AMELIORATIVE POTENTIAL OF QUERCETIN AND BERBERINE ON EXPERIMENTALLY INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

To date, an overwhelming production of oxidative stressors resembles the most considerable effector in doxorubicin (DOX) induced nephrotoxicity. A solid body of evidence indicates that DOX-driven oxidative stress, if remain unopposed, can undoubtedly upset the cellular redox balance initiating apoptosis and inflammatory cascades that eventually threaten the cell's survival.Over years, silencing these DOX-initiated oxidative stressors and their undesirable consequences was considered as one of the most aggressively pursued goals to overcome its use associated drawbacks. Although individual phytochemical remediesachieved partial successes in this respect, a satisfactory outcome has not reached yet. In the current study, quercetin (QUR) and berberine (BER) were investigated individually and in combination to withstand against DOX-induced nephrotoxicity in rats which was induced by single intravenous DOXinjection (7.5 mg/kg). Oral administrations of QUR (50 mg/kg) alone or BER (50 mg/kg) alone as well as a blend of them in the same doses were started one week before DOX injection and continued concomitantly for another 14 consecutive days. Results of our study revealed that mixed administration of both QUR and BER worked synergistically to restore the DOX-induced impaired renal functions with approximately three-fold decreases in serum creatinine and BUN coupled with ten-fold decrease in microalbuminuria in comparison to DOX group. Also, the use of this blend succeeded efficiently to demolish the DOX-driven oxidative stress with significant increases in GSH level and SOD activity and significant decreases in MDA and NO levels compared to DOX-treated rats.These data collectively concluded, for the first time, that QUR and BER binary administration works synergistically to attenuate DOX-induced kidney injury through antioxidant mechanisms.

Keywords: *Doxorubicin; Nephrotoxicity; Oxidative stress; Quercetin; Berberine*

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INTRODUCTION

Doxorubicin (DOX) is an anthracycline antibiotic that has enjoyed considerable popularity in the last few decades as a prominent chemotherapeutic agent in the management of a variety of solid and hematologic malignancies**[1]**. Nonetheless, its clinical applications are constrained by numerous dose related organotoxic potentials including nephrotoxicity **[2, 3]**. The vulnerability of the kidney can be attributed predominantly to the toxic potential of DOX towards the proximal convoluted tubule and the glomerulus because a significant portion of the unchanged intravenously administered DOX typically arrives at the kidneys for clearance where DOX and its more toxic metabolites (13-OH-doxorubicinol, doxorubicinone, doxorubicinolone and 7-deoxydoxorubicinone) enjoy ample opportunity to interact with kidney cells motivating serious harms **[4]**.

During the past decades, several research groups overshadowed looking into numerous supposed potential pathways that could explain the mechanisms of the abovementioned versatile DOX-induced cell injuries. The majority of these studies tend to agree on a common view that the DOX's organotoxic potential could emerge from the substantial increase in oxidative stress emanating from overproduction of free radicals that interact with molecular oxygen to synthesize superoxide. This in turn precipitously depletes tissue antioxidants and reacts with hydrogen peroxide to form highly reactive hydroxyl radicals that can cause severe macromolecular injury and initiate uncontrolled lipid peroxidation, ultimately inducing structural and functional injury to a wide variety of tissues which may have been the case in the kidney **[5]**.

It is an interesting paradox that some natural occurring compounds such as plant-derived flavonoids and polyphenols showed promise in this area through their antioxidant potential and its ability to protect against various oxidative stressors without notable side-effects **[6]**. As an example, quercetin (QUR) represents one of the most widely distributed flavonoids in the plant kingdom **[7]**. Previous studies have shown that QUR possess a broad range of pharmacological properties such ascarcinostatic, antioxidant, antiviral and anti-inflammatory activities, suppress cell proliferation, modify eicosanoid synthesis, protect low-density lipoprotein from oxidation, prevent platelet aggregation, stabilize immune cells, and promote relaxation of cardiovascular smooth muscle**[5, 8, 9]**.

In the same context, berberine (BER), an isoquinoline alkaloid that is found in several plant species, has extensively been reported in numerous studies in a variety of experimental animal models to exert a broad array of pharmacological activities, including anti-inflammatory and anti-apoptotic activities, mainly through a potent antioxidant potential **[10-12]**. Modernly, extensive *in vitro* and *in vivo* trials have revealed that combinatorial administration of more than one antioxidant herbal products can be considered as a good strategy to synergistically alleviate oxidative stress and enhance the free radical scavenging capacity that would provide better outcomes than either agent administered alone **[13-15].**

In light of above knowledge, the current study aimed to investigate the modulatory effects ofthese phytochemicals, i. e., QUR and BER against DOX-induced toxic kidney injury in *a wistar rat*model.Along with that, our study aimed importantly to examine the potential synergistic effect of the two compounds against DOX-induced toxic nephrotoxicity, and to assume their possible modes of actions.

