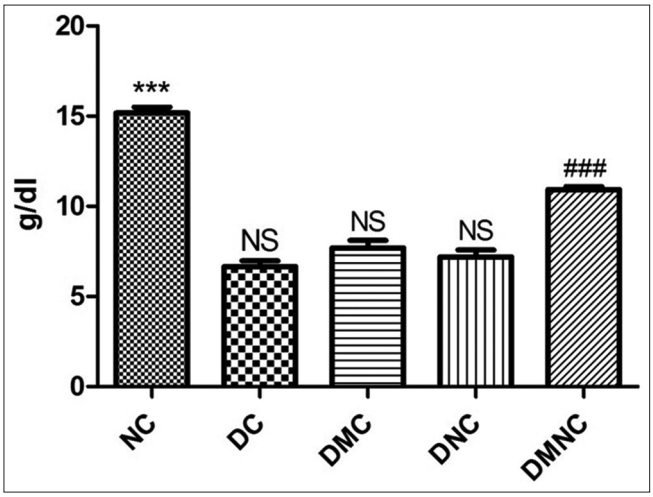

Figure 1: Shows Graphical representation of the effect of the combined treatment on hemoglobin levels; where NC-Normal control, DC-DENA control, DMC-DENA+MTX control, DNC-DENA+Np control and DMNC-DENA+MTX+Np control. *and# shows data compared to NC and DC, respectively. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. NS→ non-significant data

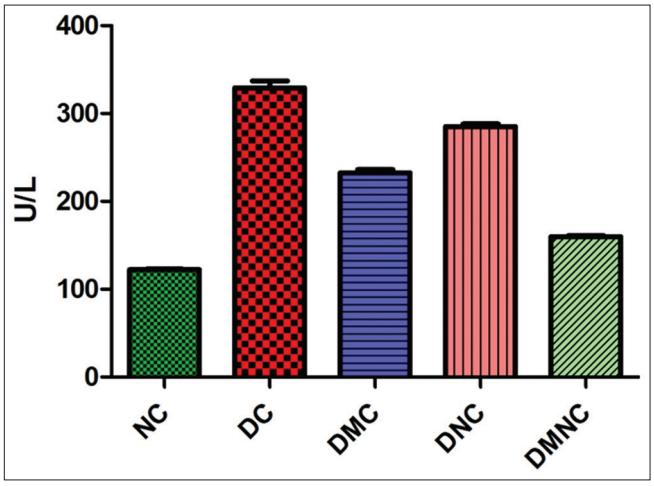

Figure 3: Shows Graphical representation of the effect of the combined treatment on ALP levels; where NC- Normal control, DC- DENA control, DMC- DENA+MTX control, DNC- DENA+Np control and DMNC- DENA+MTX+Np control. *and# shows data compared to NC and DC respectively. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. NS→ non-significant data

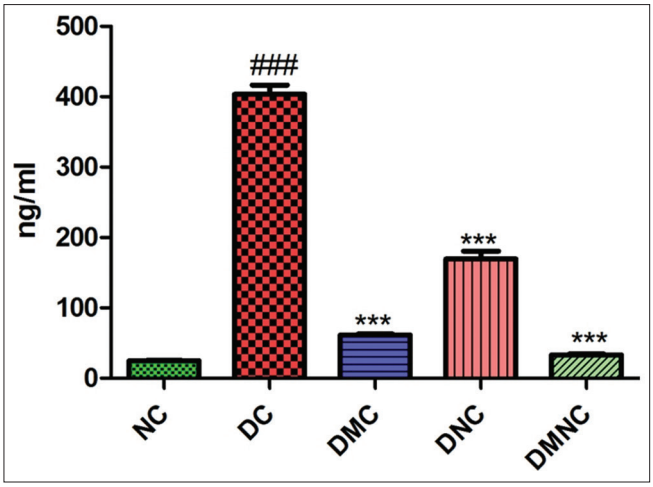

Figure 2: Shows Graphical representation of the effect of the combined treatment on AFP levels; where NC- Normal control, DC- DENA control, DMC- DENA+MTX control, DNC- DENA+Np control and DMNC- DENA+MTX+Np control. *and# shows data compared to NC and DC respectively. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. NS→ non-significant data

