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Original article

Azadircta indica as a modulator of membrane stability parameters and surface changes during 1,2 dimethylhydrazine-induced colorectal carcinogenesis

Azadircta indica *comme un modulateur de la stabilité de la membrane et changements de surface au cours des paramètres 1,2 diméthylhydrazine induit la cancérogenèse colorectale*

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ABSTRACT

Objective. – The aim of the present study was to study the modulatory potential of *Azadirta indica* on colonic surface abnormalities and membrane fluidity changes following 1,2 dimethylhydrazine-induced [DMH] colon carcinogenesis.
Materials and methods. – Brush border membranes [BBM] were isolated from the colon of rats and the viscosity as well as fluidity parameters were assessed by using the membrane extrinsic fluorophore pyrene.
Results. – DMH treatment resulted in a significant increase in lipid peroxidation [LPO]. Reduced glutathione levels [GSH] and the activities of glutathione reductase [GR], glutathione transferase [GST], superoxide dismutase [SOD], catalase [CAT] and glutathione peroxidase [GPx] were found to be significantly decreased following DMH treatment. On the other hand, supplementation with AI, DMH-treated rats resulted in a significant decrease in the levels of lipid peroxidation but caused a significant increase in the levels of GSH as well in the activities of GR, GST, SOD, CAT and GPx. The results further demonstrated a marked decrease in membrane microviscosity following DMH treatment. On the other hand, a significant increase was observed in the excimer/monomer ratio and fluidity parameter of DMH-treated rats when compared to normal control rats. However, the alterations in membrane microviscosity and the fluidity parameters were significantly restored following *A. indica* treatment. Further, histological as well as colon surface alterations were also observed following DMH treatment, which however were greatly prevented upon AI co-administration.
Conclusions. – The study, therefore, concludes that *A. indica* proves to be useful in modulating the colonic surface abnormalities and membrane stability following DMH-induced colon carcinogenesis.

Mots clés :
Diméthylhydrazine
Membrane dynamics
Azadirta indica
Changements de surface

RÉSUMÉ

Le but de la présente étude était d'étudier le potentiel de modulateurs de *Azadirta indica* sur surface colique des anomalies et des variations de fluidité membranaire après 1,2 diméthylhydrazine (DMH) induit la cancérogenèse du côlon. Les membranes de la bordure en brosse (BBM) ont été isolées dans le côlon de rats et de la viscosité, ainsi que la fluidité des paramètres ont été évaluées à l'aide de la membrane d'extrinsèque fluorophore pyrène. Le traitement DMH a entraîné une augmentation significative de la peroxydation lipidique (POL). De glutathion réduit (GSH) et l'activité de la glutathion-réductase (GR), de glutathione transférase (GST), superoxyde dismutase (SOD), de la catalase (CAT) et de

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la glutathion peroxydase (GPx) se sont avérés être significativement diminué après le DMH traitement. D'autre part, la supplémentation avec IA, DMH rats traités, ont entraîné une diminution importante dans les niveaux de la peroxydation des lipides mais a causé une augmentation significative dans les niveaux de GSH ainsi que dans les activités de GR, la TPS, la SOD, la CAT et de la GPx. Les résultats de plus, ont démontré une diminution marquée de microviscosité membranaire suite au traitement DMH. D'autre part, une augmentation importante a été observée dans l'excimère/monomère ratio et fluidité paramètre de rats traités lorsque comparé DMH à chez des rats témoins normaux. Toutefois, les altérations de la membrane et la fluidité microviscosité paramètres étaient significativement rétablis Azadirta suivantes indica traitement. En outre, histologiques ainsi que du côlon d'altération de surface ont aussi été observés après l'DMH traitement qui ont cependant empêché sur IA coopération grandement l'administration. L'étude conclut, par conséquent, que *A. indica* prouve qu'un utile dans la modulation de la surface colique anomalies la stabilité de la membrane et suite DMH la cancérogenèse du côlon induite.

1. Introduction

Colorectal carcinogenesis ranks third among all cancers, the world over [1]. 1,2 dimethylhydrazine [DMH] is a carcinogen which specifically induces tumors in the descending colons of experimental animals [2]. Earlier studies from various other laboratories; have proved the specificity of the DMH for induction of colorectal carcinogenesis [3–6]. The prime focus of the present study was to investigate biophysical modulatory potential of *Azadirachta indica*, if any, by studying membrane stability parameters and surface changes during 1,2 dimethylhydrazine-induced colorectal carcinogenesis.

