RESEARCH ARTICLE

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Synergistic Antigenotoxic and Antioxidant Action of Gum Arabic and Eugenol in Rat Liver Following Induction of Colorectal Carcinogenesis

Nayanna de Oliveira Ramos Melo[1]*, Matheus De Sousa Silva[2], João Pedro Navarro Ribeiro[2] , Weslei Pires Lima[2], Francisco Vagnaldo Fechine Jamacaru[3], Bruno Coêlho Cavalcanti[4], Antônio Adailson De Sousa Silva[4], Conceição Aparecida Dornelas[5]

Abstract

Objective: Much research has been conducted to identify natural antioxidant and antimutagenic compounds capable of preventing, reverting or treating conditions caused by oxidative stress and genotoxicity. In this study we evaluated the effects of 10% gum arabic (GA) and eugenol (EUG) on hepatic oxidative stress and genotoxicity induced by dimethylhydrazine (DMH) in rats. **Methods:** The prevention arm of the study included 4 control groups and 4 experimental groups. Once a week for 20 weeks, the controls received saline s.c. while the experimental groups received DMH at 20 mg/kg s.c. During the same period and for an additional 9 weeks, the animals received either water, 10% GA , EUG or 10% GA + EUG by gavage. The treatment arm of the study included 4 control groups and 4 experimental groups. Once a week for 20 weeks, the controls received saline s.c. while the experimental groups received DMH at 20 mg/kg s.c. During the subsequent 9 weeks, the animals received either water, 10% GA, EUG or 10% GA + EUG by gavage. Finally, the livers were harvested for histopathological study with HE, measurement of genotoxicity and oxidative stress. **Result:** Genotoxicity and oxidative stress were found to be significantly lower in Group XII (animals treated concomitantly with GA and EUG). This is the first study to observe the synergistic action of GA and EUG administered concomitantly in this scenario. **Conclusion:** Indicating a synergistic antigenotoxic and antioxidant effect on liver cells in rats with DMH-induced colorectal carcinogenesis.

Keywords: Eugenol- Arabic gum- Liver- Oxidative stress- Genotoxicity

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Introduction

1,2-dimethylhydrazine (DMH) is an indirect and specific inductor of carcinogenesis through the activation of its metabolite, azoxymethane (AOM). DMH is commonly used in animal colorectal cancer models (Sivaranjani et al., 2016; Juca et al., 2014) but is also known for its hepatocarcinogenic potential (Shebbo et al., 2020).

DMH is metabolized in the liver mediated by cytochrome P450, resulting in the release of free radicals (Hrycay and Bandiera, 2015). The imbalance between the release and clearance of free radicals through antioxidant systems (Bucher, 2019) leads to the accumulation of free radicals (Wang et al., 2021) and excessive oxidative stress.

The final carcinogenic metabolite of DMH (the highly reactive methyldiazonium ion) methylates the DNA, forming O6-methyl-deoxyguanosine and N7-methyl-deoxyguanosine. These nucleosides are capable of inducing mutations, especially in genes involved in the Wnt signaling pathway, which is crucial for the development of liver tumors (hepatocarcinogenic potential (Shebbo et al., 2020).

Based on the known association between oxidative stress, genetic mutations and their systemic repercussions, much research has been conducted to identify natural

*[1]Postgraduate Program in Medical-Surgical Sciences, School of Medicine, Federal University of Ceará, Fortaleza, Brazil. [2]School of Medicine, Federal University of Ceará, Fortaleza, Brazil. [3]Researcher at NRDM (Nucleus of Research and Development of Medicines), Laboratory of Pharmacology and Preclinical Research, School of Medicine, Federal University of Ceara, Fortaleza, Brazil. [4]Nucleus for Research and Development of Medicines (NPDM), National Laboratory of Experimental Oncology, Federal University of Ceará, Fortaleza, Brazill. [5]Permanent Professor of the Postgraduate Program stricto sensu in Pathology and Medical-Surgical Sciences, School of Medicine, Federal University of Ceara Fortaleza, Brazil. *For Correspondenc e:nayannaoliveira16@hotmail.com*

antioxidant and antimutagenic compounds capable of preventing, reverting or treating conditions such as cancer, in which oxidative stress and genotoxicity are important pathophysiological factors (Wang et al., 2021). Among the advantages of most natural compounds with pharmacological action are effectiveness, safety, easy access and low cost (Amir Rawa et al., 2022).

Gum arabic (GA) is a gummy exudate harvested from the acacia tree Senegalia senegal through incisions in the trunk. Widely employed in the food and pharmaceutical industry, GA has a range of pharmaceutical properties (antibacterial, antifungal, analgesic, immunomodulating) of which the most important is antioxidant and anticarcinogenic action (Ashour et al., 2022) .

The antioxidant activity of GA is protective against nephrotoxicity (Gado and Aldahmash , 2013) and oxidative stress caused by contrast radiography (Garawani et al., 2021) and has been shown to reduce oxidative stress and boost antioxidant response in sickle cell anemia (Kaddam et al., 2017). It also protects against hepatoxicity induced in mice (Gamal el-din et al., 2003) and rats (Pal et al., 2014).

Eugenol (EUG) is a volatile phenylpropanoid found in clove oil, with a characteristic smell and light yellow color (Haro-González et al., 2021) . It has several biological properties (analgesic, anti-inflammatory, antimicrobial, antipyretic), with emphasis on antioxidante 7 and anticarcinogenic action (Petrocelli et al., 2021). The antioxidant action of EUG effectively reduces oxidative stress in the pancreas (Oroojan et al., 2020), provides neuroprotection in Alzheimer disease (Amir Rawa et al., 2022), protects rat liver tissue by strengthening antioxidant response (Niazi et al., 2021) and reduces oxidative stress induced by arsenic trioxide (Binu et al., 2018) .

In an attempt to improve the therapeutic response of a drug for neoplastic diseases, the amount administered, its concentration or even association with other drugs which have already been studied a good response for a better effectiveness of the treatment can be increased. It was with this in mind that we proposed to evaluate the antineoplastic potential of the association of gum arabic and eugenol. The purpose of this study was to evaluate the effects 10% GA and EUG on oxidative stress and genotoxicity in the liver of rats submitted to colorectal carcinogenesis.

Materials and Methods

The study protocol complied with the guidelines of the National Board for the Control of Animal Testing (CONCEA) and was approved by the Animal Research Ethics Committee (CEUA) of the Federal University of Ceará (UFC) (protocol #1675020519). The study is divided into two parts (prevention and treatment) to test the potential of gum arabic and eugenol on oxidative stress and genotoxicity hepatic induced by dimethylhydrazine (DMH) in rats.

In prevention, the use of substances concomitantly with the carcinogen tests whether the substances act to prevent oxidative stress and genotoxicity. In treatment, (substance use after exposure to carcinogens) evaluates whether substances can treat oxidative stress and genotoxicity previously induced by the carcinogen. The prevention was evaluated using, 4 control groups (Ia, IIa, IIIa and IVa each group with n = 6) and 4 experimental groups (V, VI, VII and VIII, each group with n = 10), the study used 64 female Wistar rats. Once a week, for 20 weeks, the control groups received saline solution (s.c), while the experimental groups received DMH at 20 mg/kg s.c. During 29 weeks, the animals received water (groups Ia and V), GA 10% (groups IIa and VI), EUG (groups IIIa and VII) and GA 10% + EUG (groups IVa and VIII) by gavage.