MATERIALS AND METHODS

Animals and experimental design

Animal experiments were performed after approval by the Institutional Animal Care and Use Committee of Faculty of Medicine, Assiut University, Assiut, Egypt. All experiments were performed using 50 healthy male wistar rats (weighing 200 ± 10g) that were purchased from the laboratory animal colony, Assiut University, Assiut, Egypt. Rats were housed (5 per cage) in wire-floored cages at a regulated environment (temperature, 22 ± 2°C; humidity, 50 ± 5%; night/day cycle, 12 hours) with free access to standard pellet diet and tap water *add libitum*.

After a two weeks acclimatization period, rats were randomly divided into 5 groups of 10 animals each. In group I (control group), rats received only the suspending vehicle that consist of a mixture of 1% sodium carboxy methyl cellulose and 1% Tween-80, (daily by oral gavage) throughout the experimental periodto resemble the negative control group. In group II (DOX group), rats were injected intravenously with a single dose (7.5 mg/kg) of a commercially available doxorubicin (DOX) vials (10 mg adriamycin hydrochloride from Pharmacia Italia S.P.A. Gruppo Pfizer Inc., Nerviano, Italy) *via* tail vein **[16]**. In group III (QUR+DOX), rats received orally 50 mg/kg QUR (Sigma-Aldrich Gmbh, Munich, Germany) **[17]**, suspended in the above mentioned vehicle, once daily for 7 days before administering the previously mentioned DOX injection and continued for another 14 consecutive days. In group IV (BER+DOX), rats received orally 50 mg/kg BER (Sigma-Aldrich Gmbh, Munich, Germany) **[18]**, suspended in the above mentioned vehicle, once daily for 7 days before administering the previously mentioned DOX injection and continued for another 14 consecutive days. In group V (QUR+BER+DOX), rats received orally a mixture incorporating both QUR(50 mg/kg) and BER (50 mg/kg) suspended in the above mentioned vehicle, once daily for 7 days before administering the previously mentioned DOX injection and continued for another 14 consecutive days.

On day 15 after DOX administration, each animal was housed in an individual metabolic cage for urine collection. The obtained urine samples were collected, centrifuged, and then stored at –20°C for subsequent evaluation of urinary biochemical indices. One day later, blood samples were collected from retro-orbital plexus for serum preparation and the animals were sacrificed by cervical decapitation under isoflurane anesthesia and autopsy was performed. Both kidneys of each animal were excised, purified from adhering fat and connective tissues and washed in ice-cold isotonic saline. One kidney of each animal was stored in 10% neutral buffered formalin solution and subjected for histopathological examination. The other kidney was instantly flash frozen in liquid nitrogen and stored separately at –80 °C for subsequent biochemical assays.

Assessment of nephrotoxicity indices

Serum creatinine was assayed by kinetic procedure using a kit provided from Human Diagnostic (Wiesbaden, Germany). Blood urea nitrogen (BUN) was assayed by colorimetric procedure using a kit provided from Biomerieux Sa (Lyon, France). Microalbuminuria was estimated by colorimetric procedure using a kit provided from BioSystems (Barcelona, Spain).

Assessment of oxidative stress markers

Lipid peroxidation was determined spectrophotometrically in renal tissues as thiobarbituric acid reacting substance (TBARS) and is expressed as equivalents of malondialdehyde (MDA), using *1,1,3,3* tetramethoxypropane as standard **[19]**. Reduced glutathione (GSH) was assayed spectrophotometrically in renal tissues using Ellman assay method **[20]**.SOD activity in renal tissues was estimated according to the method was described by **[21]**. Nitric oxide (NO) was assayed spectrophotometrically in renal tissues by measuring its stable metabolites, in particular, nitrite and nitrate **[22]**.

Histopathological examinations

For histopathological examination, kidney tissues were fixed in 10% neutral buffered formalin solution. Washing was carried out in sterile tap water, and then in serial dilutions of alcohols (methyl, ethyl and absolute ethyl) which were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °C in a hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by a sledge microtome and the obtained tissue sections were placed on glass slides, deparaffinized, stained by hematoxyline & eosin (H&E) stain, and then examined under light microscope for assessment of histopathological changes **[23]**.

