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Vanillin mitigates potassium bromate-induced molecular, biochemical and histopathological changes in the kidney of adult mice

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ABSTRACT

The present study aimed to explore the ability of vanillin to ameliorate the adverse effects induced by potassium bromate ($KBrO_3$) in the renal tissue. Our results showed a significant increase in hydrogen peroxide, superoxide anion, malondialdehyde, advanced oxidation protein product and protein carbonyl levels in the kidney of $KBrO_3$ treated mice, compared with the control group. Nephrotoxicity was evidenced by a decrease in plasma uric acid and kidney glutathione levels, Na^+-K^+-ATPase, lactate dehydrogenase and catalase activities. Additionally, creatinine and urea levels significantly increased in the plasma and declined in the urine. Also, Kidney glutathione peroxidase, superoxide dismutase, metallothionein (MT1 and MT2) mRNA expression remarkably increased. These modifications in biochemical and molecular values were substantiated by histopathological data. Co-treatment with vanillin restored these parameters to near control values. Interestingly, vanillin proved to possess, *in vitro*, a stronger scavenging radical activity than vitamin C and Trolox. Thus, vanillin inhibited $KBrO_3$-induced damage via its antioxidant and antiradical activities as well as its capacity to protect genes expression and histopathological changes.

1. Introduction

Oxidative stress may occur following an imbalance between the cells' detoxification systems and oxidant production. It is involved in the alteration of physiologically critical molecules such as proteins, lipids, carbohydrates and nucleic acids, leading to tissue damage. Thus, it is implicated in the pathology observed in several chronic diseases such as cancer [1].

Various exogenous stresses, among which potassium bromate ($KBrO_3$), may cause oxidative stress. $KBrO^3$ is a halogen frequently detected in tap and even bottled water. It is also the major by-product generated during the ozonation of surface water. This compound is frequently used in panification and in cosmetics as a hair weaving solution [2]. Previous experimental evidence demonstrated that $KBrO_3$ induced oxidative stress [3–5]. Generally, the toxic effects of $KBrO_3$ are attributed to its ability to enhance the production of reactive oxygen species (ROS) [6–8]. When ingested, $KBrO_3$ is rapidly absorbed by the digestive tract, reduced to bromide in the tissues and then partly excreted in the urine as a bromide ion.

Since the kidney is the primary target organ of $KBrO_3$ [9,10], the toxic effects of this compound in humans arise from acute poisoning causing renal failure [7]. Yet, the exact mechanism(s), particularly the interplay among several endogenous concurrent contributing factors behind $KBrO_3$ renal toxicity and the ways to overcome its toxic effects by natural products remain hardly known [11]. Therefore, the assessment of molecular damage induced by $KBrO_3$ is currently of great importance to elucidate its mechanism action of in the kidney.

Besides, the metallothioneins (MTs) genes expression in the renal tissue are considered as an important used marker for the evaluation of organ response to $KBrO_3$ cytotoxicity. MT is known as a sulfur-rich protein with a low molecular weight and can act as a free radical scavenger, especially with O_2^- and OH^- [12]. It is synthesized mainly in the hepatic and renal cells after exposure to

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Abbreviations

ABTS	2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid
AOPP	advanced oxidation protein product
ATP	adenosine triphosphate
CAT	catalase
DNPH	dinitrophenyl hydrazine
DPPH	1-diphenyl-picrylhydrazyl
DTNB	5-5′-dithio-bis-2-nitrobenzoic acid
GPx	glutathione peroxidase
GSH	glutathione
H_2O_2	hydrogen peroxide
$KBrO_3$	potassium bromate
LDH	lactate dehydrogenase test
LOOHs	lipid hydroperoxides
LPO	lipid peroxidation
MDA	malondialdehyde
MT	metallothionein
NADPH	nicotinamide adenine dinucleotide phosphate reduced
NBT	nitro blue tetrazolium
PCO	protein carbonyls
Pi	inorganic phosphate
ROS	reactive oxygen species
RT-PCR	reverse transcript polymerase chain reaction
SOD	superoxide dismutase
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TCA	trichloroacetic acid

toxic chemicals, and is believed to bind and neutralizes metals [13]. Among the four known isoforms of MT, MT1 and MT2 have a ubiquitous tissue distribution with particular abundance in the liver and kidney.

