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

Atif Abdulwahab A. Oyouni[a],[*], Shalini Saggu[a],[*], Ehab Tousson[b], Hasibur Rehman[a]

[a] *Department of Biology, Faculty of Sciences, University of Tabuk, Tabuk, 71491, Saudi Arabia*
[b] *Department of Zoology, Faculty of Sciences, Tanta University, Tanta, 31527, Egypt*

A B S T R A C T

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

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 $13,100 M^{-1}/cm^{-1}$.
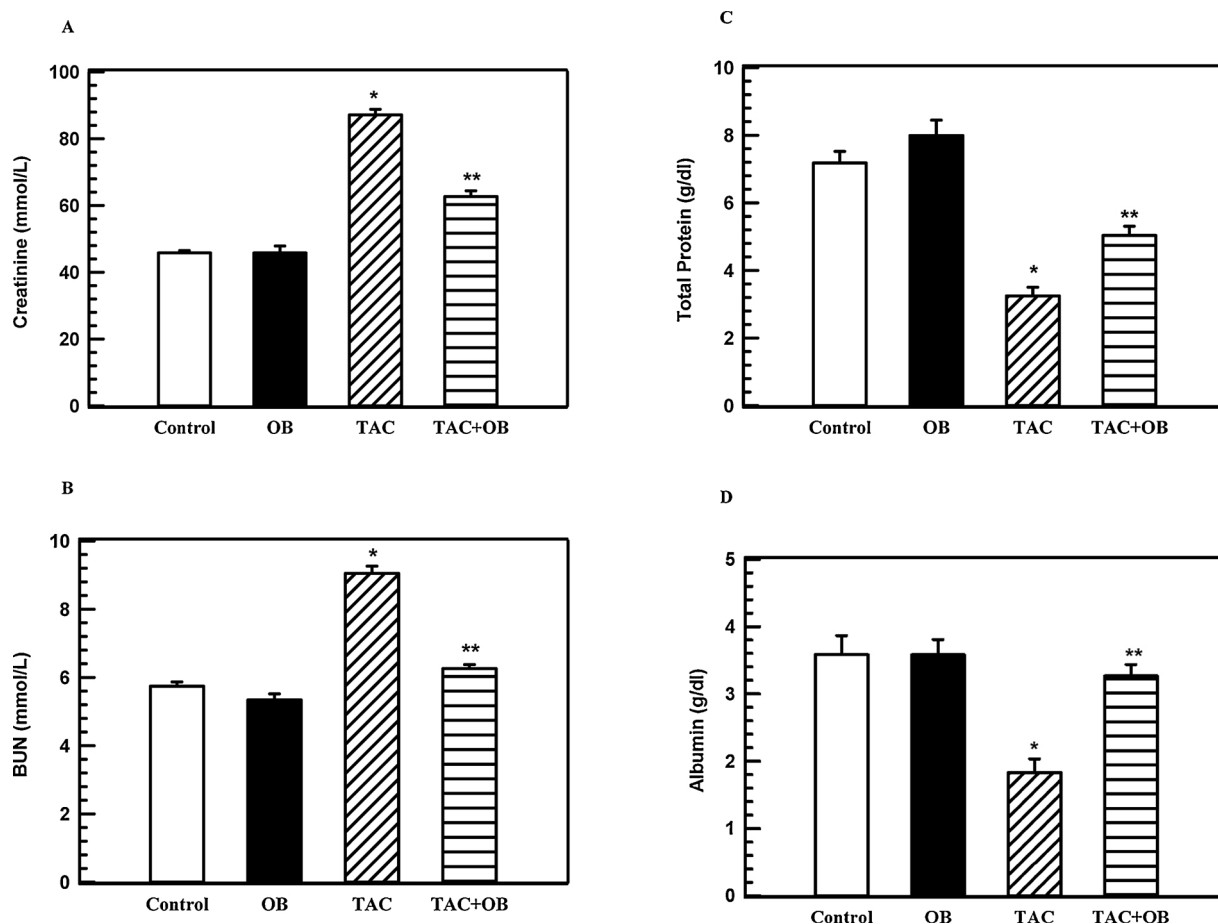
Fig. 1. Effect of OB leaves extract administration on kidney function test: Creatinine (A), BUN (B), total protein (C) and albumin (D) levels in TAC-induced nephrotoxicity in CD1 mice. Bar represents mean ± S.E.N = 10 animals per group. Statistical significances: (*P < 0.001, compared with normal control group; **P < 0.01, compared with TAC treated group).

2.9. Enzymatic and non-enzymatic antioxidants

2.9.1. Quantitative determination of MnSOD activity

The activity of MnSOD was determined by the method as described by Flohe and Otting [23]. Briefly, mitochondrial pellet suspended in 0.05 M buffer (KH_2PO_4-$NaHPO_4$/0.1 mM EDTA, pH 7.4) was disrupted by sonication. The supernatant was collected after centrifugation, and 50 μL of the sample was added to 2.9 mL of the reaction mixture (500 mM xanthine, 0.1 mM NaOH and 2 M cytochrome c in the above buffer. The reaction was started by addition of xanthine oxidase(50 μL of 0.2 U/mL in 0.1 mM EDTA), and the change in absorbance was measured at 438 nm and expressed as U/mg of mitochondrial protein.

