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PROTECTIVE EFFICIENCY OF CURCUMIN AND BONE MARROW MESENCHYMAL STEM CELLS OF NEPHROTOXICITY INDUCED BY BROMATE IN RATS

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Potassium bromate ($KBrO_3$) is used in many countries in cosmetic and food industries. In this research, we study the possible renoprotective effect of curcumin (CUR) and bone marrow meschenymal stem cells (BM-MSCs) on the actions of $KBrO_3$ in female rats. Thirty two female rats were categorized into four group, the first work as control, the second was exposed to $KBrO_3$ (100 mg/kg/day for 28 days in drinking water), the third and fourth where exposed to $KBrO_3$ like group 2 and co-treated with either CUR (100mg/kg, ip) twice a week or BM-MSCs ($2x10^6$, ip for each rat) once a week for 4 weeks, respectively. Kidney function and oxidative stress parameters were measured calorimetrically in plasma. Expression of caspase-3 in kidney by real time PCR was measured by the comparative Ct (2-ΔΔCt) method. Apoptosis in kidney was evaluated by TUNNEL assay. The results indicated that treatment with KBrO3 caused nephrotoxicity, as evident by the measured renal structural and functional indices and oxidative stress markers in plasma. CUR and BM-MSCs co-treatment significantly abated most of the indices and biomarkers of the renal toxicity caused by KBrO3, suggesting their beneficial effects with the priority of CUR due to their antioxidant effect.

INTRODUCTION

Kidneys are vital tissues that clean the blood from toxins and metabolic wastes and maintain homeostasis. Various environmental agents may impact kidney functions. Potassium bromate (KBrO3) is an oxidizing agent that is commonly used in cosmetic products and as food additive caused nephrotoxicity due to its ability to trigger the production of reactive oxygen species (ROS) (**Kurokawa *et al.*, 1987; Spassova *et***

***al*., 2015**). The oxidative stress induced by $KBrO_3$ far exceeds the cellular antioxidative defense capacity leading to marked nephrotoxicity in humans and animals. Hence, several authors investigated the oxidative injuries and probable mechanism of $KBrO_3$-induced nephrotoxicity in experimental models (**Deangelo *et al*., 1998, Murata *et al*., 2001, Ali *et al*., 2018).**

A number of studies have demonstrated that stem cells can prevent and repair damage to renal tubular cells induced by chemicals such as cisplatin (**Shaohua and Dongcheng, 2013**), glycerol (**Herrera *et al*., 2004**), and $KBrO_3$ (**Ali *et al.*, 2018**). Mesenchymal stem cells (MSCs) are multipotent stem cells that have the potential to self-renew and differentiate into a variety of specialised cell types (**Sanchez-Ramos, 2002**). MSCs are easily accessible, expandable, immune-suppressive and they do not elicit immediate immune responses **(Kassem and Abdallah, 2008)**. Therefore, MSCs are an attractive cell source for tissue engineering and vehicles of cell therapy. Bone marrow (BM) is the most common source of MSCs from many species including mouse, rat, rabbit, dog, sheep, pig, and human **(Meirelles and Nardi, 2003**). Many authors used BM-MSCs to treat acute kidney injury in animal models and have found that renal function and structure can be improved by infusion with BM-MSCs (**Yadav *et al*., 2012; Morigi and Benigni, 20013**). For example BM-MSCs partially protect cisplatin-treated rats from acute renal injury by inhibiting tubular cell apoptosis (**Shaohua and Dongcheng, 2013**).

Despite evidence for the therapeutic potential of BM-MSCs, the mechanisms underlying the improvement in kidney function and structure remain unclear. Therefore, the search for safe and effective synthetic and/or naturally occurring ROS scavengers and antioxidants is of major clinical importance. Curcumin and related compounds have the ability to inhibit free radical generation and act as a free radical scavengers and antioxidants (**Daniel *et al*., 2004**). Moreover, CUR reduces inflammation related factors (**Sun *et al*., 2017**), suppress cancer cell growth by interfering with the tumor cell cycle (**Schwertheim *et al*., 2017**) and inhibits tumor cell invasion through regulation of growth factors and their

receptors (**Bachmeier *et al.*, 2018**). Therefore, the present study was carried out to insight into the preventive role of CUR supplementation and BM-MSCs on nephrotoxicity induced by $KBrO_3$ in female rats.