Statistical analysis

Statistical analyses of the data were carried out using GraphPad prism version 5.0 (Graph pad software San Diego, USA). Data comparisons were performed using analysis of variance (ANOVA) followed by Tukey's t-test. The levels of significance were accepted with $p < 0.05$ and all relevant results were graphically displayed as mean ± SEM.

RESULTS

Effect of tested compounds on animal body weight and mortality rate

In the current study, all animals in the five groups remained alive throughout the experimental period except for a single mortality which was recorded in the DOX group on day 31, i. e., the 10th day after DOX injection. We have also observed that, after DOX administration on day 21 and throughout the remaining experimental period, animals in DOX group seemed extremely lethargic and lost significant weight (~25%) compared to their control counterparts which gained weight over (~20%). This DOX-induced decrease in the body weight was moderately attenuated to some extent in both (QUR+DOX) and (BER+DOX) groups with minor weight losses of (~6%) and (~3.2%) respectively. On the other hands, this DOX-forced attenuation in the body weight disappeared markedly in animals of the combination group (QUR+BER+DOX) which seemed spirited and even gained weight over (~1%) throughout the remaining experimental period as seen in figure-1.

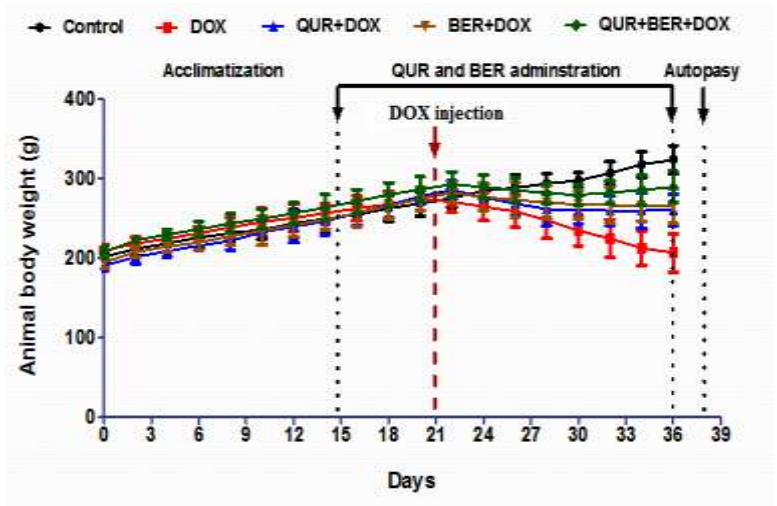

Figure-1: illustration of the effect of tested compounds on changes in the animal body weight during the experimental period.

Effect of the tested compounds on biochemical indices of renal performance

As observed in table 1, in comparison to the control group, administration of DOX resulted in significant increases in serum creatinine ($p < 0.001$), BUN ($p < 0.001$), and microalbuminuria ($p < 0.001$). Conversely, in comparison to the DOX group, pre- and co-treatments with either QUR or BER alone in (QUR+DOX) and (BER+DOX) groups were able to attenuate these signs of renal impairment as indicated by significant decreases in serum creatinine ($p < 0.001$ and $p < 0.001$, respectively), BUN ($p < 0.001$ and $p < 0.001$, respectively) and microalbuminuria ($p < 0.001$ and $p < 0.001$, respectively). More advantageously, combinatorial pre- and co-treatment with both QUR and BER in

(QUR+BER+DOX) group was efficiently able to buffer the above mentioned alterations in the overall estimated biochemical indices of renal function to match its corresponding levels in the healthy control group without any observed significant differences, see figure 2 A-C.

