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The potential ameliorating effect of 5-FluorouraciL nanoparticles on 1,2-Dimethylhydrazine- induced hepatotoxicity in rats

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ABSTRACT

Dimethylhydrazine (DMH) is very hazardous to the body's organs, especially the liver. 5-fluorouracil (5-FU) is used to treat breast, brain, liver, and colorectal tumors. The potential ameliorating effect of 5-fluorouracil nanogel (5-FUNG) against 1,2-dimethylhydrazine (DMH) induced hepatotoxicity in rats was evaluated. Forty rats were separated into 4 groups. Group I: act as normal control. Group II (DMH): injected with DMH (20 mg/kg/body weight) s.c once a week for eight weeks. Group III (DMH + 5-FU): rats injected with DMH as group II then treated with 5-FU (12.5 mg/kg body weight) three rimes weekly by intrapertoneal injection for a month. Group IV (DMH+ 5-FUNG): rats administrated DMH as group II then treated with 5-FUNG (2.5 mg/kg body weight) three rimes weekly by intrapertoneal injection for a month. Blood samples and liver specimens were taken for determination of some biochemical and molecular biomarkers. DMH treatment caused significant increase in serum liver enzymes and upregulation of hepatic inflammatory mediator (IL-1β) gene expression in addition to marked increase in liver oxidative stress MDA and marked decrease in hepatic SOD activity and GSH concentration when compared to normal control group. Rats treated with 5 FUNG display a potent effect than 5-FU in decreasing the activity of serum ALT and AST, IL-1β and MDA levels with obvious increase in liver GSH and SOD when compared with the DMH group. From the obtained results, it could be concluded that 5-FUNG has a potent effect rather than 5-FU against 1,2-dimethylhydrazine (DMH) induced hepatotoxicity in rats.

1. INTRODUCTION

The liver is the biggest organ in the body and plays an important role in metabolism of vital component (Starr and Hand, 2002). Even though the liver has a significant ability for regeneration, repeated and varied exposure to xenobiotics, environmental contaminants, and chemotherapy drugs that may decrease and even override the liver's natural defenses, which resulted in hepatic failure (Sisein et al., 2016).

1-2 ,dimethylhydrazine (DMH) is extremely hazardous substance, which affects the liver among other organs. After being metabolized in the liver, it releases alkyl free radicals and extremely reactive carbonium ions, which seriously harm the liver by producing necrosis and fatty infiltration (El-Bagoury et al., 2019). Also, the carcinogenicity and preferential toxicity of DMH for the colon and rectum in animal models have been extensively reported. Additionally, it is a potent hepatocarcinogen that, when metabolized in the liver, causes oxidative stress, hepatotoxicity, and hepatocellular cancer (Shebbo et al., 2020).

Azoxymethane (AOM), a metabolite of DMH, and other procarcinogens need metabolic activation to convert into DNA-reactive compounds. A number of metabolic enzymes are involved in the metabolism of these procarcinogenic substances. The primary route is the hepatic conversion of DMH to AOM and azoxymethanol, which is then excreted by the biliary system and conjugated with glucuronic acid. However, high amounts of azoxymethanol are toxic to the liver and can damage cell membranes and other organelles (Venkatachalam et al., 2013).

5-fluorouracil (5-FU) is a potent chemotherapeutic treatment that can be used to treat various cancer (Reddy et al., 2016). Despite a number of benefits, the emergence of drug resistance following chemotherapy restricted the clinical use of 5-FU. Therefore, in order to battle medication resistance and increase drug response rates, novel therapeutic techniques are urgently needed (Sethy and Kundu, 2021). By utilizing biodegradable and biocompatible materials, Nano particulate drug delivery methods offer a more effective but safer way around some of these obstacles. The importance of controlled drug delivery mechanism is to create novel Nano medicines. Consequently, hard inorganic porous matrices and biodegradable polymers are typically employed as drug release carriers. Biodegradable polymers have garnered a lot of interest lately because of their possible uses as carriers in medication delivery systems (Reddy et al., 2016). Accordingly, this study aimed to explore whether the hepatotoxicity effect of DMH was lower after treatment with 5-FUNG than with 5-FU alone.

