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Immunosuppressant drug tacrolimus induced mitochondrial nephrotoxicity, modified PCNA and Bcl-2 expression attenuated by *Ocimum basilicum* L. in CD1 mice

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ABSTRACT

Tacrolimus (TAC) is used sporadically as an immunosuppressive agent for organ transplantation, but its clinical used is limited due to its marked nephrotoxicity. *Ocimum basilicum* L. (Lamiaceae) (OB) had been shown to possess antioxidant, anti-inflammatory and nephroprotective activity, and effective at improving renal inflammation and glomerular. In our study, we aim to evaluate the efficacy of the OB against TAC-induced mitochondrial nephrotoxicity in CD1 mice. Mice were randomly divided into four groups. Group 1 (control group); administered orally with normal saline (1 mL/kg) for two weeks; Group 2 (OB extract treated-group) (500 mg/kg b.wt) gavaged once/day for two weeks; Group 3 (TAC-treated group) (3 mg/kg b.wt, administered ip once a day for two weeks); and Group 4; (TAC plus OB extract treated-group). Tacrolimus-induced nephrotoxicity was assessed biochemically and histopathologically. The OB extract was high in phenolic content (50.3 mg/g of gallic acid equivalent), total flavonoids (14.5 mg/g CE equivalent). The potential antioxidant efficacy of the extract (IC_{50}) was 24.5 µg/mL. OB pretreatment significantly improved the TAC-induced changes in biochemical markers of nephrotoxicity for instance blood urea nitrogen (BUN), creatinine, total protein, and albumin ($P < 0.01$, when compared with TAC treated group). Also, it significantly restored the increase activities of TBARS, protein carbonyl (PC) ($P < 0.001$, when compared to healthy control group) and decreased activities of nonprotein thiol (NP−SH) levels, Mn-superoxide dismutase (Mn-SOD) and glutathione peroxidase (GPx) antioxidants of mitochondria. The nephroprotective efficacy of the OB leaves extract was further evident by histopathological analysis together with the PCNA-ir and Bcl2. The upshot of the present study revealed that the OB possessed significant antioxidant and nephroprotective activity and had a preventive effect on the biochemical alterations and histological changes in TAC-treated mice.

1. Introduction

The kidney is an excretory organ that executes a critical function in the body. Notably, the metabolism and abolition of therapeutic drugs, xenobiotics, as well as environmental exposures, are remarkable functions. They are exposed to a substantial proportion and a high concentration of drugs and toxins than the other organs [1,2]. Indeed, drug-induced kidney damage is problematic and accounts for 19–26% of cases with acute kidney injury among hospitalized patients [3].

Graft rejection and autoimmune diseases can be prevented by using immunosuppressive drugs. TAC is now an accepted primary immunosuppressive agent that could modulate neutrophil infiltration during inflammation [4]. There may be several factors associated with the mechanism of TAC-induced nephrotoxicity for instance augmentation of vasoconstriction factors, such as endothelin or thromboxane, and a decrease in vasodilation factors like prostacyclin, prostaglandin E2, and nitric oxide. TAC can also produce reactive oxygen species (ROS) via triggering of NADPH oxidase pathway and a fracas in antioxidant defense which may be responsible for nephrotoxicity [5].

It has been found that the green tea extract and polyphenols abrogated TAC-induced nephrotoxicity in mice, rats, and LLC-PK1 cells. Also, they significantly suppressed the increased intracellular reactive

Abbreviations: ABC, Avidin-Biotin- Peroxidase; ANOVA, analysis of variance; BUN, blood urea nitrogen; DNPH, dinitrophenylhydrazine; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; EOBPV, Egyptian Organization for Biological Products and Vaccines; GPx, glutathione peroxidase; GSH, glutathione; H&E, hematoxylin and eosin; H_2O_2, hydrogenperoxide; IAEC, Institutional Animals Ethics Committee; IC, inhibitory concentration; Ip, intraperitoneal; mLPO, mitochondrial lipid peroxidation; Mn-SOD, Mn-superoxide dismutase; NP-SH, nonprotein thiol; OB, *Ocimum basilicum*; OPA, orthophosphoric acid; PC, protein carbonyl; PCNA-ir, proliferating cell nuclear antigen immunoreactivity; ROS, reactive oxygen species; TAC, tacroliums

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oxygen species levels as well as caspase-3 activation [6–8]. Studies on natural products that may minimize TAC-induced nephrotoxicity are still marginal. It has been found that the Ocimum group of species possess a wide range of chemopreventive and therapeutic activities [9,10]. Furthermore, extracts of the leaves exhibit-dominant antioxidant activity in several assays models [11,12]. It has been found to contain linalool, eugenol, methyl chavicol, methyl cinnamate, ferulate, methyl eugenol, triterpenoids and steroidal glycoside known to display antioxidant activities [13–15]. Therefore, it is entirely possible that the extracts may serve as a remedy for disrupting the activity of environmentally acquired toxins, drug-induced perturbations or toxicity. Thus, the primary goal of the study is to assess the protective efficacy of the OB extract against TAC-induced mitochondrial nephrotoxicity in CD1 mice.