2.9.2. Measurement of mitochondrial glutathione peroxidase activity

The activity of glutathione peroxidase was assayed by the method described by Flohe and Gunzler [24]. Briefly, Mitochondria (0.1 mg protein/mL) were suspended in reaction mixtures containing (1 mM GSH, 0.4 U of glutathione reductase, 0.22 mM NADPH, 5 mM EDTA and 50 mM Tris − HCl buffer, pH 7.6). t-Butylhydroperoxide was added to start the reaction, and the absorbance was measured at 340 nm for 3 min. One unit of the enzyme is defined as one nanomole of NADPH consumed per minute per mL. Results were expressed as U/mg of mitochondrial protein.

3. Histopathological examination

The histopathology was carried out according to Scheuer [25]. Briefly, after excision of kidneys, instantly they were washed using chilled saline solution and treated for further bioassays. A small section was straightaway fixed in 10% formalin and later embedded in paraffin, 5 μm sectioned were cut and stained with hematoxylin and eosin (H&E), and examined under a light microscope.

## 3.1. PCNA immunoreactivity and anti-apoptotic Bcl2 assessment

Proliferating cell nuclear antigen immunoreactivity (PCNA-ir) and anti-apoptotic Bcl2 were performed according to Tousson et al., [26,27]. PCNA stained nuclei and cytoplasmic Bcl2 distribution were examined in deparaffinized sections (5 μm) using an Avidin-Biotin-Peroxidase (ABC) immunohistochemical method (Elite–ABC, Vector Laboratories, CA, USA) with PCNA monoclonal antibody or with anti-rabbit Bc1-2 monoclonal antibody (Dako, Japan; 1:100 and 1:2000, respectively) and mounted on poly-L-lysine-coated glass slides, and viewed using Olympus microscope. Sections were examined blindly at high power. Microscopic fields were randomly selected, and five fields per slide were assessed. Nuclear labeling indices for PCNA (positive nuclei/total number of counted nuclei) were determined.

4. Statistical analysis

Data are presented as means ± SE for at least six replications for each prepared sample. For statistical analysis, one-way analysis of variance (ANOVA) followed by Student–Newman–Keul's test was done using SigmaPlot, Systat Software program version 11, to assess significant differences among different groups. They were considered to be significant when P < 0.05.
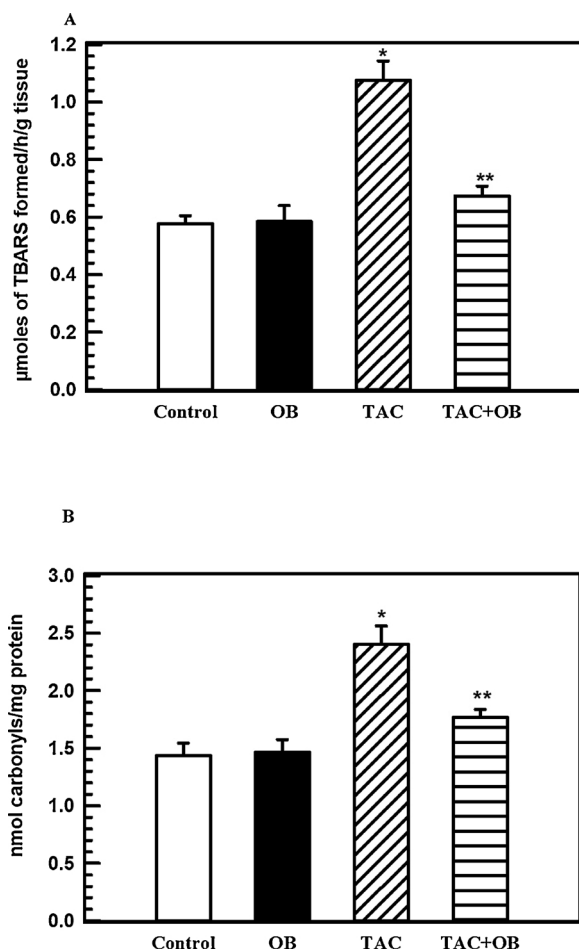
Fig. 2. Efficacy of OB leaves extract on kidney mitochondrial LPO (A) and PC (B) levels in TAC-induced nephrotoxicity in mice. Bar represents mean ± S.E.N = 10 animals per group. Significance differences indicated by *P < 0.001, compared with normal control group; **P < 0.01, compared with TAC treated group.
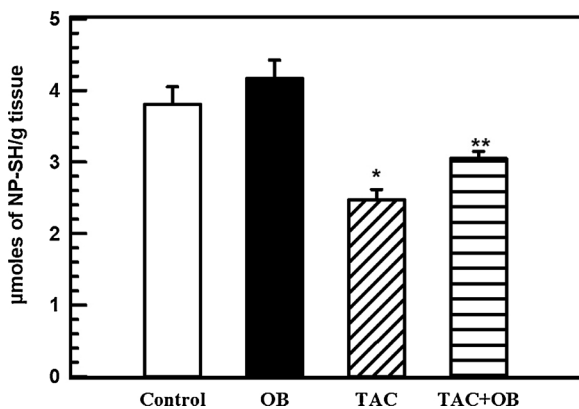
Fig. 3. TAC pretreatment affects mitochondrial NP−SH content in mice and ameliorated by OB extract. Bar represents mean ± S.E.N = 10 animals per group. Significance differences indicated by *P < 0.01, compared with normal control group; **P < 0.05, compared with TAC treated group.