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2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals, including acrylic acid (AAc) and gelatin (G), and 1,2 Dimethylhyrdrazine were purchased from Sigma Aldrich (USA).

2.2. Irradiation Process

A 60Co Gamma cell was utilized by the Egyptian Atomic Energy Authority's National Center for Radiation Research and Technology to irradiate a polymer or monomer in order to create a cross-linked nanogel with regulated (physical, chemical, and biological) properties. 5KGy of radiation were administered at a rate of 0.732 kGy/h.

2.3. Poly (G/AAc)/5-FU Nanogel synthesis by gamma irradiation according to (Rattanawongwiboon et al., 2018)

2.4. Characterization of Gelatin-based p(AAc/AAm)/5-fluorouracil according to (Gyawali et al., 2018)

2.5. The LD50 of 5-Fu Nanogel: According to (Bass et al., 1982)

2.6.-1,2 – Dimethylhydrazine (DMH)

DMH was diluted with sterile saline. Rats received weekly doses of DMH (20 mg/kg) by subcutaneous (s.c.) injections once a week for 8 weeks (Umesalma and Sudhandiran., 2010)

2.7. Experimental Animals

The Nile Company for Pharmaceuticals and Chemical Industries in Cairo, Egypt, provided forty male Wister rats (100-120 g) 5 weeks old. The animals were fed a regular pellet diet and were given tap water. Rats were cared for in accordance with the Clark et al. (1997) and recommendations of the National Centre for Radiation Research and Technology animal care committee. The current study has also been authorized by the Institutional Animal Care and Use Committee Research Ethic Board. The Experimental protocol was conducted according to the guide for institutional Animal Care and Use Committee approved by Research Ethics Board, Faculty of Veterinary Medicine, Benha University (BUFVTM 04-04-022).

2.8. Experimental design

Rats were randomly divided into four equal groups as follows :
Group I (Control): Rats were injected intraperitoneally (i.p) with saline.
Group II (DMH): Rats injected with DMH (20 mg/kg b wt) subcutaneously (s.c) once a week for eight weeks.
Group III (DMH+ 5-FU): Rats injected with DMH (20 mg/kg b wt/s.c) once a week for eight weeks then it treated with 5-FU (12.5 mg/kg b. wt/ i.p) 3 times weekly for one month.
Group IV (DMH+ 5-FUNG): Rats injected with DMH (20 mg/kg b wt/s.c) once a week for eight weeks then it treated with 5-FUNG (2.5 mg/kg b. wt/ i.p) 3 times weekly for one month.

2.9. Sampling
Blood and Liver samples:

Blood samples for serum separation were taken by heart puncture. Serum was separated by centrifugation at 3000 rpm for 10 minutes. The separated serum was stored at -80 C until used for determination of liver marker enzymes [Alanine transaminase (ALT), Aspartate transaminase (AST)]. After blood sample collection rats were scarified by cervical decapitation, then liver was removed, cleaned with saline, weighted, and stored at -80 C till RNA extraction for determination of Interleukin -1β (IL-1β) gene expression by reverse transcription polymerase chain reaction (RT-PCR) .

2.10. Analysis :
2.10.1. Biochemical analysis:

Serum AST (AST, EC 2.6.1.1) and ALT (ALT, EC 2.6.1.2) activities were determined according to the assay protocols outlined in the commercial kits obtained from Spectrum Diagnostic Company in Cairo, Egypt.
Liver MDA and SOD were determined by a commercial kit from Bio-diagnostic Company in Cairo, Egypt according to (Nishikimi et al., 1972) and (Satoh, 1978) respectively. GSH in liver tissue was determined according to the methods described by Moron et al .(1979) ,.

2.10.2. Quantitative RT-PCR analysis

Interleukin-1β (IL-1β) gene expression was detected in liver tissues after the extraction of RNA with the RNA Purification Kit and synthesis of the complementary DNA (cDNA) by Reverse Transcription Kits. Real-time PCR was executed with a StepOnePlus thermal cyclerand SYBR Green PCR Master Mix. β-actin as internal reference was used to normalize the expression of the target genes (table 1). The relative mRNA expression of the target genes was calculated (Livak and Schmittgen, 2001) .