Group	Creatinine (mg/dl)	B U N (mg/dl)	Microalbuminuria (mg/L)
Control	0.62 ± 0.03	8.2 ± 0.5	30.9 ± 2.6
D O X	1.69 ± 0.05***	23.0 ± 1.03***	330.8 ± 16.6***
QUR + DOX	0.82 ± 0.05$^{*,\circ\circ\circ}$	11.7 ± 0.5$^{*,\circ\circ\circ}$	76.4 ± 9.4$^{*,\circ\circ\circ}$
BER + DOX	0.73 ± 0.04$^{\circ\circ\circ}$	9.6 ± 0.7$^{\circ\circ\circ}$	72.0 ± 6.5$^{*,\circ\circ\circ}$
QUR+BER+DOX	0.61 ± 0.04$^{\circ\circ\circ,\$}$	8.3 ± 0.8$^{\circ\circ\circ,\$}$	33.5 ± 4.5$^{\circ\circ\circ,\$,\Phi}$

Table-1: Effects of QUR and BER and their combination on DOX-induced changes in the biochemical indices of renal performance including serum creatinine, BUN, and microalbuminuria. Data are presented as mean ± SEM (n = 10). *, ○, $ and Φ indicate significant change from control, DOX, (QUR+DOX) and (BER+DOX) respectively. *, ○, $ and Φ indicate significant change at $p < 0.05$; **, ○○, $$ and ΦΦ indicate significant change at $p < 0.01$; ***, ○○○, $$$ and ΦΦΦ indicate significant change at $p < 0.001$.

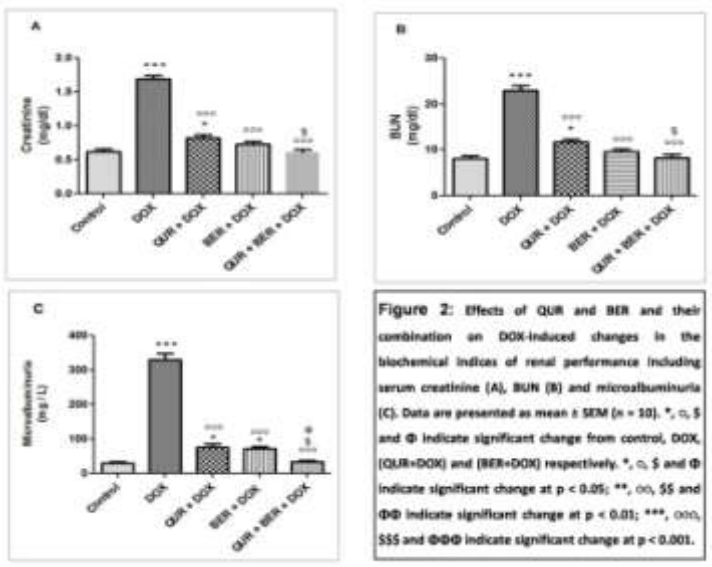

Figure 2: Effects of QUR and BER and their combination on DOX-induced changes in the biochemical indices of renal performance including serum creatinine (A), BUN (B) and microalbuminuria (C). Data are presented as mean ± SEM (n = 10). *, ○, $ and Φ indicate significant change from control, DOX, (QUR+DOX) and (BER+DOX) respectively. *, ○, $ and Φ indicate significant change at $p < 0.05$; **, ○○, $$ and ΦΦ indicate significant change at $p < 0.01$; ***, ○○○, $$$ and ΦΦΦ indicate significant change at $p < 0.001$.

Effect of the tested compounds on the oxidant/antioxidant system

As summarized in table-2, in comparison to the control group, administration of DOX caused significant decreases in renal tissue content of GSH ($p < 0.001$) and SOD activity ($p < 0.001$) in addition to significant increases in renal MDA ($p < 0.001$) and NO ($p < 0.001$) levels. In comparison to the DOX group, pre- and co-treatments with either QUR or BER alone in (QUR+DOX) and (BER+DOX) groups were able to significantly increase renal tissue contents of GSH ($p < 0.001$ and $p < 0.001$, respectively) and SOD activity ($p < 0.001$ and $p < 0.001$, respectively) with significant decreases in renal tissue contents of MDA ($p < 0.001$ and $p < 0.001$, respectively) and NO ($p < 0.001$ and $p < 0.001$, respectively). It is of note that, in comparison to the healthy control group, neither QUR nor BER individual treatment was able to completely abolish the DOX-induced oxidative stress status, whereas combinatorial pre- and co-treatment with both QUR and BER in (QUR+BER+DOX) group was more aptly able to withstand against these DOX-induced alterations in the estimated markers of oxidative stress without any significant differences with its corresponding values in the healthy control group as seen in figure 3 A-D.