5. Results

5.1. Quantifiable phytochemical analysis

The potential efficacy of the OB extract used in this study was partly assessed by estimating the content of total phenols, flavonoid and its

Table 2
Effect of *OB* extracts on Mn-SOD and GPx activities in TAC-treated mice. Values are means ± S.E. N = 10 animals per group. Mn-SOD and GPx were expressed as U/mg mitochondrial protein. Statistical significances are indicated by *P < 0.01 when compared with normal control group; **P < 0.05, compared with TAC-treated groups.

Group	Parameter	
	Mn-SOD	GPx
Control	4.33 ± 0.36	0.33 ± 0.02
OB extract	4.63 ± 0.46	0.37 ± 0.03
TAC	2.07 ± 0.28*	0.14 ± 0.01*
TAC + OB extract	3.21 ± 0.27**	0.28 ± 0.03**

antioxidant capacity (IC_{50}). The extract was found to be high in phenolic content (50.28 mg/g of gallic acid equivalent), total flavonoids were around 14.54 mg/g of CE equivalent) which might be accountable for its observed antioxidant activity (Table 1).

DPPH test using the parameter IC50 quantified the free radical scavenging or the potential antioxidant efficacy of the plant extract. The extracts had dose-dependent activity, and the IC_{50} value of the extract was found to be 24.5 µg/mL (Table 1).

5.2. Toxicity

During the study, no mortality was recorded in animals. The OB extract was found to be safe and did not produce any side effects in the animals.

5.3. Effect on renal damage indicators

Levels of serum creatinine, BUN, total protein and albumin in control, TAC, TAC + OB leaves extract treated mice after two weeks are shown in Fig. 1A, B, C, and D. The level of serum creatinine, BUN was significantly elevated in mice treated with TAC (Fig. 1A and B). Pre-treatment with the OB extract attenuated the changes in these levels, showing the protective efficacy of the extract. However, the level of total protein and albumin were strikingly altered in TAC-treated group mice as compared to control group (Fig. 1C and D). The pre-exposure to OB defended the TAC-induced impediment of total protein and albumin.

5.4. OB impedes an increase in LPO and PC content in the TAC-exposed group

TAC treated group led to significant augmentation in the content of TBARS (*$p < 0.001$)(Fig. 2A) and PC (Fig. 2B) compared to control group. In our results, OB pre-exposure (group IV) notably dwindle the level (**$p < 0.001$-0.001) of LPO and PC contents when compared with TAC- exposed group (group III).

5.5. Effect on non-protein thiol (glutathione)

The TAC-treated group (group III) caused a significant diminution ($P < 0.001$) in NP−SH product in kidney mitochondria when compared with the control group (Fig. 3). OB pretreatment significantly elevated the content in NP−SH levels as compared to control ($p = 0.016$). This articulated to the fact that cells acclimatizing pre-protection by OB are susceptible to TAC-induced mitochondrial oxidative stress.

5.6. OB extracts enhance enzymatic antioxidants after TAC treatment

TAC-treatment (group III) significantly (**$p < 0.01$) decreased Mn-SOD (Table 2) activity in kidney mitochondria as compared to control. There was a -0.43-fold decrease in a TAC-treated group whereas, OB
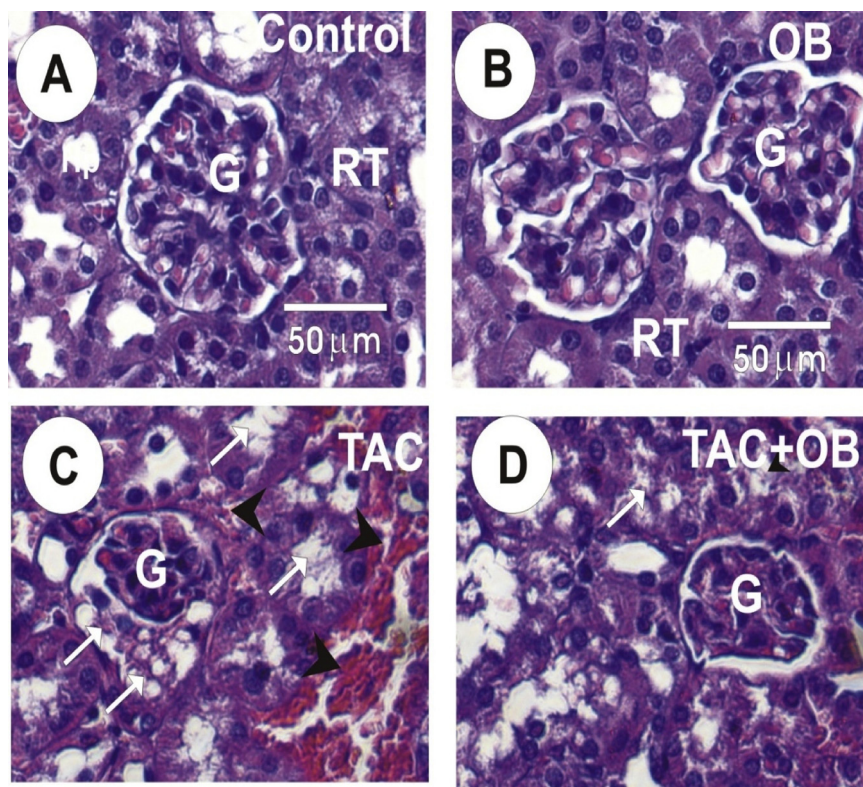
Fig. 4. (A−D): Photomicrographs of mice kidney sections of different experimental groups stained with hematoxylin and eosin. (A and B): kidney sections of control (A) and OB extract (B) groups. (C): sections of the TAC-treated group and (D) TAC and OB extract group. The bar is 50 μm.