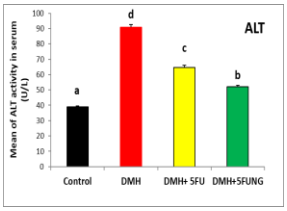
Table (1): Forward and reserve primers sequences for genes that used for qPCR:

Gene	Forward primer (′5 ------ ′3)	Reverse primer (′5 ------ ′3)
IL-1β	CACCTCTCAAGCAGAGCACAG	GGGTTCCATGGTGAAGTCAAC
β-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

2.11. Statistical analysis
Statistical analysis of the data and perform tests of significance was carried out using SPSS ver. 20.0 . A one-way ANOVA test was used, followed by a post hoc test for multiple comparisons. P< 0.05 is used to determine significant difference between groups.

3. RESULTS

3.1. Nanoparticle characterization:
Cited in previous our research (Abo-Zaid et al., 2023).
3-2 LD50 of gelatin-based P(G/AAc)/5-FU nanogel:
Cited in previous our research (Abo-Zaid et al., 2023).
Effects of 5-FU and 5-FU nanogel on serum liver marker enzymes activity
The obtained results presented in figure (1) showed significant increase in serum ALT and AST activities were observed in DMH group when compared with control group. The rats treated with 5-FU or 5-FU nanogel exhibited significant decreased in the liver enzymes activity when compared to DMH group .



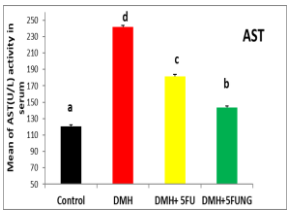


Fig. (1): Effect of 5-FU and 5 –FU nanogel, on the liver marker enzymes of DMH treated rats

Effect of 5-FU and 5-FU nanogel on hepatic IL-1β gene expression level .

Compared to control group, rats treated with DMH exhibited a significantly upregulation in liver IL-1β gene expression level .However, the 5-FU or 5-FU nanogel treated groups showed significantly down-regulation in IL-1β gene expression compared to DMH treated group (figure 2).

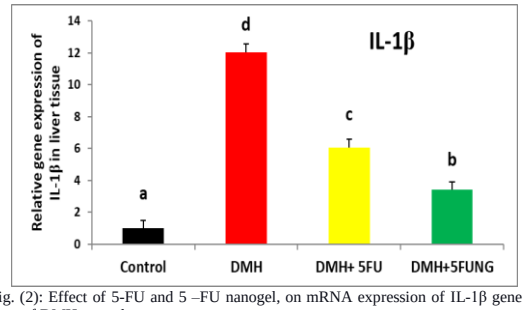


Fig. (2): Effect of 5-FU and 5 –FU nanogel, on mRNA expression of IL-1β gene in the liver of DMH treated rats.

Effect of 5-FU and 5-FU nanogel on hepatic oxidative stress and antioxidant markers .

DMH exhibits a significant decreased in GSH concentration and SOD activity when compared with control. Moreover, DMH exhibits a significantly increased in the concentration of MDA when compared to the control group. Treatment with 5-FU or 5 FU nanogel significantly increased GSH concentration and SOD activity with a significant decrease in MDA level Compared with DMH group (Figure 3).

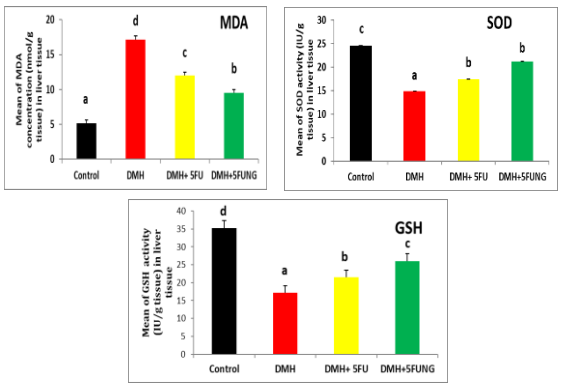


Fig. (3): Effect of 5-FU and 5 –FU nanogel, on hepatic oxidative stress and antioxidant markers of DMH treated rats.