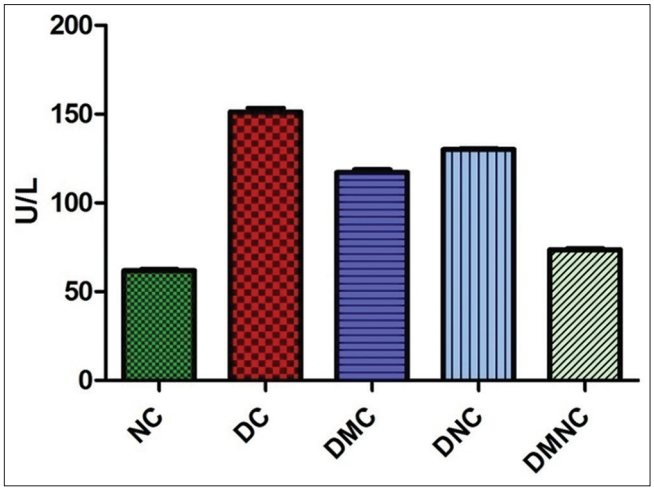

Figure 4: Shows Graphical representation of the effect of the combined treatment on SGOT levels; where NC- Normal control, DC- DENA control, DMC- DENA+MTX control, DNC- DENA+Np control and DMNC- DENA+MTX+Np control. *and# shows data compared to NC and DC respectively. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. NS→ non-significant data

SGOT

Administration of DENA rats, produced a significant increase in SGOT level in rats as compared to normal control. Treatment with Methotrexate Naproxen in rats showed significant decrease in SGOT level as compared to DENA control group. [Table 4] [Figure 4]

SGPT

Administration of DENA rats, produced a significant increase in SGPT level in rats as compared to normal control. Treatment with Methotrexate Naproxen in rats showed significant decrease in SGPT level as compared to DENA control group [Table 5] [Figure 5].

Tb

Administration of DENA rats, produced a significant increase in Tb level in rats as compared to normal control. Treatment with Methotrexate Naproxen in rats showed significant decrease in Tb level as compared to DENA control group [Table 6] [Figure 6].

Tc

Administration of DENA rats, produced a significant increase in Tc level in rats as compared to normal control. Treatment with Methotrexate Naproxen in rats showed significant decrease in Tc level as compared to DENA control group [Table 7] [Figure 7].

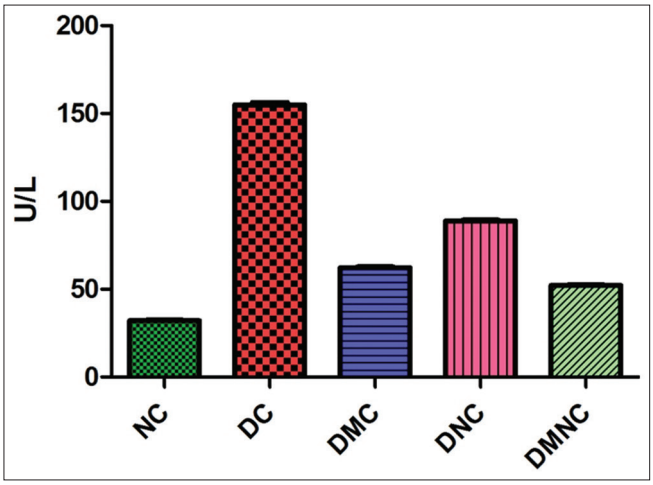

Figure 5: Shows Graphical representation of the effect of the combined treatment on SGPT levels; where NC- Normal control, DC- DENA control, DMC- DENA+MTX control, DNC- DENA+Np control and DMNC- DENA+MTX+Np control. *and# shows data compared to NC and DC respectively. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. NS→ non-significant data