Group	G S H (µmol/g pro	S O D (U/g protein	M D A (nmol/g pro	Nitric oxide (µmol/g prot
Control	6.20 ± 0.40	0.11 ± 0.010	18.0 ± 1.6	1.0 ± 0.13
D O X	1.55 ± 0.20***	0.013 ± 0.001***	58.0 ± 2.4***	4.4 ± 0.30***
QUR + DOX	5.00 ± 0.30$^{\circ\circ\circ}$	0.08 ± 0.003$^{**,\circ\circ\circ}$	28.8 ± 2.8$^{*,\circ\circ\circ}$	2.0 ± 0.15$^{*,\circ\circ\circ}$
BER + DOX	4.70 ± 0.30$^{*,\circ\circ\circ}$	0.09 ± 0.003$^{*,\circ\circ\circ}$	28.5 ± 2.5$^{*,\circ\circ\circ}$	1.7 ± 0.17$^{\circ\circ\circ}$
QUR+BER+DOX	6.10 ± 0.40$^{\circ\circ\circ,\Phi}$	0.11 ± 0.010$^{\circ\circ\circ,\$\$,\Phi}$	19.0 ± 2.2$^{\circ\circ\circ,\$,\Phi}$	1.1 ± 0.10$^{\circ\circ\circ,\$}$

Table-2: Effects of QUR and BER and their combination on DOX-induced changes in markers of oxidative stress including renal tissue GSH contents, SOD activity, MDA and NO levels. Data are presented as mean ± SEM (n = 10). *, ○, $ and Φ indicate significant change from control, DOX, (QUR+DOX) and (BER+DOX) respectively. *, ○, $ and Φ indicate significant change at $p < 0.05$; **, ○○, $$ and ΦΦ indicate significant change at $p < 0.01$; ***, ○○○, $$$ and ΦΦΦ indicate significant change at $p < 0.001$.

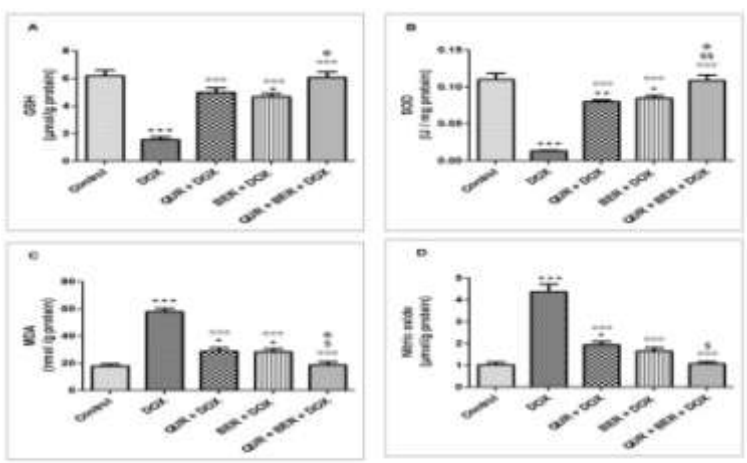

Figure 3: Effects of QUR and BER and their combination on DOX-induced changes in markers of oxidative stress including renal tissue GSH contents (A), SOD activity (B), MDA (C) and NO (D) levels. Data are presented as mean ± SEM (n = 10). *, ○, $ and Φ indicate significant change from control, DOX, (QUR+DOX) and (BER+DOX) respectively. *, ○, $ and Φ indicate significant change at $p < 0.05$; **, ○○, $$ and ΦΦ indicate significant change at $p < 0.01$; ***, ○○○, $$$ and ΦΦΦ indicate significant change at $p < 0.001$.

Effect of tested compounds on histological findings

Histological examinations revealed that DOX administration induced apparent alterations in the kidney tissues compared to the control group in the form of congestion of renal blood vessel and focal haemorrhage that was detected in between the tubules associated with degeneration in the tubular lining epithelium at the cortex as well as focal inflammatory cells

infiltration in the cortical portion as appear in figure-4B. Individual administrations of QUR and BER in (QUR+DOX) and (BER+DOX) groups were able, to some extent, to calm down the DOX-induced pathological alterations in the kidney tissues architecture, although abnormal findings were still observed in kidney tissues of these groups in the form of minor focal haemorrhage that was detected in between the degenerated tubules at the cortex in addition to mild degeneration and desquamation that were observed in the tubular lining epithelial cells in the cortex as well as in the corticomedullary portions – see figure-4C-D. Combined administration of both QUR and BER in (QUR+BER+DOX) group, on the other hand, resulted in absence of any histopathlogical alteration in the renal tissues with normal histological structure of the glomeruli and tubules at the cortex which were approximately identical to those observed in the renal tissues of the control group which showed uniform staining intensity that clearly reflect intactness of the renal tissues as recorded in figure 4A-E.