4. DISCUSSION

1,2 Dimethylhydrazine (DMH) is extremely toxic to the liver and other body organs. As a result of its metabolism in the liver, it produces alkyl free radicals and extremely reactive carbonium ions, which seriously harm the liver and induce fatty infiltration and necrosis (El-Bagoury et al., 2019).

Chemotherapy is used to stop the spread of cancer cells; however, it cannot tell the difference between healthy and malignant cells (El-Naggar et al., 2018). Two main barriers to effective cancer chemotherapy are toxicity and therapeutic resistance after treatment.

One common chemotherapeutic medication for treating different cancer, is 5-fluorouracil (Lee et al., 2016) .

Drug delivery currently in development, the use of nanoparticles to transport pharmaceuticals, to cancer cells is one way that nanotechnology is being applied in medicine. Drugs or genes have been delivered to different malignancies using nanoparticles (Wang et al., 2013 and Reddy and Kumar., 2019).

In the current study, hepatotoxic effects were brought on by DMH therapy via the free radical pathway (El-Bagoury et al., 2019 and Al-Zharani et al., 2022). The biochemical manifestation of DMH-induced hepatotoxicity was demonstrated by notable increases in Alanine transaminase (ALT) and Aspartate transaminase (AST) activities, indicating liver injury. In cases of necrosis or membrane injury, the increases in serum ALT and AST activities are typically caused by these enzymes leaking from the liver cytosol into the blood, making them powerful indicators of hepatic damage (McGill, 2016 and (Shebbo et al., 2020). Treatment with 5-fluorouracil nanogel (5-FUNG) exhibited a more potent effect than 5-fluorouracil (5-FU) in decreasing liver enzyme activities. This may be due to the role of the nanogel drug delivery system in delivering the drugs in the site of action with low dose (Abd El-Karim et al., 2015).

In the existing study the obtained results showed significant increase in liver MDA level and marked decrease in GSH and SOD in DMH treated rats. One possible explanation for the decline in SOD and GSH level in rats treated with DMH alone may be due to the dangerous effect caused by DMH on liver. This damage causes free radicals to multiply and increase, which in turn causes the cells that store GSH to become stressed out and less able to fight off the free radicals produced by the injury (Sisein et al., 2016). Also, DMH increased the level of MDA and this can be explained by the increasing tissue lipid peroxidation levels suggesting that oxidative stress was elevated during DMH-induced carcinogenesis (Karthik Kumar et al., 2010 and El-Bagoury et al., 2019). 5- FU nanogel treated group showed a significant decreased in MDA and increased in GSH and SOD when compared to DMH group alone and this may be due to the ability of nanogel to decrease the side effect of DMH on liver tissue by decreasing the oxidative stress in the liver tissue. This may be due to the antioxidant effect of the nanogel which may be attributed to the presence of Gelatin in the component of nanogel. Gelatin is a natural biomaterial consisting of various amino acids (hydroxyproline, serine, arginine, lysine and aspartic and glutamic acids) thus displaying various health biological activities such as reducing oxidation (Shiao et al., 2021) .

This study showed that DMH increased the inflammatory marker of liver IL-1β gene expression when compared to the control group. This can be explained by the hepatocarcinogen action of DMH that induces oxidative stress, hepatotoxicity, and hepatocellular carcinoma upon its metabolism in the liver (Shebbo et al., 2020). Also many studies demonstrate the relation between the oxidative stress and inflammatory reactions in promotion and initiation of tumors (Giftson et al., 2010 and Perše & Cerar, 2011). On the other hand, 5-FU nanogel decreased the inflammation produced by DMH and had a more potent effect than 5 FU alone by decreasing oxidative stress in the liver tissue due to the presence of gelatin, a natural component of the nanogel that has anti-inflammatory and antioxidant properties.

5. CONCLUSIONS

In conclusion, the injection of DMH exhibits severe liver damage and treatment with 5-FU nanogel improved the toxic effect of the DMH due to the antioxidant and anti-inflammatory activity of nanogel which minimizes the damaging effect of DMH toxicity.

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