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The Pharmacological Effect of Gum Arabic on Liver Hyperplasia in the Presence or Absence of Laser Beam

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Abstract:
Background: The pharmacological effect of Gum Arabic with and without laser exposure is largely unknown. Objective: This study aimed to explore the pharmacological effect of GA on hepatic hyperplasia induced by diethylnitrosamine (DENA) in Balb-C mice and the changes in lipid peroxidation, apoptosis and histopathological changes in hepatic hyperplasia mice with or without laser irradiation. Results: In vivo studies showed that GA was biologically inactive expect when combined with laser irradiation in healthy and in hepatic hyperplasia mice, it leds to elevation in lipid peroxidation level. Conclusion: GA is safe and did not induce biological alterations. Co-administration of GA with laser induced lipid peroxidation.

Keywords*: hepatic hyperplasia mice, gum Arabic, apoptosis, MDA, histopathology*

1. Introduction

Hepatic cancer is the third leading cause of cancer deaths worldwide due to prevalence of chronic hepatitis B and C which considered the highest risk factor for incidence of hepatic cancer (1, 2). Diethylnitrosamine (DENA) is often used as a carcinogenic agent in combination with carbon tetrachloride (CCl_4) as a tumor promoter. DENA is used to persuade liver cancer in experimental animal models (3) through formation of preneoplastic foci, neoplastic nodules, and ultimately HCC nodules of various sizes (4). The distinct initiation-promotion steps of the multistage hepato-carcinogenesis model are well recognized but the time of initiation depends on the promoters ability to effectively facilitate the cloned expansion of preneoplastic cells (5).

GA is a naturally stirring exudate from Acacia senegal and Acacia seyal trees, and acquires a highly branched polysaccharide structure consisting of a complex mixture of potassium, calcium and magnesium salts from Arabic acid, with residues of galactose, rhamnose, glucuronic acid and arabinose [6]. GA acquires a remarkable surface-active and rheological properties [7, 8], therefore being recognized as non-toxic for humans [9]. Recently, the use of GA has been extended to nanomedicine fields, due to its biocompatibility for in vivo and in vitro applications [10].

The current study is intending to estimate, in presence and absence of near infrared (NIR) laser irradiation, the therapeutic effects of GA on hepatic hyperplasia induced by DENA/CCl_4 in mice.

2. Material and methods

All chemicals were purchased from (Sigma/Aldrich, VA, USA) except mentioned.

2.1. In vivo experiments

2.1.1. Laser irradiation conditions for mice

The laser source was a diode array laser from Quanta System (Milan, Italy) emitting at 800 nm. The nominal output energy (continuous wave) was 0.4 W. Laser spotlight was performed under the following conditions: wavelength: 807 nm, average power: 50 W for a spot size of 2-cm diameter. Mice chest and abdomen were irradiated by NIR laser for 10 min. Mice received one session in each laser irradiated mouse. The laser energy was delivered to the treatment site in a non-contact mode from the skin surface.

2.1.2. Design of mice experiments

Male wild type Balb-c mice (Theodor Bilharz institute, Cairo, Egypt) were used in all experiments. Six to eight weeks old mice (18-20 g) were maintained in a temperature controlled environment at 24 °C with a 12 h light/dark cycle, and were provided with drinking water and feed ad libitum. Animal experiments were performed according to the guidelines for the animal care of the Ethical Committee, National Research Center, Cairo, Egypt. We follow the international guidelines.

Mice were subdivided into 9 different groups (n = 16 / group), as the following:

- Untreated Group: Untreated control mice.
- Vehicle Group: Mice were feed by oral gavage of 100 µl corn oil dissolved in saline every 2 w for 16 w.
- Laser Group: Mice were irradiated by NIR laser for 10 min.
- GA Group: Mice were IV- injected with a single dose of GA (0.2 mg/100 µl/mice) through tail vein.
- GA + Laser Group: Mice were IV- injected with a single dose of GA (0.2 mg /100 µl/mice), then after 2 h, mice were irradiated by NIR laser for 10 min.
- DENA/CCl_4 Group: Mice were injected intraperitonealy (IP) by DENA (50 mg/kg) at the same time mice were feed by oral gavage CCL_4 (2 ml/kg) dissolved in com oil (1: 2 V/V), each were administrated once for 16 w every 2 w.
- DENA/CCl_4+ Laser Group: Mice received DENA/CCl_4 for 16 w and then irradiated by NIR laser for 10 min.
- DENA/CCl_4 + GA Group: Mice received DENA/CCl_4 for 16 w and then IV-injected with a single dose of (GA 0.2 mg/100 µl/mice).
- DENA/CCl_4+ GA + laser group: Mice received DENA/CCl_4 for 16 w then IV-injected with a single dose of GA (0.2 mg/100 µl/mice), then after 2 h, mice were irradiated by NIR laser for 10 min. After one month from first treatment, mice were scarified by cervical dislocation and immediately dissected. The liver were excised from all groups and rinsed multiple times in PBS, and part of it preserved in 4% para-formaldhyde for histochemical analysis and the other part homogenized following the method described by Blalock et al., [11] for biochemical estimations.

2.2. Histological analysis

2.2.1. Haematoxyline and eosin

Histopathological examination by haematoxyline and eosin (H & E) staining Paraffin-embedded lung tissue sections on slides were stained with H & E stain for histopathological analysis and examined by a pathologist.

2.2.2. Apoptosis and necrosis

Mode of cell death that predominates in liver tissues was scrutinized in sections using double staining with acridine orange and ethidium bromide (AO/EB). Liver sections were deparaffinized, rehydrated and then rinsed in PBS before stained with equal volumes of AO (100 µg/ml) and EB (100 µg/ml) in PBS. Images were visualized using a micro-scope (Axiostar plus, Zeiss, Goettingen, Germany) equipped with digital camera (PowerShot A20, Canon, USA). Live cells display a normal green nucleus, early apoptotic cells have bright green nucleus with condensed or fragmented chromatin; late apoptotic cells display condensed and fragmented bright green to yellow chromatin and cells that have died from direct necrosis have a structurally normal orange to red nucleus [12].

2.2.3. Lipid peroxidation

The level of malondialdehyde (MDA), which is the end product of lipid peroxidation, was measured in the lung homogenates using commercially supplied kits (Biodiagnostic). In which thiobarbituric acid reactive substances detected spectrophotometrically at 535 nm [13].

2.2.4. Statistical analysis

Differences between non-treated cells and treated cells were analyzed using an unpaired student's t-test. $p < 0.05$ was considered significant. Data were statistically analyzed using the Statistical Package for Social Scientists (SPSS) 10.00 for windows (SPSS Inc., Chicago, USA). The data were expressed as mean ± SE.

3. Results

3.1. In vivo Experiments

3.1.1. Histopathological examination by H&E staining

Liver tissue sections from the subsequent five groups (untreated, Vehicle, GA, laser and GA + laser) showed normal histopathology structure of liver tissue (H&E x400) as shown in fig 1.

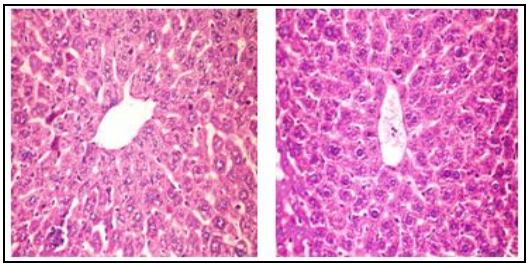

Figure 1: Representative photomicrographs of a normal histopathology structure of hepatic lobule (H&E x 400) in untreated, Vehicle, GA, laser and GA + laser groups

DENA/CCl_4 group showed karyomegaly of hepatocytic nuclei and oval cells hyperplasia, focal hepatic necrosis associated with leucocytic cell infiltration as seen in fig 2.

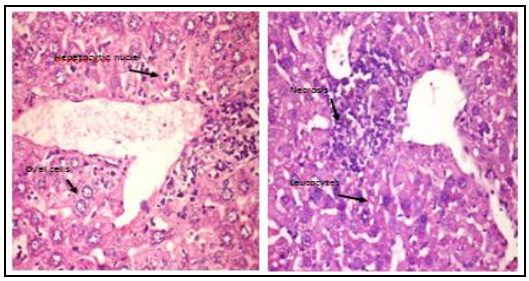

Figure 2: Representative photomicrographs of histopathology structure of hepatic lobule (H&E x 400) in DENA/CCL_4.