Table 3
The severity of damage in the kidney sections in different treated groups.

Histological changes	Control	OB	TAC group	TAC + OB group
Glomerular atrophy	–	–	+ + +	+ +
Degeneration in renal tubules	–	–	+	+
Focal necrosis	–	–	+ +	+ +
Glomerular congestion	–	–	+ +	+
Interstitial infiltrations	–	–	+ +	+
Vacuolation	–	–	+ + +	+
Apoptosis	–	–	+	±
Hemorrhage	–	–	+ +	+

Grade: -, Negative; ± , Very slight; +, Slight; + +, Moderate; + + +, Severe or marked.

extracts pretreatment upturned the observed decreased activity of Mn-SOD (Table 2). Our result signifies that the observed reduction may be due to the enhancement of superoxide radical formation and the OB pretreatment revitalized Mn-SOD action in kidney mitochondria.

Mitochondria from TAC-treated animals showed a significant decrease in the activity of GPx ($p < 0.01$) when compared to control animals. However, the OB pretreatment resulted in noteworthy augmentation in GPx activity when compared to TAC-treated animals. Alone OB treatment showed no substantial effect in the activities of both the antioxidant enzymes in the mitochondria (Table 2).

5.7. Histopathological alterations in kidney

The histopathological changes in the kidney sections in the different groups are shown in Fig. 4(A–D) and Table 3. The histological structure of mice kidney sections in control and basil groups revealed the normal structure of cortex and medulla (Fig. 4A & B). Examination of kidney sections after intraperitoneal injection of tacrolimus at the concentration 3 mg/kg body weight for two weeks revealed severe

histopathological changes in the renal cortex and medulla (Fig. 4C, Table 3). These changes were increased with increasing the time of injections and concentrations. Treatment of tacrolimus with basil revealed a good improvement (Fig. 4D). A semiquantitative rating for each slide ranging from normal (or minimal) to severe (extensive damage) was assigned to each component. Severity was graded as absent/normal (-), very slight (±), slight (+), moderate (+ +) and severe (+ + +) (Table 3)

5.7.1. Proliferating cell nuclear antigen (PCNA) immunohistochemical changes

Cells were PCNA positive if there were brown nuclear staining and negative nuclei not stained and appear blue. The distribution and detection of PCNA expression in kidney sections in the different groups were shown in Fig. 5(A–D) and Table 4. Strong positive reaction (grade 6) for PCNA expression was observed in the kidney in control and basil groups (Fig. 5A and B). A significant decrease in the expression of PCNA was observed in the kidney after intraperitoneal injection of TAC (3 mg/kg b.wt) when compared with the control group (Fig. 5C). PCNA expression was faint or very low (grade 1) positive reaction in kidney section in treated mice with TAC for two weeks (Fig. 5C and Table 4). A significant increase in PCNA expression was observed in kidney section in treated mice with when pre-treated with OB (Fig. 5D).

5.7.2. Anti-apoptotic Bcl2 immunohistochemical changes

Cells were Bcl2 positive if there was brown cytoplasm staining of the cells and negative nuclei not stained and appear blue. The distribution and detection of Bcl2 expression in kidney sections in the different groups were shown in Fig. 6(A–D), and Table 4.Strong positive reaction (grade 6) for Bcl2 expression was observed in the kidney in control and basil groups (Fig. 6A and B). A significant decrease in the expression of Bcl2 was observed in the kidney after intraperitoneal injection of TAC at the dose of 3 mg/kg b.wt for two weeks when compared with the control group (Fig. 6C). Bcl2 expression was mild
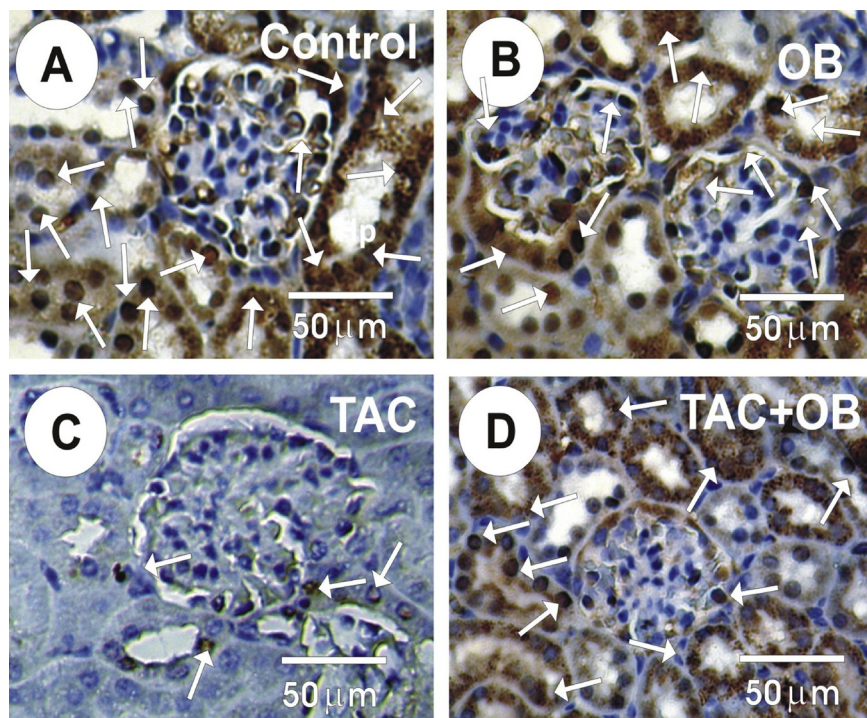
Fig. 5. Photomicrographs of PCNA-ir (immunoperoxidase labeling) in the kidney cross-sections in control rats (A) and OB extract (B), showed a strong positive reaction (grade 6) for PCNA expression. (C) And (D) are the sections of the TAC-treated group (decrease in expression of PCNA) and TAC plus OB extract group (slight increase in the staining compared to the TAC-treated group). PCNA indicates proliferating cell nuclear antigen.