The treatment was evaluated using, 4 control groups (Ib, IIb, IIIb , IVb each group with n = 6) and 4 experimental groups (IX, X, XI and XII, each group with n = 10), the study used 64 female Wistar rats. Once a week for 20 weeks, the control groups received saline s.c., while the experimental groups received DMH at 20 mg/kg, s.c. During the subsequent 9 weeks, the animals received water (groups Ib and IX), 10% GA (groups IIb and X), EUG (groups IIIb and XI) or 10% GA + EUG (groups IVb and XII) (Figure 1).

Carcinogen (DMH)

To induce cancer, we used symmetrical 1,2-dimethylhydrazine dihydrochloride (Sigma-Aldrich Brasil Ltda) dissolved in a previously prepared 0.9% NaCl solution containing 1.5% EDTA as vehicle, adjusted to a final pH of 6.5 using a NaOh solution (Larangeira et al., 1998) .The carcinogen was administered s.c at 20 mg/kg body weight once a week for 20 weeks (Ravnik-Glavac et al., 2000).

Gum arabic (GA)

GA (Dinâmica Química Contemporânea Ltda) was diluted in distilled water at 10% (Nasir et al., 2010) and administered by gavage at 5 mL/kg body weight 4 times a week for 9 weeks.

Eugenol (EUG)

EUG (Laboratório Quinari) was administered orally using a pipette at 100 mg/kg body weight 3 times a week for 9 weeks (Manikandan et al., 2010).

Surgical procedure

By the end of the experiment, the animals were anesthetized with ketamine (100 mg/Kg body weight) and xylazine (10 mg/Kg body weight) i.p. and submitted to longitudinal xyphopubic laparotomy and the livers were harvested.

Histopathological study

The livers were fixed in 10% buffered formalin and then taken to histotechnical processing. After embedment in paraffin making 5 µ thick cuts were made and stained with hematoxylin and eosin (HE) and evaluated microscopically.

Preparation for biological assays

To evaluate oxidative stress and genotoxicity we macerated liver fragments in phosphate buffered saline

(PBS) at 4ºC. Cells were obtained by filtering.

Oxidative stress measurement
ROS dosage

The production of intracellular reactive oxygen species (ROS) in colon, liver and blood was quantified by exposing cell preparations to 20 µM 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) at 37ºC for 30 min, in the dark. H2DCFDA is oxidized by intracellular ROS into the highly fluourescent compound 2',7'-dichlorodihydrofluorescein (DFC) (Crow, 1997; Hempel et al., 1999). The larger the amount of ROS in the cells, the greater the fluorescence observed (Lebel et al., 1992). In our experiment, ROS included the radicals hydoxyl (HO•), peroxyl (ROO•), peroxynitrite (ONOO•), hydrogen peroxide (H2O2) and singlet oxygen (1O2) (Bartosz, 2006). The preparations were subsequently washed, resuspended in PBS and submitted to flow cytometry (Guava Technologies, Inc., Hayward, CA, USA) to quantify the fluorescence and ROS percentage of each sample. The assays were performed as three independent experiments in triplicate so that ten thousand events were analyzed per sample.

Glutathione dosage (GSH)

To measure the level of GSH, 3 mL blood was collected from each anesthetized animal, centrifuged and frozen in liquid nitrogen at -70ºC. The model of Sedlak and Lindsay (Sedlak and Lindsay, 1968) is based on reacting 2-nitrobenzoic acid with free thiol to produce mixed disulfide + 2-nitro-5-thiobenzoic acid quantified with a Beckman spectrophotometer at an absorbance of 412 nm.

Dosage of the concentration of thiobarbituric acid substances (TBARS)

Using peripheral blood, lipid peroxidation was quantified with the TBARS Assay. Bernheim et al., 1948).

Genotoxicity tests
Comet assay

The level of DNA damage was determined by comet assay under alkaline and neutral conditions, as described by Hartmann and Speit (1997) and Wojewodzka, Buraczewska, Kruszewski (2002), respectively.

Modified alkaline comet assay

The modified alkaline comet assay was used to increase the sensitivity and specificity of the comet assay. The method consists of adding the enzyme DNA-formamidopyrimidine glycosylase (FPG) which recognizes oxidized nitrogenated bases, as described for the alkaline comet assay with minor modifications.

Micronucleus assay

Using the acridine orange technique, the frequency of micronuclei suspended in hepatocytes was determined, as described by Hayashi et al. (1990).

Statistical analysis

The quantitative variables were also analyzed using the Shapiro-Wilk test to verify the normality of the distribution. After meeting this requirement, two-way analysis of variance was used to evaluate the effects of treatments (factor 1: AD, GA, EUG, and GA+EUG) and exposure to carcinogen (factor 2: DMH or SF) on genotoxicity and oxidative stress quantification parameters, considering both prevention and treatment protocols. The analysis was complemented by the Tukey's multiple comparisons test (comparisons between treatments in the exposed and non-exposed to carcinogen groups, as well as comparisons between exposed and non-exposed to carcinogen for each treatment).

All analyses used two-tailed tests, with a significance level established at 0.05 (5%), therefore, a P value less than 0.05 was considered statistically significant. GraphPad Prism software version 8.0 (GraphPad Software, San Diego, California, USA) was used for both statistical procedures and graph preparation.

Results

Histopathological study

The histopathological examination of the liver showed premalignant lesions (clear cell foci, steatosis, foci of tigroid cells and cysts containing fine flocculent eosinophilic material (hematoxylin crystals) (Figure 2). As for hepatocellular carcinoma in median lobe (Figure 3), hepatocelullar carcinoma (Figure 4) , hepatocellular carcinoma acinar (Figure 5) and – cholangioma (Figure 6) were observed in a few animals in the different groups subjected to the carcinogen, but no statistical differences were observed among them.

Oxidative stress measurement and genotoxicity tests
Prevention (use of substances concomitantly with the carcinogen)

The evaluation of oxidative stress for prevention in groups V, VI, VII, and VIII, through the measurement of reactive oxygen species (EROS), showed that it was significantly lower (**P < 0.01) in the groups treated with arabic gum GVI (DMH + GA 10%), eugenol GVII (DMH + EUG), and arabic gum and eugenol GVIII (DMH + GA 10% + EUG) compared to the untreated group GV (DMH + WATER) (Figure 7). The evaluation of oxidative stress for prevention in groups V, VI, VII, and VIII, through the measurement of GSH, showed that exposure to the carcinogen DMH significantly reduced the amount of GSH (+++P<0.001), regardless of the substance used (WATER, GA, EUG or GA+EUG) (Figure 7). The evaluation of oxidative stress for prevention in groups V, VI, VII, and VIII, through the measurement of TBARS, showed that it was significantly lower (*P<0.05) in group GVIII (DMH+ GA 10% + EUG) compared to the untreated group GV (DMH + WATER) (Figure 7).