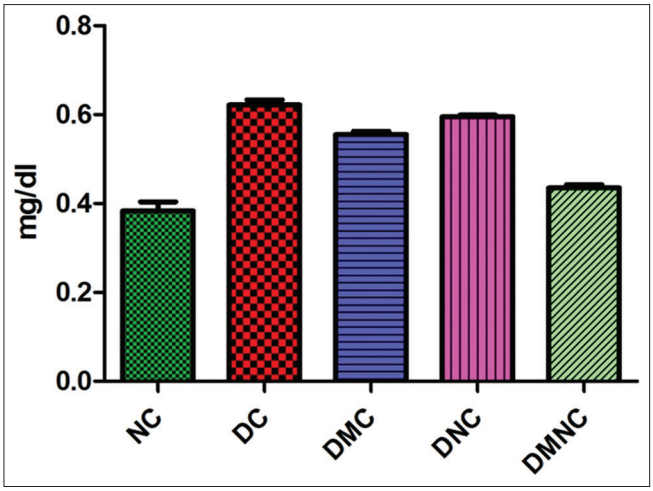

Figure 6: Shows Graphical representation of the effect of the combined treatment on Tb levels; where NC- Normal control, DC- DENA control, DMC- DENA+MTX control, DNC- DENA+Np control and DMNC- DENA+MTX+Np control. *and# shows data compared to NC and DC respectively. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. NS→ non-significant data

Discussion and Summary

HCC is considering perhaps the most continuous and deadliest diseases in the entire world, which has been expanding significantly on the planet step by step because of specific reasons.[12] In the shortfall of compelling treatment for HCC, novel chemopreventive specialists are critically needed to bring down the impact of HCC.[13,14] As we probably are aware DENA is a hepatotoxin and hepatocarcinogenic specialist. In the accompanying investigation, the stamped height in serum AFP unmistakably shows the hepatic cell harm prompted by DENA causing HCC. Different boundaries like SGPT, SGOT, ALP, Hb, and so on likewise show the outcome for DENA initiated HCC. Pharmacological assessment of MTX and Np show the ideal outcome in the details of bringing down HCC induced by DENA.

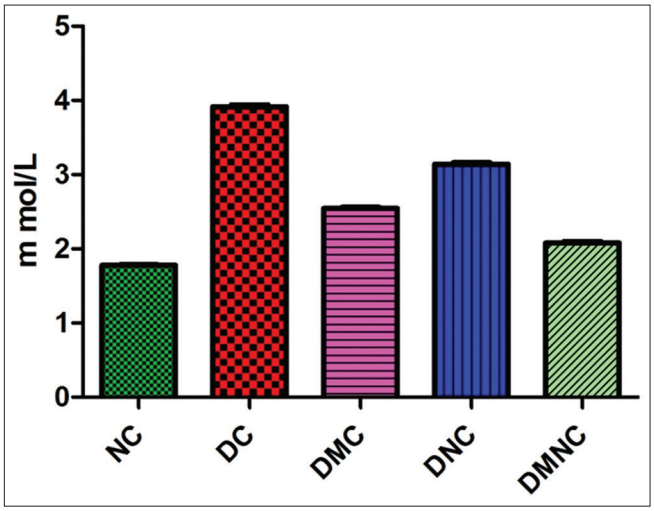

Figure 7: Shows Graphical representation of the effect of the combined treatment on Tc levels; where NC- Normal control, DC- DENA control, DMC- DENA+MTX control, DNC- DENA+Np control and DMNC- DENA+MTX+Np control. *and# shows data compared to NC and DC respectively. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. NS→ non-significant data

Table 1: Effect of combined treatment on hemoglobin (g/dl) of animals.

S.no	*Groups*	*Hemoglobin (g/dl)*
1	Normal control	15.17±0.34
2	DENA control	6.68±0.31
3	DENA+Methotrexate control	7.70±0.42
4	DENA+Naproxen control	7.20±0.42
5	DENA+Methotrexate+Naproxen control	10.92±0.18

Table 2: Effect of combined treatment on AFP (ng/ml) of animals

S.no	*Groups*	*AFP (ng/ml)*
1	Normal control	24.83±0.53
2	DENA control	403±13.08
3	DENA+Methotrexate control	61.50±1.09
4	DENA+Naproxen control	169.2±11.29
5	DENA+Methotrexate+Naproxen control	32.92±1.34

Table 3: Effect of combined treatment on ALP (U/L) of animals

S.no	*Groups*	*ALP (U/L)*
1	Normal control	122±1.06
2	DENA control	329±7.71
3	DENA+Methotrexate control	232.5±3.8
4	DENA+Naproxen control	285±3.02
5	DENA+Methotrexate+Naproxen control	159.7±1.36