The membrane fluidity is one of important aspects in cell physiology, which may be influenced by diet. Improper dietary habits are the prime concerns as these exert influence on the key molecular events associated with onset of colon carcinogenesis. Many dietary compounds are being investigated for their prominent roles in chemoprevention of colorectal cancer. *A. indica [neem]* is the photochemical of interest in the present study as it possess potent ability to remove cancerous phenotype as observed in various earlier studies, the world over [7,8]. Though *A. Indica* has desirable biological activities against cancer but there is a paucity of information with regard to its modulatory role on membrane parameters and surface changes during colon carcinogenesis. So, the present study was focused to evaluate the efficacy of *A. indica* as a modulator of membrane parameters as well as surface changes during DMH-induced colon carcinogenesis in rats.

2. Materials and methods

2.1. Chemicals

All the chemicals used in this study were of analytical grade and were obtained from Sigma Chemical Co., St. Louis, Mo, USA.

2.2. Description of A. indica

A. indica, also known as Neem, Nimtree, and Indian Lilac is a tree in the mahogany family *Meliaceae.*

2.3. Aqueous A. indica leaves extract

Capsules of spray dried aqueous *A. indica* leaf extract [AAILE] (Lot of March 2014) were obtained from a recognized herbal company [Dabour India Pvt. Ltd]. Each capsule continued 100 mg of AAILE. The contents of the capsule were reconstituted into double distilled water immediately before its oral administration to the animals. The solution/suspension obtained after dissolving AAILE was covered with dark paper in order to protect the same from direct exposure of light.

2.4. Experimental design and animal treatment

Animal care procedures followed in the current study were approved by the University Ethical Committee on Experimental Animals for Biomedical Research.

Male Wistar rats weighing 120–150 g were procured from the Central Animal House of the Hubei University of Medicine, China. All the animals were housed in polypropylene cages under hygienic conditions, and were provided with pellet diet and drinking water *ad libitum*. Fourty animals were segregated randomly and equally (10 animals each) into four treatment groups. Animals in Group I served as normal controls and were given water and diet *ad libitum.* Rats in this group also received 1 mM EDTA-saline subcutaneously per week, which was used as a vehicle for giving DMH treatment to group II animals. Animals in Group II were given a weekly subcutaneous injection of DMH at a dose level of 30 mg/Kg body weight dissolved in 1 mM EDTA-normal saline [pH 6.5], for the time duration of 20 weeks [9]. Group III animals were given *A. indica* in the form of aqueous *A. Indica* leaf extract [AAILE] which was administered orally at a dose rate of 100 mg/Kg/Body weight on alternate days three times a week for the entire duration of the study. Animals in Group IV were given a combined treatment of DMH as well as *A. indica* in a similar manner as was given to Group II and Group IV animals, respectively.

2.5. Lipid peroxidation and antioxidant defense system enzymes

Lipid peroxidation assay was done by the method of Wills [10].

Reduced Glutathione [GSH]: estimation of GSH was performed in the tissue homogenates of colon by the method of Ellman [11].

Catalase: the method of Luck was used for the estimation of catalase [12].

Superoxide Dismutase [SOD]: the activity of SOD was estimated by using the method of Kono [13].

Glutathione Reductase [GR]: the enzyme was assayed by the method of Carlberg and Mannervik [14].

Glutathione-S-Transferase [GST]: this enzyme was assayed by the method of Habig et al. [15].

Glutathione Peroxidase [GPx]: this enzyme was assayed by the method of Pagila and Valentine [16].

Protein: protein assay was done by the method of Lowry et al. [17].

2.6. Isolation of colonic brush border membrane

Animals were sacrificed ether anesthesia, and the colons were excised. Intestinal BBM was isolated by homogenizing the tissue in chilled sodium maleate buffer [10% homogenate] in a waring blender fitted with a Teflon pestle and using a glass homogenizing tube with 0.05-mm clearance. All the procedures were done strictly at 4 °C including the excision of the colon, flushing with physiologic saline, and dissection into several small pieces for homogenization. The homogenate was passed through two layers of surgical bandage, and to the filtrate, anhydrous $CaCl_2$ was added with constant stirring on a magnetic stirrer to a final concentration of 10 mM. The tubes were centrifuged at 2000 × *g* for 10′ at 4 °C and the supernatant so obtained was further recentrifuged at 42,000 × *g* for 20′. The pellet was suspended in 20 volumes of 50 mM sodium maleate buffer [pH 6.5–6.8] and was recentrifuged at 42,000 × *g* for 20 min. The final pellet obtained was suspended in the 50 mM sodium maleate buffer. The method has been based on the procedure of Schmitz et al. [18].

2.7. Membrane stability studies in BBM

Pyrene fluorescence excimer [dimer] formation was used to study the lateral diffusion in the membrane following the method of Massey et al. [19]. The fluorescence intensity was read in a fluorescence spectrophotometer by using the excitation wavelength of 365 nm and emission wavelengths of 515 nm and 475 nm for the excimer and monomer fluorescences, respectively. The viscosity [η] was calculated from the monomer/excimer fluorescence intensity ratios by using the following relationship:

E/M (excimer/monomer) = (Pyrene)Tk/η,