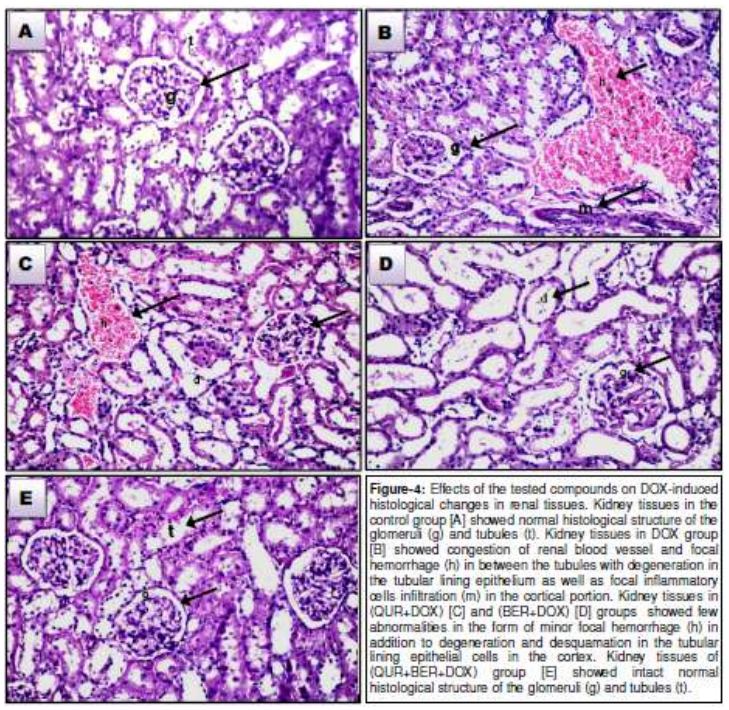

Figure-4: Effects of the tested compounds on DOX-induced histological changes in renal tissues. Kidney tissues in the control group [A] showed normal histological structure of the glomeruli (g) and tubules (t). Kidney tissues in DOX group [B] showed congestion of renal blood vessel and focal hemorrhage (h) in between the tubules with degeneration in the tubular lining epithelium as well as focal inflammatory cells infiltration (m) in the cortical portion. Kidney tissues in (QUR+DOX) [C] and (BER+DOX) [D] groups showed few abnormalities in the form of minor focal hemorrhage (h) in addition to degeneration and desquamation in the tubular lining epithelial cells in the cortex. Kidney tissues of (QUR+BER+DOX) group [E] showed intact normal histological structure of the glomeruli (g) and tubules (t).

DISCUSSION

Cancer chemotherapy usually demolishes the normal physiological homoeostasis and affects multiple organs during the course of treatment. Despite its extensive clinical utilization in the fight against a variety of human malignancies, treatment with the conventional DOX is limited because of its multi organ toxicities including renal damage and nephrotoxicity **[24, 25]**. Although, the exact mechanism by which DOX produces renal toxicity remains largely uncertain. Among the suggested mechanisms is free radical formation that initiate an overwhelming case of oxidative stress which intuitively, if remaining unopposed, can undoubtedly upset the redox balance and functionality of the intracellular organelles, induce inflammatory cascades, and promote programmed cellular death that eventually threaten the cell's survival **[26-28]**.

Historically, attenuation of this DOX-induced nephrotoxicity has been a fundamental goal in several studies. Traditional herbal medicines received a lot of attention as promising candidates in this area and some of them were reported to achieve a partial success in preserving relatively normal renal function and structure probably through their antioxidant effects, such as caffeic acid phenethyl ester **[29]**, Zingiber officinale Roscoe **[30]**, and Solanum torvum **[31]**.

In the current study we evaluate the potential benefit of two naturally occurring bioactive phytochemicals, QUR and BER, either individually or, more importantly, in their novel combination as a possible strategy to withstand against the aforesaid DOX-induced nephrotoxicity. Both of these phytochemicals, i.e., QUR and BER, have been individually reported in other occasions to exhibit antioxidant, anti-inflammatory and anti-apoptotic properties **[7, 11, 12, 32, 33]**. To our knowledge, no previous study has been conducted on the co-effect of both QUR and BER on DOX-induced nephrotoxicity in wistar rats. Therefore, the nephroprotctive activities of these phytochemicals, i.e., QUR and BER were assessed in our current work at histopathological and biochemical levels.