The evaluation of genotoxicity for prevention, through the comet assay (without enzyme) (Figure 7) in groups V, VI, VII, and VIII, demonstrated that it was significantly lower in the eugenol-treated groups GVII (DMH + EUG) (***P<0.001) and GVIII (DMH + GA 10% + EUG) (***P<0.001) when compared to the untreated group GV (DMH + WATER). Additionally, group VII (DMH +

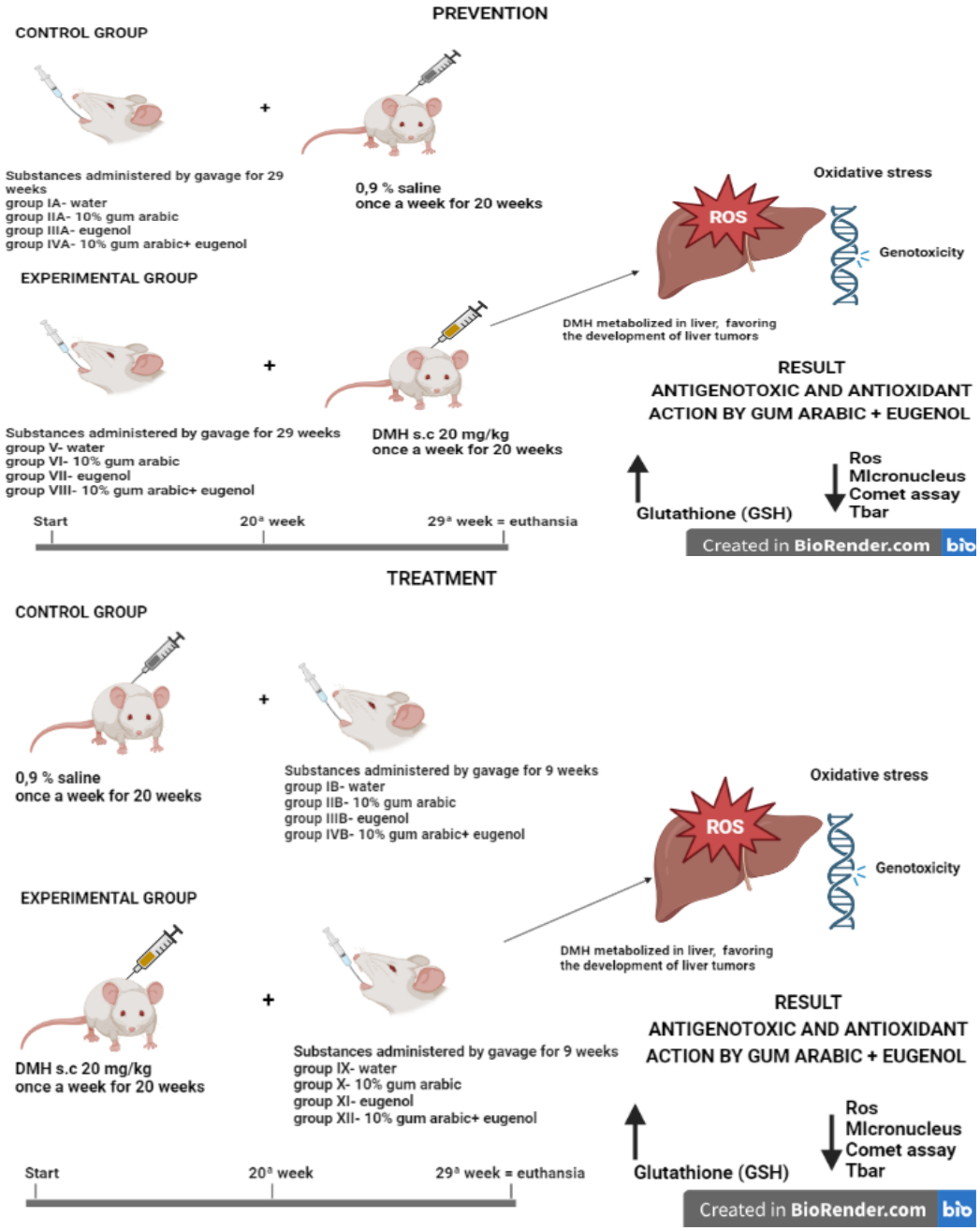

Figure 1. Design Experimental

EUG) significantly reduced ($$$P<0.001) when compared to group VI (DMH + GA 10%). Group VIII significantly reduced when compared to groups GVI (DMH + GA 10%) (###P<0.001) and GVII (DMH + EUG) (##P<0.01).

The evaluation of genotoxicity for prevention, through the comet assay (with enzyme) (Figure 7) in groups V, VI, VII, and VIII, demonstrated that it was significantly lower (***P<0.001) in the treated groups with gum arabic GVI (DMH + GA 10%), eugenol GVII (DMH + EUG), and gum arabic and eugenol GVIII (DMH+ GA 10% + EUG) when compared to the untreated group GV (DMH + WATER). Additionally, group VII (DMH+ EUG) significantly reduced ($P<0.05) when compared to group VI (DMH + GA 10%). Group VIII significantly reduced when compared to groups GVI (DMH + GA 10%) (###P<0.001) and GVII (DMH+ EUG) (#P<0.05).

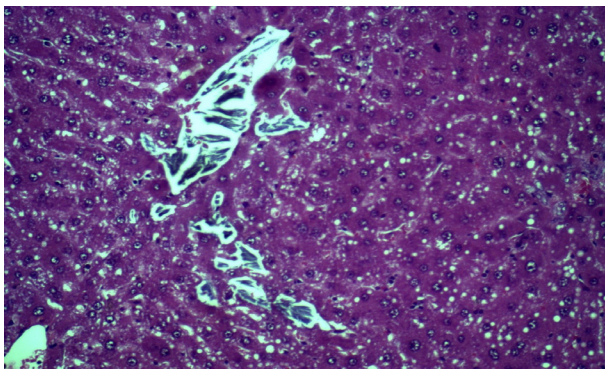

Figure 2. Cysts Containing Fine Flocculent Eosinophilic Material (Hematoxylin Crystals). The cysts are not lined by endothelial cells and do not compress the surrounding liver parenchyma. Legend : Stained with H&E (magnification: 100X) (Group GIX R5)

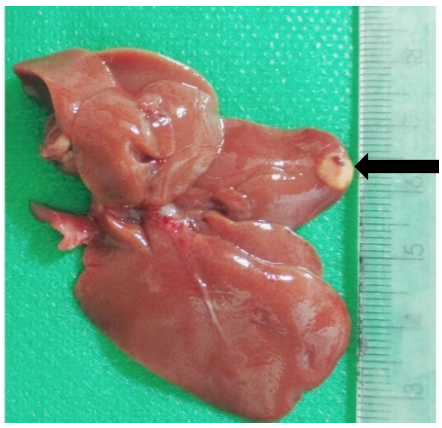

Figure 3. Hepatocellular Carcinoma in Median Lobe. Legend: The black arrow (Group XII R5)

The evaluation of genotoxicity for prevention, through the micronucleus test in groups V, VI, VII, and VIII, demonstrated that groups VII (DMH+ EUG) (**P<0.01) and VIII (DMH+ GA 10%+EUG) (***P<0.001) significantly reduced the amount of micronuclei compared to the untreated group GV (DMH + WATER) (Figure 7).

Treatment (use of substances after carcinogen exposure)

The evaluation of oxidative stress in the treatment, in groups IX, X, XI, and XII, through the measurement of ROS, showed that it was significantly lower (***P<0.001) in the treated groups with gum arabic GX (DMH + GA 10%), eugenol GXI (DMH + EUG), and gum arabic and eugenol GXII (DMH+ GA 10% + EUG) compared to the untreated group GIX (DMH + WATER). However, the group treated with gum arabic and eugenol GXII (DMH+ GA 10% + EUG) showed a significantly higher reduction (###P<0.001) when compared to the group treated only with gum arabic GX (DMH + GA 10%) (Figure 8).

The evaluation of oxidative stress in the treatment, in groups IX, X, XI, and XII, through the measurement of GSH, showed that it was significantly higher (***P<0.001) in the treated groups with gum arabic GX (DMH + GA 10%), eugenol GXI (DMH + EUG), and gum arabic and eugenol GXII (DMH+ GA 10% + EUG) compared to the untreated group GIX (DMH + WATER) (Figure 8).