Table 4
Grades of PCNA and Bcl2 changes in kidney sections in the different groups.

	Control	OB	TAC group	TAC + OB group
PCNA	6	6	1	3
Bcl2	6	6	2	5

(grade 2, Table 4) positive reaction in kidney section of the TAC-treated group. A significant increase in Bcl2 expression was observed when pretreated with the OB extract as compared with a TAC-treated group (Fig. 6C and D).

6. Discussion

TAC, a calcineurin inhibitor widely used in the transplantation but its adverse effects are a significant obstacle in long-term immunosuppressive therapy [28,29]. The mechanism of TAC-induced nephrotoxicity is less known, but evidence shows that the calcineurin pathway might mediate it. Therefore, in kidney TAC binding proteins are present at a high concentration compared to liver and spleen.

Our preliminary phytochemical finding in this study revealed the presence of phenolic compounds and flavonoids in the alcoholic extract obtained from OB leaves. Redox properties of the phenols permit their action as reducing agents, hydrogen donors and singlet oxygen quenchers [30]. Hence, the OB extract is of value for quantifying the
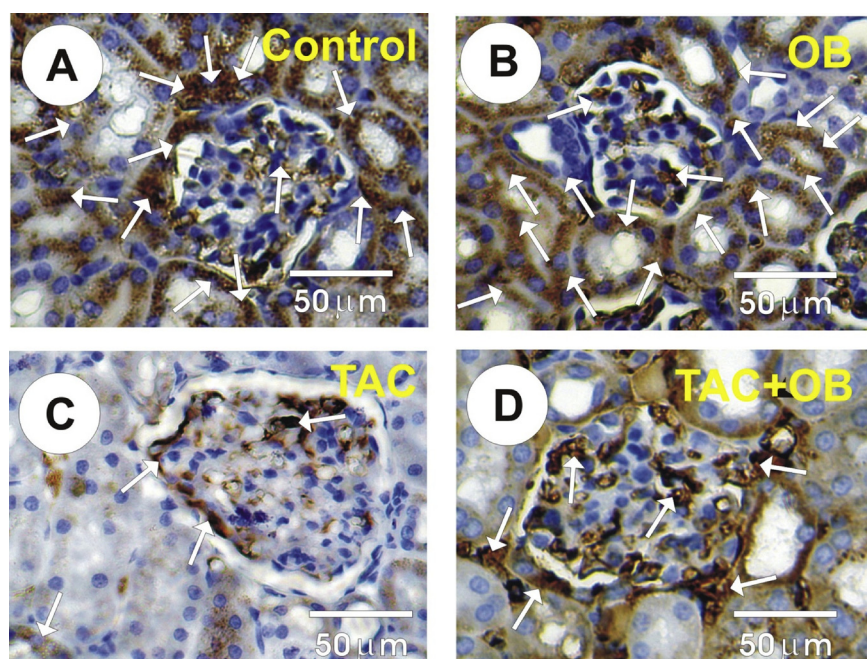
Fig. 6. Representative mice kidney sections processed for anti-apoptosis assay by the Bcl-2 reaction. A. Control group. B. OB group. The strong positive reaction for Bcl-2 expression was observed in the kidney in control, and OB treated mice. TAC treated (C) group showed marked decrease in Bcl-2 ir Whereas, TAC plus OB treated (D) group shows a significant increase in the expression of Bcl-2-ir (arrows).

putative role of them as antioxidant agents, for confronting the oxidative stress. In our study, the alcoholic extract of the OB contains proper magnitude of total antioxidant activity, total phenolic and total flavonoids content (Table 1).

Oral administrations of OB extract showed no signs of toxicity and mortality after 48 h in doses up to 3000 mg/ kg, asserting a possibility that the extracts were nontoxic and are safe. Plants with LD values past than 5000 mg/kg are nontoxic.