Table 4: Effect of combined treatment on SGOT (U/L) of animals

S.no	*Groups*	*SGOT (U/L)*
1	Normal control	61.67±0.88
2	DENA control	151.3±1,87
3	DENA+Methotrexate control	117±1.75
4	DENA+Naproxen control	130±0.53
5	DENA+Methotrexate+Naproxen control	73.50±0.076

Table 5: Effect of various pharmacological interventions on SGPT (U/L) of animals

S.no	Groups	SGPT (U/L)
1	Normal control	32±0.59
2	DENA control	155±1.27
3	DENA+Methotrexate control	62.17±0.57
4	DENA+Naproxen control	88.83±0.61
5	DENA+Methotrexate+Naproxen control	52±0.59

Table 6: Effect of combined treatment on Tb (mg/dl) of animals

S.no	Groups	Tb (mg/dl)
1	Normal control	0.38±0.02
2	DENA control	0.62±0.01
3	DENA+Methotrexate control	0.56±0.01
4	DENA+Naproxen control	0.59±0.01
5	DENA+Methotrexate+Naproxen control	0.53±0.01

Table 7: Effect of combined treatment on Tc (mmol/L) of animals

S.no	Groups	Tc (mmol/L)
1	Normal control	1.78±0.01
2	DENA control	3.92±0.02
3	DENA+Methotrexate control	2.54±0.01
4	DENA+Naproxen control	3.14±0.02
5	DENA+Methotrexate+Naproxen control	2.07±0.01

Rodents in the NC group created typical urine and showed critical puts on in weight, great gleam, and top-notch hide. Although rodents in the DC group and other treatment gatherings, subsequent to inciting sickness give the indications of chronic frailty, including apathy, dull coats, deficiency of weight, loss of craving, decreased movement, and moderate development. For screening the possible enemy of – HCC compounds, DENA induced HCC in rodents' model are broadly utilized in the entire world and of the best described exploratory models to research hepatocarcinogenesis.[15,16] AFP has been utilized as a clinical marker in the finding and checking of HCC.[17] In our investigation, there was a checked expansion in serum AFP level show in DC group show the portrayed HCC in rodents' other boundary likewise raised by DENA induced HCC like Hb, ALP, SGOT, SGPT, Tb, and so on.

Utilization of anticancer drug MTX in treatment group DENA + MTX control (DMC) group shows a positive reaction toward DENA induced HCC, serum AFP lower down subsequent to giving MTX in DMC group in contrast with DC group which show the viability of medication. Other biological parameters are likewise influenced by MTX and show positive reaction toward the MTX treatment.

In additional examination, we likewise study the anticancer activity of NSAIDs with the goal that mix impact of anticancer medication and NSAIDs ought to be utilized as novel compound in the therapy of HCC. Here we use Np a nonselective COX inhibitor which likewise has an anticancer action in GI tract malignancies. We utilized Np in the fourth group, i.e. DENA + Np control (DNC) group rodents having DENA induced HCC to noticed the impact of Np on HCC. As results show AFP and different markers are further down not as much in DMC group but rather show an anticancer movement toward HCC which shows a positive reaction toward our theory.

In last group i.e., DENA + MTX + Np control (DMNC) group we study the blend impact of MTX and Np on DENA induced HCC in rodents and result show that mix of anticancer medication and a NSAIDs specialist have a stamped decline in AFP when contrasted with other group and other boundary likewise raised in certain way. In this way subsequent to contrasting DC group and other treatment and NC group it is come out that blend impact of medication is more dependable at that point single medication impact.

From the aftereffects of the examination, it was presumed that pharmacological assessment of MTX and Np show positive reaction toward treating a DENA induced HCC in animals and this theory can be used in additional investigations in treating HCC. As we probably are aware therapy of disease is exorbitant and not effectively manage by a large portion of patient because of which it becomes deadly for them thusly this blend impact medication may decrease the expense of medication just as increment the adequacy and intensity of medication.[18-21] Thus, mix impact of MTX and Np has a positive reaction toward the treating DENA-induced HCC.

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