The evaluation of oxidative stress in the treatment, in groups IX, X, XI, and XII, through the measurement of TBARS, showed that it was significantly lower (***P<0.001) in the treated groups with gum arabic GX (DMH + GA 10%), eugenol GXI (DMH + EUG), and gum arabic and eugenol GXII (DMH+ GA 10% + EUG) compared to the untreated group GIX (DMH + WATER). Furthermore, the eugenol GXI (DMH + EUG) (##P<0.01) and gum arabic and eugenol GXII (DMH+ GA 10%+ EUG) (###P<0.001) groups were significantly lower when compared to the GX group (DMH+ GA 10%) (Figure 8).

The evaluation of genotoxicity in the treatment, through the comet assay (without enzyme) (Figure 8), in groups IX, X, XI, and XII, showed that it was significantly lower (***P<0.001) in the treated groups with gum arabic GX (DMH + GA 10%), eugenol GXI (DMH + EUG), and gum arabic and eugenol GXII (DMH+ GA 10% + EUG) compared to the untreated group GIX (DMH + WATER).

Additionally, group XI (DMH+ EUG) presented a significantly higher damage index ($$$P<0.001) than observed in the GX group (DMH + GA 10%). However, the gum arabic and eugenol GXII (DMH+ GA 10%+

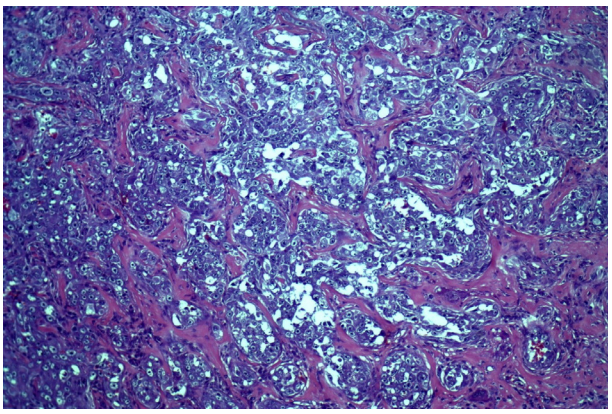

Figure 4. Hepatocelullar Carcinoma - Morphological Features of Clear Cell Hepatocellular Carcinoma with Trabecular/Solid Growth, Enlarged Phytoid Like Tumor Cells with Prominent Nuclei, Predominantly Pale Cytoplasm. Legend: Stained with H&E (magnification: 100X) (Group GVIII R1)

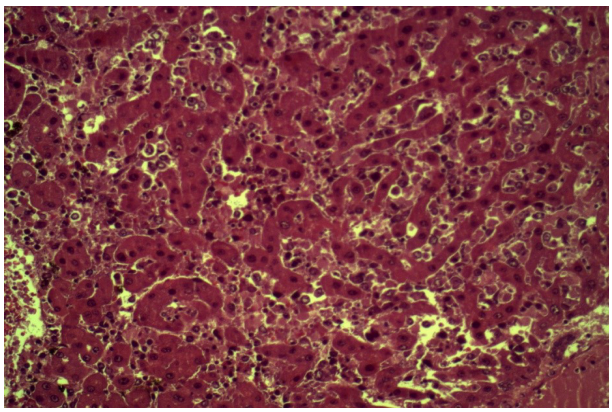

Figure 5. Hepatocellular Carcinoma Acinar. Neoplastic hepatocytes form a single layer some around a central clear space. Legend: Stained with H&E (magnification: 100X) (Group VII R2)

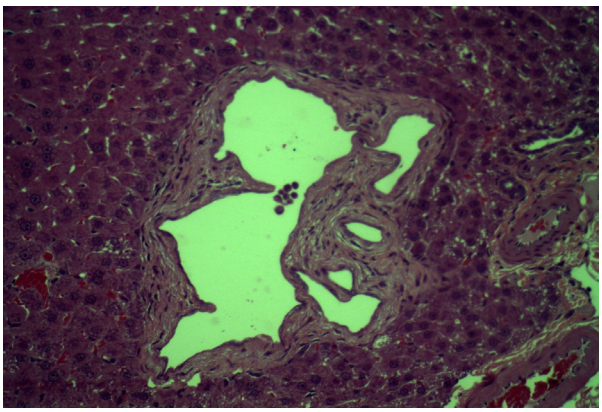

Figure 6. Cholangioma – Cysts with Single Layer of Flattened or Low Cuboidal Epithelium with Peripheral or Sub-Basilar Collagen Deposition and Compress the Adjacent Hepatic Parenchyma. Legend : Stained with H&E (magnification: 100X) (Group V R3)

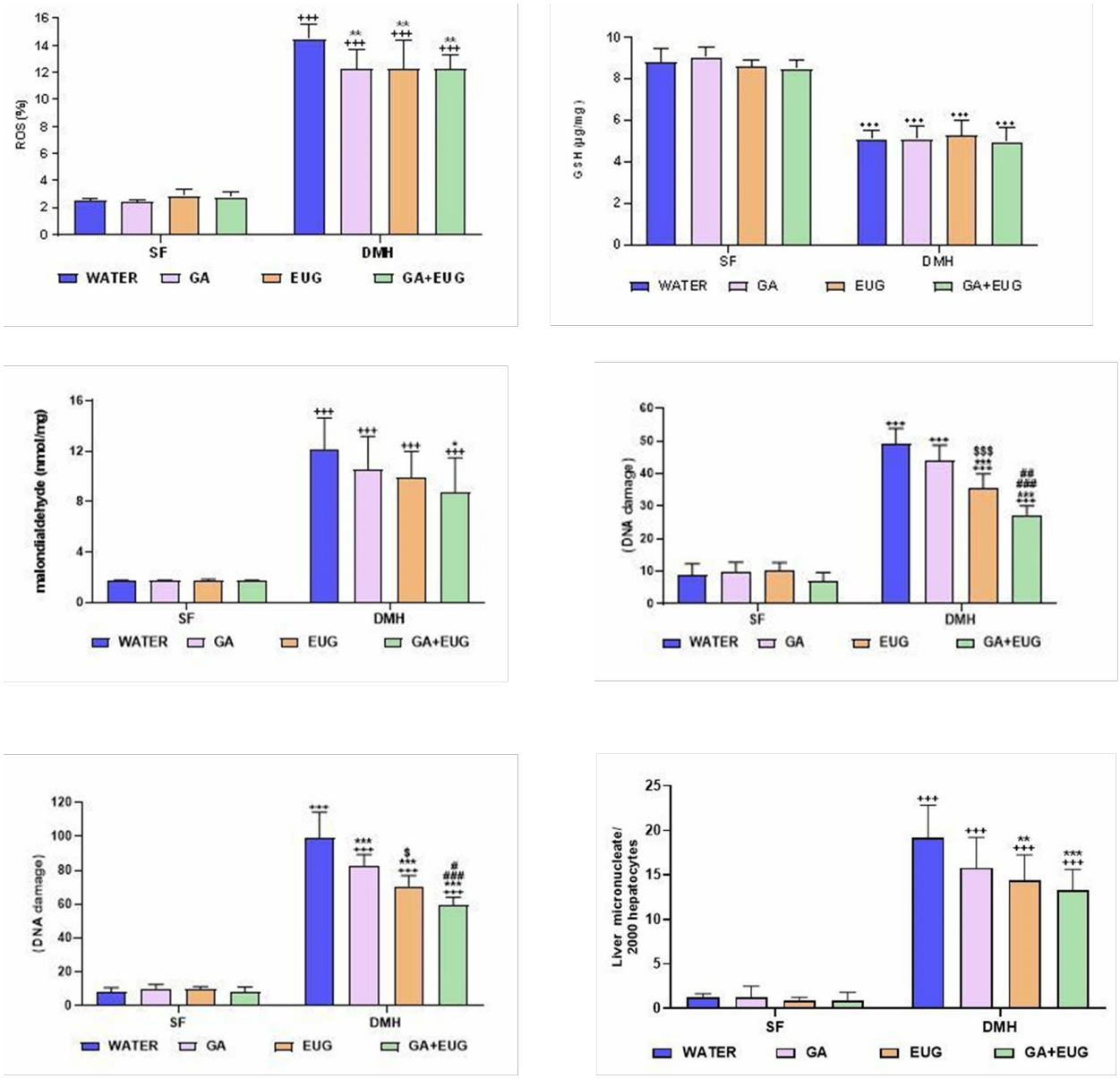

Figure 7. ROS Dosage, GSH Dosage, TBARS Dosage, Comet Test and Micronucleus Test in the Liver (prevention)

EUG) group was significantly lower than the XI group (DMH+ EUG) (###P<0.001) (Figure 8).

