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Original article

Protective effect of *Raphanus sativus* on D-galactosamine induced nephrotoxicity in rats

Effet néphroprotecteur de Raphanus sativus *chez le rat après agression toxique à la D-galactosamine*

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Abstract

The aim of the present study was to evaluate nephroprotective effect of *Raphanus sativus* ethanolic extract (RSEt) on tissue defense system in galactosamine (GalN) induced renal damage in rats. GalN was administered intraperitoneally at a dose of 400 mg/kg/b.w for three alternate days and the renal toxicity was manifested by a significant ($P < 0.05$) increase in the levels of renal markers such as urea, creatinine and uric acid. This was found to be associated with decreased activities of renal antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) and depletion of renal reduced glutathione (GSH), vitamin C and vitamin E. Administration of the RSEt (850 mg/kg/body weight, oral) for 15 days to rats reduced the levels of renal markers and significantly increased the level of antioxidants. The activities of gamma glutamyl transpeptidase (GGT) and thiobarbituric acid reactive substances (TBARS) were also decreased in the kidney of RSEt treated group. Renal histology examination confirmed the damage to the kidney as it reveals severe necrosis of the proximal renal tubules with haemorrhage which was ameliorated by the treatment with RSEt. These results suggest that the *R. sativus* has protective effects on GalN-mediated nephrotoxicity and this may be related to the action of the antioxidant content of the extract.

Keywords: *Raphanus sativus*; Galactosamine; Antioxidants; Free radicals; Kidney damage

Résumé

Introduction. – Le but de l'étude était d'évaluer l'effet néphroprotecteur d'un extrait éthanolique de *Raphanus sativus* (RSEt ; radis) dans un modèle de lésions rénales induites par la galactosamine (GalN) chez le rat.
Méthodes. – Quatre groupes de rats étaient testés. Un groupe recevait per os du sérum physiologique (groupe I), un groupe recevait GalN par voie intra-péritonéale à une dose de 400 mg/kg de poids corporel pendant trois jours alternés (groupe II), un groupe recevait par la même voie GalN et par voie orale RSEt à raison de 850 mg/kg de poids corporel pendant 15 jours (groupe III), et un groupe recevait seulement RSEt (groupe IV) per os durant 15 jours.
Résultats. – Pour le groupe II, la toxicité rénale se manifestait par une augmentation significative ($p < 0,05$) des concentrations de marqueurs sériques rénaux tels que l'urée, la créatinine et l'acide urique. Ces variations étaient associées à une réduction des activités enzymatiques antioxydantes rénales portant sur la superoxyde dismutase (SOD), la catalase (CAT), la glutathion-S-transférase (GST), la glutathion peroxydase (GPx) et la glutathion réductase (GR), ainsi qu'à une réduction au niveau rénal du glutathion réduit (GSH) et des vitamines C et E. L'administration aux rats de la RSEt (groupe III) réduisait les concentrations des marqueurs rénaux de toxicité et augmentait fortement le niveau des antioxydants. Les activités rénales de gamma glutamyl transférase (GGT) et le niveau des substances réactives à l'acide thiobarbiturique (TBARS) diminuaient également pour ce groupe traité par RSEt. L'histologie révélait sous GalN l'induction d'une nécrose hémorragique grave des tubules rénaux proximaux, améliorée par le traitement par RSEt.

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Conclusion. – Ces résultats suggèrent que *R. sativus* a des effets protecteurs sur la néphrotoxicité médiée par GalN ; ceci peut être lié à l'action de la teneur en antioxydants de l'extrait.

Mots clés : Raphanus sativus ; Galactosamine ; Antioxidants ; Radicaux libres ; Lésions rénales

1. Introduction

Nephrotoxicity is a serious health problem among all populations which may be due to hypertension, inflammatory disease, diabetes, obesity, and toxic nephropathies from environmental and occupational hazards that include heavy metals, drugs, and chemicals [1,2]. Recent studies have documented that oxidative stress is highly prevalent in patients with renal disease [3]. Amino sugar, galactosamine (GalN) with its unique properties causes many metabolic and morphological abnormalities in the liver and advanced renal failure in experimental animals [4]. The toxic effect of GalN has been reported to associate with the depletion of UTP nucleotides followed by the formation of UDP hexosamines resulted in the inhibition of transcription and consequently the translation processes [5,6]. GalN intoxication increased reactive oxygen species (ROS), reactive nitrogen species (RNS) and tumor necrosis factor-α (TNF-α) production and disturbed the antioxidant machineries in the kidney tissue [7]. Moreover GalN damages the enzymes responsible for mitochondrial substrate transportation leading to decrease in renal blood flow and vasoconstriction [8]. The detailed mechanism by which GalN induces nephrotoxicity is unclear. Nevertheless, a role has been postulated for free radical induced lipid peroxidation [9].

Many numbers of plants are extensively used in indigenous system of medicine and also have been reported for its nephroprotective effect [10,11]. The leaves of *Aegle marmelos, Ceratonia silique, Ocimum sanctum;* roots of *Cassia auriculata, Withania somnifera;* seeds of *Carica papaya, Cucurbita pepo;* fruits of *Crataeva nurvula, Morinda citrifolia, Gymnema montanum* and many other plants are proved to have protective effect against nephrotoxicity [12,13]. It has been reported that nephroprotective activities of the compounds may be well correlated with its antioxidant and free radical scavenging properties [14,15].

Raphanus sativus Linn (family - Cruciferae) is commonly called "radish", is an aromatic annual herb, which is used in traditional medicine to treat various diseases. The roots and leaves of this plant have been reported to possess various pharmacological values like hepatoprotective [16–18], cardioprotective [19], antioxidant [20], antiurolithiatic effects [21] and gastrodynic pains [22]. So far, limited information exists concerning the beneficial effects of *R. sativus* against the oxidative injuries in kidney. Hence, the present study aimed to evaluate nephroprotective effect of RSEt on tissue defense system in GalN induced renal damage in rats.

2. Materials and methods

2.1. Chemicals

D-galactosamine was purchased from Sigma Aldrich Limited (St Louis, MO, USA). All other chemicals used were of highest purity and analytical grade.

2.2. Extraction of R. sativus

R. sativus roots were collected from JSS Institute of Naturopathy and Yogic Sciences, Ooty, Tamil Nadu. The roots were dried at ambient temperature and stored in dry place prior to use. The roots were then cleaned and washed with 10% saline, shade dried and powdered. About 500 g of powder was subjected to ethanolic extraction using soxlet apparatus. The organic fraction was collected and concentrated using a rotary evaporator under reduced pressure and lyophilized to get powder and used for analysis. The final yield (w/w) of the extract was 7.30%.

2.3. Qualitative phytochemical estimation

The ethanolic extract of *R. sativus* was examined by standard methods to determine the presence of phytochemicals such as alkaloids, proteins, flavonoids, phenolics, tannins, saponins and carbohydrates.

2.4. Quantitative phytochemical estimation

2.4.1. Estimation of total phenolic content

The total phenolic content in RSEt was determined by folin-ciocalteu method [23] spectrometrically. A 0.2-mL of test extract solution was mixed with 1 mL of folin-ciocalteu reagent and then 1 mL of sodium carbonate was added followed by distilled water. The reaction mixture is allowed to stand for 2 hours at room temperature and the absorbance was measured at 760 nm. The gallic acid is used as a standard. The total phenolic content was expressed in the terms of gallic acid equivalents.

2.4.2. Estimation of total flavanoid content

Total flavonoid content in RSEt was determined colorimetrically according to the method described by Chang et al. [24]. A 0.5 mL of extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction mixture was incubated