As a very well documented phenomenon, intravenous administration of DOX on day 21 of our current study produced several signs of toxicity throughout the remaining experimental period as were manifested for example by immense weight loss (~25%) and 10% mortality in animals of DOX group compared to the healthy animals in the control group which gained weight over (~20%) and remained alive over the experimental time. Contradictory, this DOX-induced body weight loss was moderately attenuated to some extent in both (QUR+DOX) and (BER+DOX) groups with minor weight losses of (~6%) and (~3.2%) respectively without recording any mortality. Outstandingly, this DOX-forced attenuation in the body weight totally disappeared in the animals of the combination group (QUR+BER+DOX) which seemed spirited and even gained weight over

(~1%) throughout the remaining experimental period as seen in figure-1with no recorded mortalities. This significant retardation in the body weight loss could reflect the pivotal role of QUR and BER, individually and more efficiently in their combination, in revoking the direct toxicity of DOX on the animal intestinal mucosa as well as its indirect hazardous effects on the gastrointestinal tract arising from inadequate food intake and exhaustive energy expenditure causing a decrease in secretion of internal hormones and resulting in decreased trophic effects to the mucosa **[34, 35]**.

Additionally and in consistent with numerous previous reports, signs of DOX-induced nephrotoxicity was reported clearly in our current investigation with intense perturbations in biochemical nephrocyte injury markers. For instance, DOX-exposure caused tangible three-fold increases in serum creatinine and BUN levels coupled with a corresponding ten-fold increase in microalbuminuria (table-1) suggesting significant renal impairment **[36, 37]**. Conversely, in comparison to the DOX group, separate pre- and co-treatments with either QUR or BER alone were able to attenuate these signs of renal impairment as indicated by intrinsic two-fold decreases in serum creatinine and BUN levels coupled with a corresponding four-fold decrease in microalbuminuria with minimal non-significant superiority to BER over QUR (table-1). More worthy, combinatorial pre- and co-treatment with both QUR and BER in (QUR+BER+DOX) group was efficiently able to buffer the above mentioned alterations in the overall estimated biochemical indices of renal function to match its corresponding levels in the healthy control group without any observed significant differences suggesting unquestionable improvement in renal performance and indicate the reno-protective effect of these phytochemicals (table-1) **[32, 35, 38]**.

So far, much concern has been given to the possible involvement of oxidative stress in pathogenesis of DOX-induced kidney injury. This was conspicuous in the results of the current study which revealed serious increases in oxidative and nitrosative damages in the form of markedly increased lipid peroxidation products MDA and NO levels and depleted of GSH levels and SOD activity in renal tissues of DOX-treated rats. These data are corroborated by several previous researches on DOX-induced nephrotoxicity in rats which proposed that DOX and/or its short-lived toxic metabolites can interact with molecular oxygen and initiates a cascade of consecutive reactions producing reactive oxygen species including superoxide and hydroxyl radicals as well as hydrogen peroxide which in turn deplete tissue antioxidants and consequently lead to disturbance in oxidant-antioxidant systems resulting in tissue injury **[29, 39-41]**. On contrary, individual pre- and co-treatment with QUR or BER and more effectively their blend usage succeeded to improve the antioxidant capacity and reduced the oxidative and nitrosative stress as indicated by significant increases in GSH level and SOD activity and significant decreases in MDA and NO levels compared to DOX-treated rats (table-2). This can be correlated with the ability of these phytochemicals to scavenge highly reactive species, iron-chelating properties and inhibitory effects on carbonyl reductases as been pointed out in previous studies **[38, 42-44]**.

Further, evidences for the nephro-protective effects of QUR and BER are materially augmented by the outcome of our obtained histopathological investigation. In DOX group, DOX administration leaded to congestion of renal blood vessel and focal haemorrhage that was detected in between the tubules associated with degeneration in the tubular lining epithelium at the cortex as well as focal inflammatory cells infiltration in the cortical portion (figure-4B). Similar histopathological changes have been previously reported in acute DOX-induced nephrotoxicity **[45]**. Individual supplementation with QUR or BER was relatively able to calm down the DOX-induced pathological alterations in the kidney tissues architecture; although minor focal haemorrhage as well as mild degeneration and desquamation in the tubular lining epithelial cells in the cortex and the corticomedullary portions remained observable in kidney tissues of these groups (figure-4C-D). On the other hand, combined administration of both QUR and BER in (QUR+BER+DOX) group resulted in absence of any histopathlogical alteration in the renal tissues with normal histological structure of the glomeruli and tubules at the cortex reflecting intactness of the renal tissues, approximately as were observed in the control group (figure 4A-E). These results support those of earlier studies that demonstrated the

nephro-protective properties of both QUR and BER **[32, 35, 38]**.