2. Materials and methods

2.1. Plant material and extract preparation

Plant leaves were collected from Tabuk region, authenticated by botanist and the alcoholic extraction was performed. Briefly, 600–800 g of OB fresh leaves were harvested, washed, dried in the shade and subsequently crushed in a grinder. Further, in a tightly covered container, the dried powder of leaves was immersed in 90% ethyl alcohol and was allowed to put for 15 min. Later, moved to a percolator, where it was mightily packed in and allowed to macerate for 24 h at room temperature, followed by slow percolation. The process was repeated until no further extraction was possible, and the obtained residue was transferred to a vacuum desiccator.

2.2. Phytochemical screening

2.2.1. Flavonoid estimation

The presence of flavonoid in the extracts was measured by a colorimetric assay as was emphasized by [16] For the calibration curve, rutin was used as a standard and was expressed as mg rutin equivalents (RE) per gram of sample (mg/g).

2.2.2. Analysis of total phenolic content

The accumulated amounts of phenolic content were assessed as reported by [17]. Gallic acid (0.1 mg/mL) was prepared and different concentrations were used for the standard curve and were expressed as mg/g gallic acid equivalent.

2.2.3. Assessment of total antioxidant capacity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) method was used for the estimation of total antioxidant capacity as recorded by [18]. The presence of antioxidants lowers the concentration of DPPH at 515 nm, and the engrossing nature disappears as the process continues. Analysis of the samples was done in triplicates, and the outcomes were calculated as averages. A negative control was taken after adding DPPH solution to 0.1 mL of the methanol.

2.3. Animals

The experiments were performed on 40 male CD1 mice weighing 20 ± 2 g and of 10–12 weeks old were procured from the breeding unit of Egyptian Organization for Biological Products and Vaccines (EOBPV), Abbassia, Cairo. The mouse was kept in steel mesh cages and maintained for one week acclimatization period on commercial standard and pellet diet and drinking water *ad libitum*. The housing cycle was 12:12 h light -dark cycle under controlled temperature (20–22 °C). The animal use implementation was approved by the Institutional Animals Ethics Committee (IAEC) of Tanta University.

Table 1
Total flavonoids; total phenolics contents and percentage inhibition of antioxidant activity (IC_{50}) in OB leaves extract. The value represents mean ± S.D of three determinations.

Parameter	OB leaves Extracts
Total phenolic compound (mg/ g gallic acid)	50.28
IC_{50} (μg/ml)	24.5
Total Flavonoids (TF) (mg/g dry weight)	14.54

2.4. Experimental design

For *in vivo* assessment of TAC-induced mitochondrial nephrotoxicity and its modulation by OB, the overnight fasted mice were administered with intraperitoneal (ip) dose (3 mg/kg) of TAC, once a day for two weeks. They were divided into four groups of 10 animals each. Group 1 (control group)- normal saline (1 mL/kg) for two weeks; Group 2 (OB group) mice were administered daily with OB extract (500 mg/kg b.wt.) for two weeks; Group 3 (TAC treated group); and Group 4; TAC + OB extract group. For co-administration of TAC and OB extract, OB was gavaged after ip administration of TAC for two weeks. The maximal effective dose of OB was selected according to the DPPH radical scavenging activity.

2.5. Sample preparation and biochemical analysis

Samples of blood were taken from the orbital sinus, and the serum was separated by centrifugation at 3000 *g* for 10 min using capillary tubes and renal function parameters were estimated by the auto-analyzer.

Mitochondria from kidney tissues were isolated from the fasted animal by the differential centrifugation method as reported by [19,20]. Briefly, in an ice-cold isolation buffer containing 0.25 M sucrose, 1 mmol EDTA adjusted by Tris to pH 7.4 tissue was homogenized and centrifuged at 800 *g* for 5 min. Further, the supernatant was centrifuged for 10 min, and the obtained pellet was resuspended and washed in a 0.25 M sucrose medium. Finally, the pellet was resuspended in a 0.25 M sucrose medium adjusted by Tris to pH 7.4. The protein concentration of the stock suspension was determined by using the Lowry method.

2.6. Estimation of mitochondrial lipid peroxidation (mLPO)

m LPO quantification was done by the method of Waseem and Parvez [20]. The reaction mixture consisted of 10 mM BHT, 0.67% TBA and 1% chilled orthophosphoric acid (OPA). The rate was determined as μmoles of TBARS formed/h/g of tissue using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}cm^{-1}$

2.7. Protein carbonyl content

Protein carbonyl content was measured using dinitrophenylhydrazine (DNPH) as described by Sohal et al. [21]. The difference in the absorbance between DNPH treated and HCl treated sample is determined and expressed as nmoles of carbonyl groups per mg of protein, using extinction coefficient of $22\ mM^{-1}\ cm^{-1}$.

2.8. Nonprotein bound thiol

Determination of nonprotein thiol was done by the method described by Sedlak and Lindsay [22]. The results were read from a standard curve prepared from 1 mmol/L solution of reduced glutathione, and the absorbance of the supernatant was measured at 412 nm. NP-SH was expressed as μmoles NP-SH/g tissue DTNB molar extinction coefficient of $13{,}100 M^{-1}/cm^{-1}$.