Moreover, DENA/CCl_4+laser groups showed diffuse oval cells hyperplasia and sinusoidal leucocytosis together with karyomegaly of nuclei as made known in fig 3.

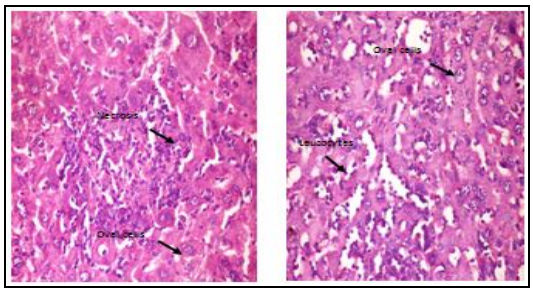

Figure 3: Representative photomicrographs of histopathology structure of hepatic lobule (H&E x 400) in DENA/CCL_4 + laser

Also in DENA/CCl_4 + GA showed cholangioma, oval cells proliferation, apoptosis of hepatocytes and oval cells hyperplasia as publicized in fig 4.

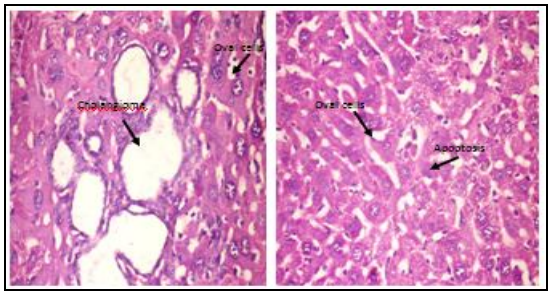

Figure 4: Representative photomicrographs of histopathology structure of hepatic lobule (H&E x 400) in DENA/CCL_4 + GA.

DENA/CCl4 + GA + laser showed hepatocytomegaly, karyomegaly, focal hepatic necrosis associated with inflammatory cells infiltration, oval cells hyperplasia, cytomegaly of hepatocytes and karyomegaly of hepatocytic nuclei as seen in fig 5.

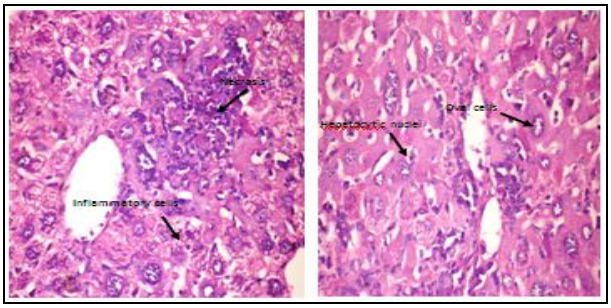

Figure 5: Representative photomicrographs of histopathology structure of hepatic lobule (H&E x 400) in DENA/CCL_4 + GA+ laser.

3.2. Apoptosis and necrosis staining

The mode of cell death was examined in the liver tissue sections using AO/EB. As shown in fig. 6, the following groups: untreated, vehicle, GA, and laser, most of the cells were viable. They have uniform green nuclei with organized structure indicates the intact DNA. Additionally, in DENA groups and in case of being treated with laser, GA, or GA+laser also showed preponderance green staining signal of viable cells and a normal level of apoptotic cells.

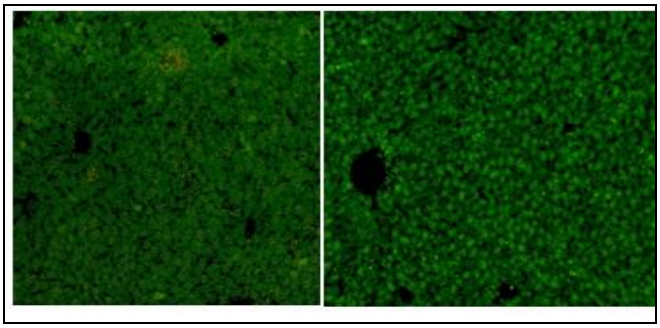

Figure 6: Representative image for the analysis of cell death type in liver section from different mice groups, as stained by AO/EB and captured under fluorescence microscope (X 200). Vital cells display a normal green nucleus