Mitochondria play an essential role in the cytotoxicities of several drugs and chemicals [31,32]. In the present work, the observed nephrotoxicity due to immunosuppressive TAC treatment was manifested by marked increases in serum creatinine, BUN clearance, and a decrease in total protein and albumin. The intensities of creatinine and BUN depend on the glomerular filtration rate (GFR). The observed increase in creatinine and BUN may be due to the decrease in the glomerular filtration rate or may be secondary due to the rise of the reactive oxygen species [33]. Previous reports have confirmed that tacrolimus-induced nephrotoxicity is unveiled by arising in the serum levels of creatinine and BUN [34–36].

In our study, we have observed a significant rise in the mLPO levels in TAC-treated mice as compared to the control group. It may be due to the enhanced production of ROS. Congruently, other authors also described the similar drift in their studies with a cumulative dose of tacrolimus [35]. OB pretreatment for two weeks instigated the decrease in mitochondrial LPO, showing a protective action against LPO. In 2004, Zhou et al. [37], reported that tacrolimus significantly enhanced the production of ROS leading to death.

PC is a universally accepted biomarker of protein carbonyl accumulation and oxidation of protein. TAC pretreatment aggravated the increase in protein oxidation and OB pretreatment restored the levels in insolated kidney mitochondria (Fig. 2).

For sustaining proper energy supply and function of tissues, mitochondrial homeostasis is crucial, and its abundance is determined by biogenesis, fission/fusion, and mitophagy. Oxidative stress-induced mitochondrial biogenesis has also been reported in various tissues and cells [38,39]. It was shown that during normal cellular processes ROS generated are instantaneously detoxified by endogenous antioxidants like GSH, catalase, GR, GRx, GST,etc., but the unnecessary accumulation of ROS produced by oxidative stress causes a disproportion in antioxidant status leading to lipid peroxidation and GSH depletion [40]. Regarding non-enzymatic antioxidants, NP-SH is the first line of defense against oxidative stress. According to our results, TAC treated mice significantly decreased the NP-SH content in isolated kidney mitochondria. OB pretreatment considerably restored the NP-SH content (Fig. 3).

Mn-SOD, one of the critical enzyme found in the mitochondrial matrix, catalyzes the dismutation of superoxide, forming hydrogen peroxide and molecular oxygen [41,42]. By scavenging superoxide, its activity limits the reaction of superoxide with nitric oxide to form the reactive nitrogen species peroxynitrite. GPx also plays an essential role in the H_2O_2 detoxification. In our study, we found that both these vital antioxidants (Mn-SOD and GPx) were affected by TAC pretreatment and their reduced activities indicate the accumulation of superoxide radical and H_2O_2. OB pretreatment restored the levels of these antioxidants showing its efficacy effect. It is a well-known fact that ROS generation plays a significant role in peroxidation of lipid membranes of the tissue, resulting in subcellular damage as evident in the histopathological examination (Fig. 4). In our study, the kidney of TAC-treated mice has shown characteristic morphological findings such as glomerular atrophy, degeneration in renal tubules, focal necrosis, hemorrhage, vacuolation, etc. (Table 3). It creates an ischemic local environment, which leads to some cellular changes such as deterioration in membrane integrity of the kidney. Similar histopathological modifications including tubular vacuolization, arteriolar hyalinosis, interstitial fibrosis and juxtaglomerular hyperplasia were reported by [36,43,44].

The synthesis of proliferating cell nuclear antigen(a non-histone

nuclear protein)directly correlates with the rates of cellular proliferation and DNA synthesis [45]. The nephroprotective efficacy of the OB leaves extract was further revealed by with the PCNA-ir and Bcl2 staining. The mice injected with TAC group showed an abundant increase in the number of PCNA and Bcl2 staining of nuclei which was reduced by OB extracts administration (Figs. 5 and 6). The pattern of Bcl-2 expression in normal and diseased glomeruli suggests and supports the reported notion that the mechanism of apoptosis may be increased in the injured glomerulus [46].

7. Conclusions

Taken together, OB pretreatment in mice protected against TAC-induced nephrotoxicity, at least in part, by enhancing through free radical scavenging activity and this may be due to the presence of antioxidants and flavonoids in the extract. Protective efficacy was complemented by a significant attenuation of oxidative stress parameters in mitochondria isolated from kidney, augmentation in renal function biochemistry as well as the reinstatement of renal structures evinced by histopathological, PCNA and Bcl2 findings.

Authors' contributions

HR, AAO, SS, ET were involved in conception or experimental design.

HR, SS, and ET were involved in the acquisition, analysis, and interpretation of data.

HR, SS were involved in drafting/revising the manuscript.

