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ORIGINAL ARTICLE

Protective effect of Genistein against N-nitrosodiethylamine (NDEA)-induced hepatotoxicity in Swiss albino rats

Fahad Ali, Rahul, Falaq Naz, Smita Jyoti, Yasir Hasan Siddique*

Section of Genetics, Department of Zoology, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh 202002, India

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Abstract In the present study, we studied the effect of Genistein against the hepatotoxicity induced by N-nitrosodiethylamine (NDEA). NDEA is present in almost all kinds of food stuff and has been reported to be a hepatocarcinogen. The male rats were exposed to NDEA (0.1 mg/mL) dissolved in drinking water separately and along with 25, 50, 100 mg/mL of Genistein for 21 days. The activities of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were measured in blood serum. Lipid peroxidation, protein carbonyl content, micronucleus frequency and DNA damage (Comet assay) were performed on rat hepatocytes. The results of the study reveal that the treatment of NDEA along with Genistein showed a significant dose-dependent decrease in the levels of blood serum enzymes *i.e.,* SGOT, SGPT, ALP and LDH ($P<0.05$). The HE staining of histological sections of the liver also revealed a protective effect of Genistein. A significant dose-dependent reduction in the lipid peroxidation and protein carbonyl content was observed in rats exposed to NDEA (0.1 mg/mL) along with Genistein ($P<0.05$). The results obtained for the comet assay in rat hepatocytes showed a significant dose-dependent decrease in the mean tail length ($P<0.05$). Thus the present study supports the hepatoprotective role of Genistein.

1. Introduction

Genistein is an isoflavone and acts as a both phytoestrogen and antioxidant [1]. It inhibits the growth of cancerous cells by binding itself with the receptor in place of estrogen [2,3]. It has also been reported to be an antihelmintic [4]. It shows protective effects against various types of cancers [5–11]. An epidemiological study showed that the consumption of isoflavones is associated with an increased risk for hepatocellular carcinoma (HCC) in women [12]. It inhibits the bone resorption in rats [13]. It does not show teratogenic potential *in vivo* even at a very high dose of 1000 mg/kg/day by oral gavage in the embryo–fetal toxicity [14]. It has been reported to be non-mutagenic in the *Salmonella typhimurium*

*Corresponding author. Tel.: +91 0571 2700920x3430.
E-mail address: yasir_hasansiddique@rediffmail.com (Y. Hasan Siddique).

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assay and non-clastogenic in the mouse and rat micronucleus test [15]. N-nitrosocompounds (NOCs) are present in a variety of food stuff and are well-known carcinogens [16]. One of the NOCs such as N-nitrosodiethylamine (NDEA) has been suggested to cause oxidative stress and cellular injury by generating free radicals [17–19]. NDEA has been reported to generate electrophilic and reactive oxygen species by activation of cytochrome P450 in the liver [20]. This leads to liver cytotoxicity, carcinogenicity and mutagenicity [21]. The high intake of natural products has been reported to be associated with a decrease in the risk of cancer and toxicity in various epidemiological and animal studies [22]. In the present study, the effect of Genistein was studied against NDEA-induced hepatotoxicity in Swiss albino rats.

2. Experimental

2.1. Chemicals and reagents

N-nitrosodiethylamine and Genistein were procured from Sigma Chemicals Co. (USA). Agarose (normal and low melting), Triton X, ethidium bromide, dimethyl sulfoxide, Tris, EDTA and all other chemicals were purchased from SISCO Research Laboratories, India. May Grunwald's stain and Giemsa stain were procured from Merck Ltd. (India).

2.2. Animals and treatment

Male rats (Wistar strain), weighing 100–120 g, were used in the study. The animals were divided into nine groups (5 rats/group). The first group was allowed to feed on N-nitrosodiethylamine (NDEA) dissolved in water (0.1 mg/mL); the second, third and fourth groups were allowed to feed on water containing NDEA (0.1 mg/mL) plus 25, 50 and 100 mg/mL of Genistein, respectively; the fifth group served as a control (normal drinking water); the sixth, seventh and eighth groups were allowed to feed on water containing Genistein at final concentration of 25, 50 and 100 mg/mL, respectively. The ninth group was taken as a negative control, and was allowed to feed on water containing a dimethysulfoxide (DMSO) (3 μL/mL). Genistein was dissolved in 0.03% DMSO and in drinking water the final concentrations of 25, 50 and 100 mg/mL were established. The rats were allowed to feed *ad libitum* for 21 days and were sacrificed under mild ether anesthesia.

2.3. Histological evaluation of the liver

A portion of the liver was removed and washed thoroughly with 0.9% saline. The tissue was kept in 10% buffered neutral formalin (BNF) for 24 h. Then the fixed liver specimens from each group were embedded in paraffin and processed for light microscopy by staining individual sections with hematoxylin–eosin stain.

2.4. Biochemical analysis

The blood samples were collected directly by cardiac puncture in a vacutainer having a clot activator (AKÜ ret, Medkit). The serum samples were collected for the biochemical analysis of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The levels of the enzymes were estimated according to the method described in the commercial kits (Crest Biosystem, India).

2.5. Preparation of liver homogenate

The homogenate was prepared according to the procedure described by Singh et al. [23]. The livers were washed thoroughly with chilled 0.9% saline. The final wash was given with chilled homogenizing buffer (pH 7.5) containing 0.024 M EDTA, 0.075 M NaCl and 10% DMSO. After weighing, the liver was cut into pieces, suspended in chilled homogenizing buffer at a concentration of 1 g/mL, and homogenized on ice using homogenizer at 1500 rpm. The homogenate was then centrifuged at 7000 rpm for 10 min at 4 °C. The supernatant was removed, resuspended in a homogenization buffer and kept at −20 °C for further analyses.

2.6. Estimation of protein carbonyl content

The protein carbonyl content was estimated according to the protocol described by Hawkins [24] and Ali et al. [25].

2.7. Estimation of lipid peroxidation

The method described by Siddique et al. [26] was used for the estimation of lipid peroxidation in liver cells. The absorbance was read at 586 nm [25].

2.8. Micronucleus assay

Micronucleus assay was performed according to the method of Igarashi and Shimada [27]. A total of 500 cells were counted per rat for the presence of micronuclei using a light microscope [25,27,28].

2.9. Comet assay

Comet assay was performed according to the method described by Singh et al. [29]; and with modification as suggested by Dhawan et al. [30]. Frosted microscopic slides were dipped in 1% normal melting agarose and the underside was wiped to remove the agarose (dissolved in PBS). About 40 μL of the cell suspension (liver cells) was mixed with 60 μL of 0.5% low melting agarose (dissolved in PBS) and was layered on the prepared base slides. Three slides were prepared per rat and a total of 50 randomly captured comets per slide were analyzed under fluorescence microscope for scoring comet tail length by using comet 1.5 software (TriTek Corporation) [25].

2.10. Statistical analysis

All data were expressed as the mean ± standard error and student's *t*-test was used for the analysis. Statistical significance was estimated at the 5% level.

3. Results and discussion

The HE staining of histological sections of the liver showed the normal structure of the hepatocytes in the control group (Fig. 1A). The rats exposed to NDEA (0.1 mg/mL) showed sinusoidal