3.3. Lipid Peroxidation

MDA levels in the liver are indication for magnitude of oxidative stress that reflects lipid peroxidation extent in liver homogenate. The control groups receiving vehicle, laser and GA, there was no significant difference detected between them. The only significant elevation in MDA level was detected in the normal and the DENA/CCl_4 groups that were treated with GA in presence of laser irradiation when compared with untreated and DENA/CCl_4 groups as shown in fig 7.

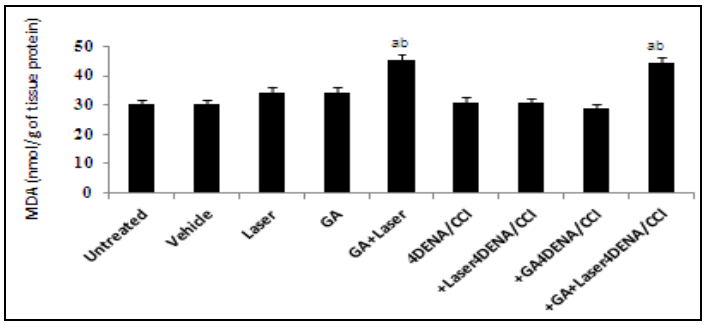

Figure 7: MDA level in liver homogenate of different groups as measured by commercial kit. Data was expressed as the mean ± SE of MDA concentration (nmol/g tissue protein). Statistical analysis was carried out using one way ANOVA followed by Post Hock LSD test. The columns label: (a) Significant at $p<0.05$ comparing with normal group, (b) Significant at $p<0.05$ comparing with DENA/CCL_4 group

4. Discussion

In the present study we investigate the effect of GA with or without laser exposure on hepatic hyperplasia mice. To the best of our knowledge, none of the previous studies detect the pharmacological effect of GA on tumor.

Our histopatological results revealed that cancer in liver tissue was initiated in mice after DENA and this result is in agreement with previous reports [14]. DENA is a DNA alkylating agent leading to the formation of mutagenic DNA adducts [15]. Moreover, DENA bioactivation by cytochrome P450 can generate reactive oxygen species (ROS) which damage DNA [15, 16]. Our histopatological findings revealed no histopathological changes due to GA and laser, both are safe. This is in agreement with El-Sayed et al., [17] and

Liu et al., [18]. It was found that found that laser beam of 51W/cm^2 did not affect the cells viability in which cancer cells were robust and the vessels were intact. DENA/CCl_4 groups treated with laser, GA and GA+laser revealed no histological alteration in liver tissue, which indicated that GA and laser did not protect or repair the liver tissue damage induced by DENA/CCl_4. Al-Kenanny et al., [19] observed no change in liver injury induced by gentamycin upon using GA in mice.

One of the hallmarks of cancer is to avoid apoptosis and continue to propagate [20]. Apoptotic cell death involves two distinct pathways, the death receptor (extrinsic) pathway and the mitochondria (intrinsic) mediated pathway [21, 22]. We observed no apoptotic cell death was induced upon treating the DENA/CCl_4 groups treated with laser, GA and GA+laser. This reflects that GA alone or in combination with laser beam did not encourage apoptotic cell death.

If the antioxidant defense mechanism fails to control the increased reactive oxygen species (ROS), they attack polyunsaturated fatty acids in cell membranes and induce the release of toxic and reactive aldehyde metabolites, such as MDA [23]. MDA reacts readily with protein or DNA forming adducts, which are considered to be highly genotoxic [24]. In the present study, MDA level significantly elevated in GA+laser group as well as hepatic hyperplasia groups treated with GA+laser. The increased lipid peroxides in hepatic hyperplasia groups may be because of uncompromised production of free radicals by DENA [14], which was reported to generate lipid peroxidation products in general [25]. Yet, further studies are required to determine GA effect on lipid peroxidation.

5. Conclusion

GA is safe and did not induce biological alterations. Co-administration of GA with laser induced lipid peroxidation.

6. References

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