Conflict of interest

There are no conflicts to be declared.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

References

[1] K. Hosohata, Role of oxidative stress in drug-induced kidney injury, Int. J. Mol. Sci. 17 (2016) 1826.
[2] Y. Tomino, Drug-induced kidney injury, Nihon Jinzo Gakkai Shi 54 (2012) 949.
[3] R. Borrows, G. Chusney, M. Loucaidou, S. Singh, A. James, J. Stichbury, J. Van Tromp, T. Cairns, M. Griffith, N. Hakim, A. Mclean, A. Palmer, V. Papalois, D. Taube, Tacrolimus monitoring in renal transplantation: a comparison between high-performance liquid chromatography and immunoassay, Transplant. Proc. Elsevier (2005) 1733–1735.
[4] K. Bendickova, F. Tidu, J. Fric, Calcineurin-NFAT signalling in myeloid leucocytes: new prospects and pitfalls in immunosuppressive therapy, EMBO Mol. Med. (2017) e201707698.
[5] A.K. Khanna, G.M. Pieper, NADPH oxidase subunits (NOX-1, p22phox, rac-1) and tacrolimus-induced nephrotoxicity in a rat renal transplant model, Nephrol. Dial. Transplant. 22 (2007) 376–385.
[6] J.H. Back, H.H. Ryu, R. Hong, S.A. Han, Y.M. Yoon, D.H. Kim, S.J. Hong, H.L. Kim, J.H. Chung, B.C. Shin, Y.E. Kwon, Antiproteinuric effects of green tea extract on tacrolimus-induced nephrotoxicity in mice, Transplant. Proc. 47 (2015) 2032–2034.
[7] F. Hisamura, A. Kojima-Yuasa, X. Huang, D.O. Kennedy, I. Matsui-Yuasa, Synergistic effect of green tea polyphenols on their protection against FK506-

induced cytotoxicity in renal cells, Am. J. Chin. Med. 36 (2008) 615–624.

[8] Z. Zhong, H.D. Connor, X. Li, R.P. Mason, D.T. Forman, J.J. Lemasters, R.G. Thurman, Reduction of ciclosporin and tacrolimus nephrotoxicity by plant polyphenols, J. Pharm. Pharmacol. 58 (2006) 1533–1543.

[9] S. Godhwani, J.L. Godhwani, D.S. Vyas, Ocimum sanctum: an experimental study evaluating its anti-inflammatory, analgesic and antipyretic activity in animals, J. Ethnopharmacol. 21 (1987) 153–163.

[10] S. Rastogi, Y. Shukla, B.N. Paul, D.K. Chowdhuri, S.K. Khanna, M. Das, Protective effect of Ocimum sanctum on 3-methylcholanthrene, 7, 12-dimethylbenz (a) anthracene and aflatoxin B1 induced skin tumorigenesis in mice, Toxicol. Appl. Pharmacol. 224 (2007) 228–240.

[11] I. Gulcin, M. Elmastas, E. Aboul, Y. Hassan, Determination of antioxidant and radical scavenging activity of basil (Ocimum basilicum L. Family lamiaceae) assayed by different methodologies, Phytother. Res. 21 (2007) 354–361.

[12] C. Jayasinghe, N. Gotoh, T. Aoki, S. Wada, Phenolics composition and antioxidant activity of sweet basil (Ocimum basilicum L.), J. Agric. Food. Chem. 51 (2003) 4442–4449.

[13] B.S. Siddiqui, H. Aslam, S.T. Ali, S. Begum, N. Khatoon, Two new triterpenoids and a steroidal glycoside from the aerial parts of Ocimum basilicum, Chem. Pharm. Bull. 55 (2007) 516–519.

[14] B.S. Siddiqui, H. Aslam, S. Begum, S.T. Ali, New cinnamic acid esters from Ocimum basilicum, Nat. Prod. Res. 21 (2007) 736–741.

[15] V.D. Zheljazkov, A. Callahan, C.L. Cantrell, Yield and oil composition of 38 basil (Ocimum basilicum L.) Accessions grown in Mississippi, J. Agric. Food Chem. 56 (2007) 241–245.

[16] A. Rohman, Y.B. Man, S. Riyanto, Authentication analysis of red fruit (Pandanus conoideus lam) oil using FTIR spectroscopy in combination with chemometrics, Phytochem. Anal. 22 (2011) 462–467.

[17] K.K. Jindal, R.N. Singh, Phenolic content in male and female Carica papaya: a possible physiological marker for sex identification of vegetative seedlings, Physiol. Plant 33 (1975) 104–107.

[18] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT-Food Sci. Technol. 28 (1995) 25–30.

[19] S. Parvez, K. Winkler-Stuck, S. Hertel, P. Schonfeld, D. Siemen, The dopamine-D2-receptor agonist ropinirole dose-dependently blocks the Ca 2 + -triggered permeability transition of mitochondria, Biochimica Et Biophysica Acta (BBA)-Bioenergetics 1797 (2010) 1245–1250.

[20] M. Waseem, S. Parvez, Mitochondrial dysfunction mediated cisplatin induced toxicity: modulatory role of curcumin, Food Chem. Toxicol. 53 (2013) 334–342.

[21] R.S. Sohal, S. Kamzalov, N. Sumien, M. Ferguson, I. Rebrin, K.R. Heinrich, M.J. Forster, Effect of coenzyme q 10 intake on endogenous coenzyme q content, mitochondrial electron transport chain, antioxidative defenses, and life span of mice, Free Radic. Biol. Med. 40 (2006) 480–487.

[22] J. Sedlak, R.H. Lindsay, Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with ellman's reagent, Anal. Biochem. 25 (1968) 192–205.

[23] L. Flohe, F. Otting, Superoxide dismutase assays, Methods Enzymol. 105 (1984) 93–104.

[24] L. Flohe, Glutathione peroxidase, oxygen radicals in biology and medicine, Springer (1988) 663–668.

[25] P.J. Scheuer, Clinical Tests Histopathology, Wolfe Medical Publications Limited, 1986.