The evaluation of genotoxicity in the treatment, through the comet assay (with enzyme) (Figure 8), in groups IX, X, XI, and XII, showed that it was significantly lower (***P<0.001) in the treated groups with gum arabic GX (DMH + GA 10%), eugenol GXI (DMH + EUG), and gum arabic and eugenol GXII (DMH+ GA 10% + EUG) compared to the untreated group GIX (DMH + WATER). Furthermore, the gum arabic and eugenol GXII (DMH+ GA 10%+ EUG) group was significantly lower (###P<0.001) compared to the GX (DMH + GA 10%) and eugenol GXI (DMH + EUG) groups (Figure 8).

The evaluation of genotoxicity in the treatment, through the micronucleus test (Figure 8), in groups IX, X, XI, and XII, showed that it was significantly lower (***P<0.001) in the treated groups with gum arabic GX (DMH + GA 10%), eugenol GXI (DMH + EUG), and gum arabic and eugenol GXII (DMH+ GA 10% + EUG) compared to the untreated group GIX (DMH + WATER). However, the eugenol GXI (DMH + EUG) group (##P<0.01) and gum arabic and eugenol GXII (DMH+ GA 10%+ EUG) group (###P<0.001) were significantly lower compared to the GX group (DMH + GA 10%) (Figure 8).

Discussion

The research is part of PhD thesis, Postgraduate Program in Medical-Surgical Sciences

DMH is widely used to induce colon cancer in animal models. Initially, DMH oxidizes into azomethane, which is converted into AOM, then hydroxylated into methylazoxymethanol (MAM). Hydroxylation takes place primarily in the liver via cytochrome P450, but also to some degree in the colon mucosa. MAM is transported to the bowel by the bile and blood circulation in the form of glucuronides. When these are hydrolyzed by bacterial enzymes, an active carcinogen is released (Weisburger, 1971).

MAM is chemically unstable and decomposes

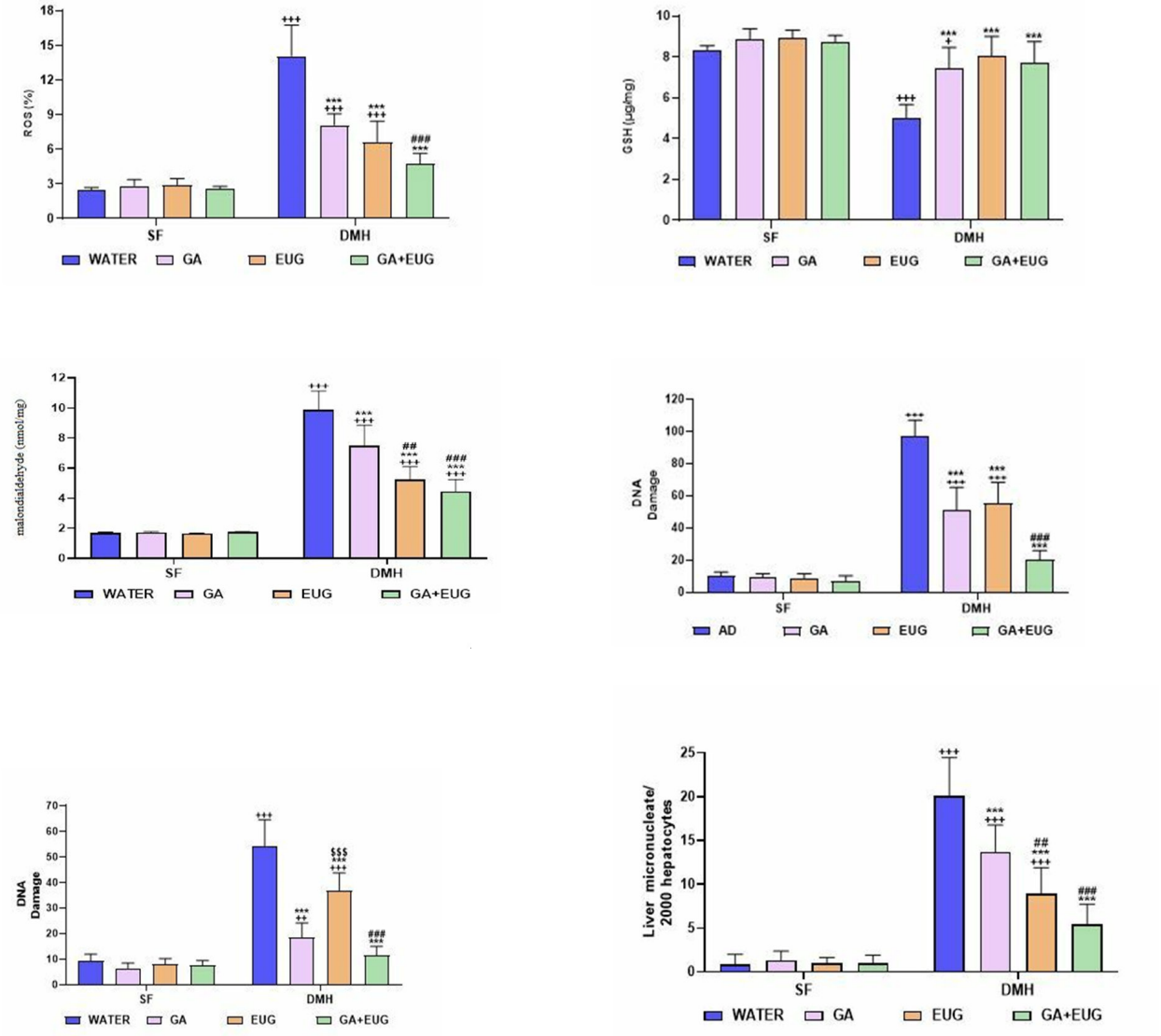

Figure 8. ROS Dosage, GSH Dosage, TBARS Dosage, Comet Test and Micronucleus Test in the Liver (treatment)

spontaneously generating methyldiazonium ions, which are capable of alkylating DNA, RNA or protein in the colon, thus playing a critical role in mutagenesis and carcinogenesis (Hawks and Magee, 1974). MAM was eventually discovered to be a substrate for NAD-dependent dehydrogenase in the colon and liver, suggesting the active metabolite of MAM is the corresponding aldehyde (Grab and Zedeck, 1977).

Due to their hepatoxicity, DMH and its metabolites cause ROS to build up, generating oxidative stress and alkylating hepatocellular DNA. This in turn produces mutations which favor the emergence of liver tumors. Thus, DMH is an effective means of inducing liver damage in animal models and testing the effect of drugs on tumor development at the molecular and enzyme level (Shebbo et al., 2020).