CONCLUSION

In conclusion, the results of present study may have identified for the first time that QUR in combination with BER shows better alleviation of DOX-induced kidney injury than their individual administration. A solid body of evidence indicates that theseunequivocal reno-protective effects could be attributed to the synergistic antioxidant activity of this dual phytochemical remedy. However further pharmacokinetic studies are required to expand understanding of this synergism and to build a foundation for later extension of the cytoprotective capacity of QUR and BER binary administration against other organotoxicities.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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الملخص العربى

تقييم التأثيرات التغيرية لبعض المركبات الطبيعية على التسمم الكلوى المستحث بمادة الدوكسوروبيسين فى فئران التجارب

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يعتبر الدوكسوروبيسين من أهم مضادات السرطان والذى يستخدم فىعلاج كثير من الأورام السرطانية فى الانسان، ولكن لهذا العقار آثار جانبية لا يمكن اهمالها على العديد من الأعضاء البشرية ومن ضمنها الكلى.

أشارت العديد من الدراسات ان سبب هذه الآثار الجانبية الضارة لعقار الدوكسوروبيسين يرجع بشكل رئيسى لإستحداثه مستويات عالية من الأكسدة ، وفى حالة إستمرارها بدون معارضة قد تؤدى الى الموت المبرمج للخلية وتوالى الالتهابات والذى فى النهاية يهدد بشكل كبير بقاء الخلايا.على مدى سنوات ، اعتبر الحد من ارتفاع مستويات الاكسدة المستحث بواسطة الدوكسوروبيسين وما يتبع هذا الإرتفاع من نتائج وآثار غير مرغوبة ، واحدا من اهم الأهداف المطلوبة للتغلب على المشاكل المرتبطة بإستخدام هذا العقار.

على الرغم من الاستخدام الفردى لبعض المركّبات النباتية قد حقق بعض النجاحات الجزئية فى هذا الشأن، الا انها لم تصل الى نتائج مرجّوة حتى الان.فى دراستنا هذه، تم بحث استخدام كل من الكوارسيتين والبربيرين بشكل فردى او عن طريق مزجهما معا وذلك لبحث إمكانية استخدامهما فى مواجهة التأثير الضار للدوكسوروبيسين على سمية الكلى. وقد تم إستحداث هذا التأثير الضار فى فئران التجارب عن طريق حقن الدوكسوروبيسين كجرعة واحدة فقط (7.5 مجم/كجم) فى وريد الذيل، وذلك فى اليوم السابع من التجربة. وقد تم إعطاء كل من الكوارسيتين (50مجم/كجم)، والبربيرين (50مجم/كجم) بشكل فردى وايضا من خلال مزجهما معا بنفس الجرعات السابقة، عن طريق الفم وذلك قبل اسبوع من حقن الدوكسوروبيسين مع الإستمرار فى إعطاء الجرعات السابقة من الكوراسيتين والبربيرين بعد ذلك لمدة اربعة عشر يوما متتابعة.

أظهرت النتائج ان الدوكسوروبيسين قد أحدث إرتفاعاً فى نسبة كل من مستوى الكرياتينين ونيتروجين اليوريا فى الدم وكذلك إرتفاع مستويات والزلال فى البول. أظهرت النتائج أيضا قدرة الدوكسوروبيسين على إحداث مستويات عالية من الأكسدة داخل الأنسجة الكلوية والتى تعتبر احد المسببات الرئيسية فى التسمم الكلوى المستحث بواسطة هذا العقار.

سجلت الدراسة ان تناول كل من الكوارسيتين او البربيرين قد أحدث تحسنا نسبيا فى معظم القياسات الكميائية ، مما يشير الى أهميتهم فى مجال الوقاية من التسمم الكلوى المستحث بواسطة الدوكسوروبيسين. كما أظهرت هذه الدراسة وللمرة الأولى ان مزج كل من الكوارسيتين مع البربيرين يعطى تحسنا ملحوظا و أفضل من إستخدام كل منهم بمفرده ، والتى يمكن ان ينسب بشكل كبير الى كفاءتهم معا كمضادات للأكسدة، وتأثيرهم الواقى على حماية الكلى بصورة كاملة من تأثير الدوكسوروبيسين الضار على وظائف الكلى.