[26] E. Tousson, E.M. Ali, W. Ibrahim, M.A. Mansour, Proliferating cell nuclear antigen as a molecular biomarker for spermatogenesis in PTU-induced hypothyroidism of rats, Reprod. Sci. 18 (2011) 679–686.

[27] E. Tousson, E. Hafez, S. Zaki, A. Gad, P53, bcl-2 and CD68 expression in response to

amethopterin-induced lung injury and ameliorating role of l-carnitine, Biomed. Pharmacother. 68 (2014) 631–639.

[28] R. Haynes, C. Baigent, P. Harden, M. Landray, M. Akyol, A. Asderakis, A. Baxter, S. Bhandari, P. Chowdhury, M. Clancy, et al., Campath, calcineurin inhibitor reduction and chronic allograft nephropathy (3C) study: background, rationale, and study protocol, Transplant. Res. 2 (2013) 7.

[29] H.U. Meier Kriesche, S. Li, R.W. Gruessner, J.J. Fung, R.T. Bustami, M.L. Barr, A.B. Leichtman, Immunosuppression: evolution in practice and trends, 1994-2004, Am. J. Transplant. 6 (2006) 1111–1131.

[30] R. Thirugnanasampandan, R. Jayakumar, Protection of cadmium chloride induced DNA damage by lamiaceae plants, Asia. Pac. J. Trop. Biomed. 1 (2011) 391–394.

[31] K. Begriche, A. Igoudjil, D. Pessayre, B. Fromenty, Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it, Mitochondrion 6 (2006) 1–28.

[32] C.M. Cardoso, J.B. Custodio, L.M. Almeida, A.J. Moreno, Mechanisms of the deleterious effects of tamoxifen on mitochondrial respiration rate and phosphorylation efficiency, Toxicol. Appl. Pharmacol. 176 (2001) 145–152.

[33] S. Noori, T. Mahboob, Antioxidant effect of carnosine pretreatment on cisplatin-induced renal oxidative stress in rats, Indian J. Clin. Biochem. 25 (2010) 86–91.

[34] A.A. Fouad, I. Jresat, Protective effect of telmisartan against cadmium-induced nephrotoxicity in mice, Life Sci. 89 (2011) 29–35.

[35] M.A. Ibrahim, O.M. Ashour, Y.F. Ibrahim, H.I. El-Bitar, W. Gomaa, S.R. Abdel-Rahim, Angiotensin-converting enzyme inhibition and angiotensin AT 1-receptor antagonism equally improve doxorubicin-induced cardiotoxicity and nephrotoxicity, Pharmacol. Res. 60 (2009) 373–381.

[36] P.S. Randhawa, T.E. Starzl, A.J. Demetris, Tacrolimus (FK506)-associated renal pathology, Adv. Anat. Pathol. 4 (1997) 265.

[37] X. Zhou, G. Yang, C.A. Davis, S.Q. Doi, P. Hirszel, C.S. Wingo, A. Agarwal, Hydrogen peroxide mediates FK506-induced cytotoxicity in renal cells, Kidney Int. 65 (2004) 139–147.

[38] M. Kitada, S. Kume, N. Imaizumi, D. Koya, Resveratrol improves oxidative stress and protects against diabetic nephropathy through normalization of Mn-SOD dysfunction in AMPK/SIRT1-independent pathway, Diabetes 60 (2011) 634–643.

[39] D.C. Wallace, Mitochondrial diseases in man and mouse, Science 283 (1999) 1482–1488.

[40] D. Kouretas, A.M. Tsatsakis, J.L. Domingo, H.A. Wallace, Mechanisms involved in oxidative stress regulation, Food Chem. Toxicol. 61 (2013) 1.

[41] L.A. Macmillan-Crow, D.L. Cruthirds, Invited review: manganese superoxide dismutase in disease, Free Radic. Res. 34 (2001) 325–336.

[42] S. Miltonprabu, V. Manoharan, Hepatoprotective effect of grape seed proanthocyanidins on cadmium-induced hepatic injury in rats: possible involvement of mitochondrial dysfunction, inflammation and apoptosis, Toxicol. Rep. 3 (2016) 63–77.

[43] A. Khanna, M. Plummer, C. Bromberek, B. Bresnahan, S. Hariharan, Expression of TGF-β and fibrogenic genes in transplant recipients with tacrolimus and cyclosporine nephrotoxicity, Kidney Int. 62 (2002) 2257–2263.

[44] H. Pavenstadt, W. Kriz, M. Kretzler, Cell biology of the glomerular podocyte, Physiol. Rev. 83 (2003) 253–307.

[45] H. Shiina, M. Igawa, H. Nagami, H. Yagi, S. Urakami, T. Yoneda, H. Shirakawa, T. Ishibe, M. Kawanishi, Immunohistochemical analysis of proliferating cell nuclear antigen, p53 protein and nm23 protein, and nuclear DNA content in transitional cell carcinoma of the bladder, Cancer 78 (1996) 1762–1774.

[46] L. Nakopoulou, K. Stefanaki, J. Papadakis, J. Boletis, P.M. Zeis, A. Kostakis, G. Vosnides, Expression of bcl-2 oncoprotein in various types of glomerulonephritis and renal allografts, Nephrol. Dial. Transplant. 11 (1996) 997–1002.