In this study, DMH was administered at 20 mg/kg body weight, a dosage shown to be efficient at inducing colorectal carcinogenesis (Venkatachalam et al., 2020). In this study, DMH was administered at 20 mg/kg body weight, a dosage shown to be efficient at inducing colorectal carcinogenesis 22. This was borne out by the fact that oxidative stress was significantly higher in animals treated with DMH than in controls ($p < 0.05$).

This was substantiated by the presence of pre-neoplastic lesions and malignant lesions in the liver, in a few animals in the different groups, without presenting significant differences between them. The pre-neoplastic lesions identified in the liver were similar to the pre-neoplastic lesions in the azoxymethane-induced colon carcinogenesis in Wistar rats (Burlamaqui et al., 2013).

GA is a safe natural product. In this study, 10% GA was administered orally (gavage). Neither oral nor intraperitoneal administration of GA has been associated with genotoxicity or carcinogenesis (Johnson, 2005). EUG is by the Food and Drug Administration considered non-mutagenic and non-carcinogenic (Nisar et al., 2021) . In this study EUG was administered orally in a microdose, using a pipette.

In this research, in prevention, it was found through ROS measurement that the GVI group (DMH + GA 10%), eugenol GVII (DMH + EUG), and gum arabic + eugenol GVIII (DMH+ GA 10% + EUG) showed a significant reduction, which was also observed in treatment.

However, it is important to note that in treatment, the concomitant use of gum arabic and eugenol G XII (DMH+ GA 10%+ EUG) significantly reduced oxidative stress when compared to the group treated with gum arabic alone GX (DMH + GA 10%). In the prevention groups, DMH significantly depleted the levels of GSH in all groups, however, in treatment, there was an increase in GSH levels in the groups treated with gum arabic GX (DMH + GA 10%), eugenol GXI (DMH + EUG), and gum arabic + eugenol G XII (DMH+ GA 10%+ EUG) when compared to the untreated group GIX (DMH + WATER).

The ROS levels observed in this study show that the combination of GA and EUG (Group XII) reduced DMH-induced oxidative stress when compared to animals treated with water (Group IX) or GA alone (Group X). GSH levels were also higher in Group XII than in Group IX.

According to some authors, GA reduces ROS and enhances GSH activity in the liver (Abu-Serie et al., 2021). Likewise, Babiker et al. (2018) reported that rats treated with 10% GA (the same concentration used in our study) displayed reduced levels of oxidative stress and greater hepatic antioxidant enzyme activity (catalase, glutathione and superoxide dismutase).

EUG is also known to reduce ROS levels and increase antioxidant response (Kumar et al., 2021). Interestingly, EUG displays dual properties: at high doses it acts as a prooxidant and at low doses it behaves as an antioxidant (Nisar et al., 2021). EUG is reported to have greater antioxidant power than Trolox (Gülçin, 2011).

The antioxidant properties of GA derive from chemical components like phenolic acids, sugars and minerals (Szwajgier et al., 2017) and antioxidant aminoacids like histidine, tyrosine and lysine (Abu-Serie et al., 2021). A member of the phenylpropanoid family, EUG is an allyl chain-substituted guaiacol which scavenges free radicals and protects against other types of toxicity. Its protective properties should be balanced dose-dependently against its hepatotoxicity (Uddin et al., 2022).

GSH is a tripeptide containing glutamic acid, cysteine and glycine. It is found in several organs, including the liver, and is responsible for immune function, clearance of free radicals and, hence, antitumoral activity (Li et al., 2021). In the liver, GSH reacts spontaneously with the active metabolite of the carcinogen, rendering it less toxic. Exposure to carcinogens leads to GSH depletion, lessening the protection against hepatocelullar damage. Substances increasing the availability of GSH are desirable as they enhance the excretion of carcinogens, thereby reducing their deleterious effects (Venkatachalam et al., 2020). In the current study, both GA and EUG reduced oxidative stress and improved antioxidant response, but the effect was greater when administered concomitantly.

When analyzing the groups through the TBARs assay, it was observed that there is lipid peroxidation in all groups that received carcinogen compared to the groups that did not receive it. In the TBARS test for prevention, a significant reduction was observed in group GVIII (DMH + GA 10% + EUG), which received concomitant gum arabic and eugenol when compared to the untreated group GV. In treatment, the isolated use of substances GX (DMH + GA 10%), eugenol GXI (DMH + EUG) significantly reduced it, however, the concomitant use of substances GXII (DMH+ GA 10%+ EUG) was significantly lower when compared to group X (DMH+ GA 10%). The concomitant use of gum arabic and eugenol suggests a more effective action in reducing oxidative stress.

Other combinations with GA have been tested for antioxidant activity, such as nanocomplexes of GA + berberine-loaded tragacanth in vitro (Bakshi et al., 2022). The same is true for EUG, which reportedly reduces titanium dioxide nanoparticle-induced toxicity in vitro when administered concomitantly with thymoquinone (Wani and Shadab, 2021).

We used the comet assay and the micronucleus assay to evaluate DMH-induced genotoxicity in hepatocytes. The former determines cell DNA damage individually by quantifying DNA migration in agarose gel (Ostling and Johanson, 1984) . The latter measures clastogenic or aneugenic damage by quantifying whole or shattered chromosomes that remain outside the nucleus of the daughter cell (MacGregor et al., 1987).The assay allows to determine whether the tested substances are able to induce or inhibit mutations in other words, to promote or prevent tumor growth.

The alcaline comet assay and the micronucleus assay revealed genotoxicity in all DMH groups. This was confirmed by the modified alkaline comet assay, which is more sensitive and detects specific lesions through the oxidation of nucleotides (Endutkin and Zharkov, 2019).

In the evaluation of genotoxicity, when comparing the different groups in prevention and treatment, a reduction was observed through the alkaline comet assay, alkaline comet assay with FPG enzyme, and micronucleus test in groups GVI and GX (DMH+GA 10%), GVII and GXI (DMH+EUG). However, G VIII (prevention) and G XII (treatment), which received concomitant gum arabic and eugenol, showed a statistically significant greater reduction compared to untreated groups GV and GIX (DMH+WATER). In the analysis of genotoxicity in the comet assay without enzyme, in prevention, it was observed that the isolated use of eugenol (GVII) was more effective than the use of gum arabic (GVI), while in treatment, the isolated use of gum arabic (GX) was more effective compared to eugenol (XI). This raises a question about whether there is some kind of competition between the use of the carcinogen and tested substances, since the concomitant use of gum arabic and eugenol in treatment is more effective than in prevention. In our findings, both gum arabic and eugenol protected hepatocytes from genotoxicity, but the concomitant use was more effective than the isolated use of the substances.

Likewise, oxidative stress and genotoxicity in the liver and other tissues of mice exposed to AOM (the metabolite of DMH) were reduced by 2.5% and 5% GA (Avelino et al., 2022). Eugenol also showed the same effects on the liver, spleen, and kidneys of rats (Wani et al., 2021).

The reduction in genotoxicity observed in both tests may be explained by the anticarcinogenic properties of GA and EUG. GA exerts an anticarcinogenic effect by modifying the mRNA expression in cancer-related genes and eliminating free radicals (Aloqbi, 2020) . The anticarcinogenic mechanism of EUG involves the

induction of apoptosis, cell cycle arrest, and inhibition of proliferation, migration, angiogenesis and metastasis (Zari et al., 2021).