Cultural knowledge about medicinal plants handling plays a vital role in the discovery of novel natural products with chemotherapeutic properties [14]. Thereby, vanillin (4-hydroxy-3-methoxybenzaldehyde), a compound isolated from the bean and pod of tropical vanilla orchid, is widely used in the food and beverage industry and is responsible for the characteristic vanilla flavor. This substance is also relevant for the synthesis of different agrochemicals, antifoaming and pharmaceutical products [15]. Although the major biological activities of vanillin are linked to its antioxidant activity, there is little data describing its influence on the expression of genes related to oxidative stress and antioxidant defence in mammalian cells *in vivo* [16–18].

For this purpose, we aimed to study the protective effects of vanillin against $KBrO_3$- induced oxidative damage by evaluating the genes expression of MT1, MT2, superoxide dismutase (SOD) and glutathione peroxidase (GPx), histopathological changes and plasma biochemical markers, since studies of vanillin ability to improve $KBrO_3$-induced nephrotoxicity remain scarce. To confirm its antioxidant power, vanillin was tested, *in vitro*, for its antiradical activity using 1, 1-diphenyl-picrylhydrazyl (DPPH) and 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$)-radical-scavenging assays.

2. Material and methods

2.1. Chemicals and reagents

Potassium bromate and vanillin, required for biochemical assays were obtained from Sigma Chemicals Co. (St. Louis, France). Some compounds like glutathione (disulfide and reduced) (purity, 99%), 1,1-diphenyl-picrylhydrazyl (DPPH) (purity, 97%), 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (purity, 98%), nicotinamide adenine dinucleotide phosphate reduced form (NADPH) (purity, 93%), 5-5′-dithio-bis-2-nitrobenzoic acid (DTNB) (purity, 91%) and thiobarbituricacid (TBA) (purity, 92%) were purchased from Sigma (St. Louis, MO, USA). Other compounds were purchased from other suppliers.

2.2. In vitro antioxidant properties of vanillin

2.2.1. $ABTS^{\bullet+}$-scavenging assay

The ABTS radical-scavenging activity was determined according to Re et al. [19]. The ABTS radical cation was prepared by reacting an aqueous solution of ABTS (7 mM) with potassium persulfate (2.45 mM, final concentration) which was kept in the dark at 25 °C for 12–16 h. 10 μl of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), vitamin C or vanillin were mixed with 990 μl of ethanol and the absorbance was determined at 734 nm after 60 min of initial mixing. Appropriate solvent blanks were run in each assay. The extent of decoloration was estimated by monitoring the reduction of the absorbance at 734 nm.

2.2.2. DPPH radical scavenging activity

The DPPH radical scavenging activity of the vanillin, vitamin E or BHT was evaluated according to the method of Blois [20]. The absorbance was measured spectrophotometrically at 517 nm using model JENWAY 6300 spectrophotometer. All determinations were performed in triplicate.

2.3. Animal diet and tissue preparation

Adult mice of the Swiss strain weighing approximately 30 g were obtained from Central Pharmacy (SIPHAT, Tunisia). Mice were kept in an air-conditioned room (temperature, 22 ± 3 °C; a relative humidity, 40%). They were housed in polycarbonate cages and were provided daily with standard pellet diet (SNA, Sfax, Tunisia) and water ad libitum.

The experimental procedures were carried out according to the Natural Health Institute of Health Guidelines for Animal Care and approved by the Ethical Committee of Sfax Science Faculty. All animal procedures were conducted in strict conformity with the "Institute ethical committee guidelines" for the Care and Use of laboratory animals [21].

The mice were randomly divided in to four groups, with 12 per group. The first group of mice served as the controls. The second group received $KBrO_3$ (2 g/L) via drinking water, group III received $KBrO_3$ (2 g/L) by the same route as group II and 100 mg/kg of vanillin, by intraperitoneal injection. Animals in the fourth group (vanillin) were given daily a single intraperitoneal dose (0.5 ml) of vanillin dissolved in distilled water (100 mg/kg b.w).

The present study was designed to investigate the toxicity of $KBrO_3$ administrated to mice via oral route as 2 g/L. This dose was selected on the basis of previous studies [22] and checked before the setting of the experiment. In fact, in our experimental study and before the setting of the experiment, we have tested 4 doses of $KBrO_3$ (0.5, 1, 2 and 2.5 g/L). No toxic effects and no oxidative stress in the kidney tissue were observed in adult mice treated with $KBrO_3$ at doses of 0.5 and 1 g/L. At dose corresponding to 2 g/L, few clinical signs of toxicity and oxidative stress were observed without