Anticarcinogenic effects have been described for GA combined with other compounds, including GA-encapsulated gold nanoparticles used to prepare anti-cancer nanodrugs (based on the cytotoxic action of GA in tumor cells) (Gamal-Eldeen et al., 2017) and GA-stabilized selenium nanoparticles (based on the antioxidant action of GA) (Kong et al., 2014) .Similarly, anticarcinogenic effects have been reported for EUG combined with other substances: EUG increased the chemotherapy potential of gemcitabine in cervical cancer by inducing apoptosis and inhibiting inflammation (Hussain et al., 2011) and enhanced cisplatin actvity by inhibiting breast tumor cells and the NF-Kb signaling pathway (Islam et al., 2018).

It should be pointed out that, to our knowldege, all previous studies evaluating the ability of GA and EUG to reduce oxidative stress and genotoxicity in liver cells have tested the compounds separately. This is the first study to observe the synergistic action of GA and EUG administered concomitantly in this scenario.

The novelty of this study is the investigation of the associated use of gum arabic and eugenol for prevention and treatment of oxidative stress and genotoxicity of rats subjected to DMH-induced colon carcinogenesis. Considering that the antioxidant and antigenotoxic effects already reported in the literature are related only to the isolated use of substances, the combined use of gum arabic and eugenol is presented as a therapeutic target, since the treatment of different malignant neoplasms is carried out through the associated use of polychemotherapy. The treatment with 10% gum arabic and/or eugenol is effective in reducing oxidative stress and genotoxicity in rats. The synergistic effect of the two substances was observed in prevention and treatment, at the doses and times used, in the livers of rats subjected to DMH-induced colon carcinogenesis.

Author Contribution Statement

All named take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published. Nayanna de Oliveira Ramos Melo: Intellectual and scientific content of the study, technical procedures, manuscript preparation; Matheus De Sousa Silva, João Pedro Navarro Ribeiro and Weslei Pires Lima: Technical procedures; Francisco Vagnaldo Fechine Jamacaru: Statistical analysis; Bruno Coêlho Cavalcanti and Antônio Adailson De Sousa Silva: Biological assays; Conceição Aparecida Dornelas: Design of the study, critical revision, final approval.

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General

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Ethical Declaration

The study protocol complied with the guidelines of the National Board for the Control of Animal Testing (CONCEA) and was approved by the Animal Research Ethics Committee (CEUA) of the Federal University of Ceará (UFC) (protocol #1675020519).

Conflict of Interest

There are no known conflicts of interest associated with this publication.

References

Abu-Serie MM, Hamouda AF, Habashy NH (2021). Acacia senegal gum attenuates systemic toxicity in CCl4-intoxicated rats via regulation of the ROS/NF-κB signaling pathway. *Sci Rep*, **11**, 20316.

Aloqbi AA (2020). Gum Arabic as a natural product with antimicrobial and anticancer activities. *Arch Pharm Pract*, **11**, 107–12.

Amir Rawa MS, Mazlan MKN, Ahmad R, Nogawa T, Wahab HA (2022). Roles of Syzygium in anti-cholinesterase, anti-diabetic, anti-inflammatory, and antioxidant: from Alzheimer's perspective. *Plants (Basel)*, **11**, 1476.

Ashour MA, Fatima W, Imran M, et al (2022). A review on the main phytoconstituents, traditional uses, inventions, and patent literature of gum arabic emphasizing Acacia seyal. *Molecules*, **27**, 1171.

Avelino ALN, Silva NVRE, Oliveira GB, et al (2022). Antioxidant and antigenotoxic actions of gum arabic on the intestinal mucosa, liver and bone marrow of Swiss mice submitted to colorectal carcinogenesis. *Nutr Cancer*, **74**, 956-64.

Babiker M, Mohammed EAM, Mohammed A, Abbas T, Dafallah MS (2018). Protective effect of long term administration of gum arabic on oxidative stress in hepatic tissue of diabetic rats. *Biomed J Sci Tech Res*, **4**, 4153- 9.

Bakshi J, Mehra M, Grewalet S, Dhingra D, Kumari S (2022). Berberine loaded tragacanth-acacia gum nanocomplexes: synthesis, characterization and evaluation of in vitro anti-inflammatory and antioxidant activity. *J Clust Sci*, **34**, 747–760.

Bartosz G (2006). Use of spectroscopic probes for detection of reactive oxygen species. *Clin Chim Acta*, **368**, 53-76.

Binu P, Priya N, Abhilash S, Vineetha RC, Nair H (2018). Protective Effects of Eugenol against Hepatotoxicity Induced by Arsenic Trioxide: An Antileukemic Drug. *Iran J Med Sci*, **43**, 305-12.

Burlamaqui IM, Dornelas CA, Almeida PR, et al (2013). Hepatic repercussions of azoxymethane-induced colorectal carcinogenesis. *Rev Col Bras Cir*, **40**, 137-41.

Bucher JR (2019). Oxidative stress and radical-induced signalling. In: Baan RA, Stewart BW, Straif K, editors.

Tumour site concordance and mechanisms of carcinogenesis. Lyon (FR): International Agency for Research on Cancer 2019 (IARC Scientific Publications, No. 165.) Chapter 15. Available from: https://www.ncbi.nlm.nih.gov/books/NBK570343/

Crow JP (1997). Dichlorodihydrofluorescein and dihydrorhodamine 123 are sensitive indicators of peroxynitrite in vitro: implications for intracellular measurement of reactive nitrogen and oxygen species. *Nitric Oxide*, **2**, 147-57.

El-Garawani I, Hassab El-Nabi S, El Kattan A, et al (2021). The ameliorative role of Acacia senegal gum against the oxidative stress and genotoxicity induced by the radiographic contrast medium (ioxitalamate) in albino rats. *Antioxidants (Basel)*, **10**, 221.

Endutkin AV, Zharkov DO (2019) Critical sites of DNA backbone integrity for damaged base removal by formamidopyrimidine-DNA glycosylase. *Biochemistry*, **58**, 2740-9.

Gado AM, Aldahmash BA (2013). Antioxidant effect of Arabic gum against mercuric chloride-induced nephrotoxicity. *Drug Design*, **7**, 1245.

Gamal el-din AM, Mostafa AM, Al-Shabanah OA Al-Bekairi AM, Nagi MN (2003). Protective effect of arabic gum against acetaminophen-induced hepatotoxicity in mice. *Pharmacol Res*, **48**, 631-5.

Gamal-Eldeen AM, Moustafa D, El-Daly SM, et al (2017). Gum arabic-encapsulated gold nanoparticles for a non-invasive photothermal ablation of lung tumor in mice. *Biomed Pharmacother*, **89**, 1045-54.

Gülçin İ (2011). Antioxidant activity of eugenol: a structure-activity relationship study. *J Med Food*, **14**, 975-85.

Grab DJ, Zedeck MS (1977). Organ-specific effects of the carcinogen methylazoxymethanol related to metabolism by nicotinamide adenine dinucleotide-dependent dehydrogenases. *Cancer Res*, **37**, 4182-9.

Hartmann A, Speit G (1997).The contribution of cytotoxicity to DNA-effects in the single cell gel test (Comet assay). *Toxicol Lett*, **90**, 183-8.

Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H (2021). Clove essential oil (Syzygium aromaticum L. Myrtaceae): extraction, chemical composition, food applications, and essential bioactivity for human health. *Molecules*, **26**, 6387.

Hawks A, Magee PN (1974).The alkylation of nucleic acids of rat and mouse in vivo by the carcinogen 1,2-dimethylhydrazine. *J Câncer*, **30** ,440-7.

Hayashi M, Morita T, Kodama Y, Sofuni T, Ishidate M Jr (1990). The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange coated slides. *Mutat Res*, **245**, 245- 9.

Hrycay EG, Bandiera SM (2015). Involvement of cytochrome P450 in reactive oxygen species formation and cancer. *Adv Farmacol*, **74**, 35-84.

Hempel SL, Buettner GR, O'Malley YQ, Wessels DA, Flaherty DM (1999). Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2',7'-dichlorodihydrofluorescein diacetate, 5 (and 6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate, and dihydrorhodamine 123. *Free Radic Biol Med*, **27**, 146-59.

Hussain A, Brahmbhatt K, Priyani A, et al (2011). Eugenol enhances the chemotherapeutic potential of gemcitabine and induces anticarcinogenic and anti-inflammatory activity in human cervical cancer cells. *Cancer Biother Radiopharm*, **26**, 519-27.

Islam SS, Al-Sharif I, Sultan A, et al (2018). Eugenol potentiates cisplatin anti-cancer activity through inhibition of ALDH-positive breast cancer stem cells and the NF-κB signaling pathway. *Mol Carcinog*, **57**, 333-46.

Johnson W (2005). Final report of the safety assessment of Acacia catechu gum, Acacia concinna fruit extract, Acacia dealbata leaf extract, Acacia dealbata leaf wax, Acacia decurrens extract, Acacia farnesiana extract, Acacia farnesiana flower wax, Acacia farnesiana gum, Acacia senegal extract, Acacia senegal gum, and Acacia senegal gum extract. *Int J Toxicol*, **24**, 75-118.

Juca MJ, Bandeira BC, Carvalho DS, Leal AT (2014).Comparative study of 1,2-dimethylhydrazine and azoxymethane on the induction of colorectal cancer in rats. *J Coloproctol*, **34**, 167-73.

Kaddam L, Fadl-Elmula I, Eisawi OA, et al (2017). Gum arabic as novel anti-oxidant agent in sickle cell anemia, phase II trial. *BMC Hematol*, **17**, 4.

Kong H, Yang J, Zhang Y, et al (2014). Synthesis and antioxidant properties of gum arabic-stabilized selenium nanoparticles. *Int J Biol Macromol*, **65**, 155-62.

Kumar A, Siddiqi NJ, Alrashood ST, et al (2021). Protective effect of eugenol on hepatic inflammation and oxidative stress induced by cadmium in male rats. *Biomed Pharm*, **139**, 111588.

Larangeira LLS, Taha MO, Ferme A, Lemos R, Plaper H (1998). Localização de lesões tumorais induzidas pela 1,2-dimetilhidrazina e seu grau de atipia no colon de ratos. *Acta Cir Bras*, **13**, 00-00.

Lebel CP, Ischiopoulos H, Bondy SC (1992).Evaluation of the probe 2, 7-dichlorofluorescin as indicator of reactive oxygen species formation and oxidative stress. *Chem Res Toxicol*, **5**, 227-31.

Li W, Li M, Qi J (2021). Nano-drug design based on the physiological properties of glutathione. *Molecules*, **26**, 5567.

MacGregor JT, Heddle JA, Hite M, et al (1987). Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. *Mutat Res*, **189**, 103-12.

Manikandan P, Murugan RS, Priyadarsini RV, Vinothini G, Nagini S (2010). Eugenol induces apoptosis and inhibits invasion and angiogenesis in a rat model of gastric carcinogenesis induced by MNNG. *Life Sci*, **86**, 936-41.

Melo NOR, Silva MS, Lima WP, et al (2023). Effect of eugenol and gum arabic on oxidative stress and genotoxicity in rat spleen, kidney and lung tissue following colorectal carcinogenesis. *Int J Herb Med*, **11**, 22-9.

Nasir O, Wang K, Föller M, et al (2010). Downregulation of angiogenin transcript levels and inhibition of colonic carcinoma by gum arabic (Acacia senegal). *Nutr Cancer*, **62**, 802-10.

Niazi AA, Kourkinejad Gharaei F, Saebinasab Z, et al (2021). Eugenol administration improves liver damage induced by a fructose-rich diet. *Adv Biomed Res*, **10**, 42.

Nisar MF, Khadim M, Rafiq M, et al (2021). Pharmacological properties and health benefits of eugenol: a comprehensive review. *Oxid Med Cell Longev*, **2021**, 2497354.

Oroojan AA, Chenani N, An'Aam M (2020). Antioxidant effects of eugenol on oxidative stress induced by hydrogen peroxide in islets of langerhans isolated from male mouse. *Int J Hepatol*, **2020**, 5890378.

Ostling O, Johanson KJ (1984). Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochem Biophys Res Commun*, **123**, 291-8.

Pal R, Hooda MS, Bias CS, Singh J (2014). Hepatoprotective activity of Acacia senegal pod against carbon tetrachloride-induced hepatotoxicity in rats. *Int J Pharm Sci Rev Res*, **26**, 165- 8.

Petrocelli G, Farabegoli F, Valerii MC, et al (2021). Molecules present in plant essential oils for prevention and treatment of colorectal cancer (CRC). *Molecules*, **26**, 885.

Ravnik-Glavac M, Cerar A, Glavac D (2000). Animal model in the study of colorectal carcinogenesis. *Pflugers Arch*, **440**, 55-7.

Sedlak J, Lindsay RH (1968). Estimation of total protein-bound, non-protein sulphydril group in tissue with Ellman's reagent. *Anal Biochem*, **25**, 192-205.

Shebbo S, El Joumaa M, Kawach R, Borjac J (2020). Hepatoprotective effect of Matricaria chamomilla aqueous extract against 1,2- dimethylhydrazine-induced carcinogenic hepatic damage in mice. *Heliyon*, **6**, e04082.

Sivaranjani A, Sivagami G, Nalini, N (2016). Chemopreventive effect of carvacrol on 1,2-dimethylhydrazine induced experimental colon carcinogenesis. *J Can Res Ther*, **12** 755-62.

Szwajgier D, Borowiec K, Pustelniak K (2017). The neuroprotective effects of phenolic acids: molecular mechanism of action. *Nutrients*, **9**, 477.

Uddin ABMN, Hossain F, Reza ASMA, Nasrin MS, Alam AHMK (2022). Traditional uses, pharmacological activities, and phytochemical constituents of the genus Syzygium: A review. *Food Sci Nutr*, **10**, 1789-1819.

Venkatachalam K, Vinayagam R, Arokia Vijaya Anand M, Isa NM, Ponnaiyan R (2020). Biochemical and molecular aspects of 1,2- dimethylhydrazine (DMH)-induced colon carcinogenesis: a review. *Toxicol Res (Camb)*, **9**, 2-18.

Wang Y, Qi H, Liu Y, et al (2021). The double-edged roles of ROS in cancer prevention and therapy. *Theranostics*, **11**, 4839-57.

Wani MR, Maheshwari N, Shadab G (2021). Eugenol attenuates TiO2 nanoparticles-induced oxidative damage, biochemical toxicity and DNA damage in Wistar rats: an in vivo study. *Environ Sci Pollut Res Int*, **28**, 22664-78.

Wani MR, Shadab GGHA (2021). Antioxidant thymoquinone and eugenol alleviate TiO2 nanoparticle-induced toxicity in human blood cells in vitro. *Toxicol Mech Methods*, **31**, 619-29.

Weisburger JH (1971).Colon carcinogens: Their metabolism and mode of action. *Cancer*, **28**, 60- 70.

Wojewodzka M, Buraczewska I, Kruszewski M (2002). A modified neutral comet assay: elimination of lysis at high temperature and validation of the assay with anti-single-stranded DNA antibody. *Mutat Res*, **518**, 9-20.

Zari AT, Zari TA, Hakeem KR (2021). Anticancer properties of eugenol: a review. *Molecules*, **26**, 7407.