MATERIALS AND METHODS

Animals:

In this study, thirty two female albino rats with weight 120-150 gm were purchased from and housed in Animal house, Faculty of Medicine, Assiut University in according to the Assiut University animal experimental regulations. Animals were fed with standard rat chow and tap water *ad libitum* in a well-ventilated room with a 12:12-hour light/dark cycle at 30°C. All efforts were made to minimize animal suffering.

BM-MSC preparation, isolation and culture:

This process was performed at the tissue culture and molecular biology center of Assiut University. Tibia and fibula bone marrow was flushed out with phosphate-buffered saline (PBS) containing 2 mM EDTA for isolation of BM-MSCs. Then, 35 ml of the sample was layered carefully on 15 ml Ficoll-Paque (Gibco-Invitrogen, Grand Island, NY), centrifuged for 35 min at 400×g, and the superior layer was aspirated without disturbing the mononuclear cell layer at the interphase. Then, the mononuclear cell layer was removed, washed twice with PBS, and centrifuged at 200×g at 10°C for 10 min. The cell pellet was re-suspended in 300µl PBS-EDTA buffer and the isolated BM-MSCs were cultured in 25-ml culture flasks in minimal essential medium (MEM) supplemented with 15% fetal bovine serum (FBS) and incubated for 2 h at 37°C with 5% humidified CO_2. Lastly, a culture of adherent MSCs was maintained in MEM augmented with 30% FBS, 0.5% penicillin and streptomycin at 37°C with 5% CO_2 and air (**Abdel Aziz *et al.*, 2010**). The mesenchymal population was isolated on the basis of its ability to adhere to the bottom of the flask according to **Bayati *et al*. (2013).**

EXPERIMENTAL DESIGN

The animals were randomly divided into 4 groups as follows (n=8 in each group):

Group 1: Treated with the vehicle as control group.

Group 2: Supplemented with $KBrO_3$ for 4 weeks with 100mg/kg body weight in drinking tap water according to **Badreldin *et al*. (2018)**.

Group 3: Supplemented with $KBrO_3$ like group 2 in addition to treatment with CUR (100 mg/k.g body weight, orally) according to **Sinha *et al.* (2012)** twice a week for 4 weeks.

Group 4: Supplemented with $KBrO_3$ like group 2 in addition to treatment with BM-MSCs ($2x10^6$, ip for each rat) once a week for 4 weeks according to **Idriss *et al.* (2018)**.

Collection of samples:

At the end of the experiments, the animals of each group were killed by decapitation. Blood samples from the rats were collected in heparinized vials and their kidneys were removed. Kidney from each rat was quickly removed, washed in a saline solution (0.9%NaCl). For histopathological investigation and immunohistochemical staining, one kidney was fixed immediately in 10 % neutral buffered formalin, dehydrated, cleared, embedded in paraffin wax blocks. Sections were de-waxed in xylene and hydrated in a graded series of alcohols and stained for hematoxalin and eosin and Masson's trichrome **(Drury and Wallington, 1980).** TUNEL assay was done to check apoptosis in 5–7-µm paraffin embedded kidney **(Ahmed *et al.*, 2015)** according to the manufacturer's protocol **(In Situ Cell Death Detection Kit, POD; Roche Diagnostics GmbH, Germany).** Small pieces of kidney were kept frozen for determination the gene expression of caspase by PCR. Serum was used to estimate Kidney function and oxidative stress parameters by using a UV-visible spectrophotometer in the Physiology Lab at Zoology Department, Faculty of Science, Assiut University, Assiut.

Oxidative stress parameters:

Malondialdehyde (MDA), the product of lipid peroxidation (LPO) was estimated according to **Ohkawa *et al.* (1979).** NO was measured as nitrite concentration using the method of **Ding *et al.* (1988).** GSH content was

determined according to **Beutler *et al.* (1963).** The activity of SOD was determined according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of **Misra and Fridovich (1972).** The activity of CAT was determined based on its ability to decompose H_2O_2 according to **Luck (1963).** Total antioxidant capacity was determined by colorimetric method by kit purchased from Biodiagnostic for diagnostic research reagents Cat No. TA 25 13.

Gene expression of caspase-3 by PCR:

Kidney samples are collected for RNA extraction using triazole kit. The concentration and ratio are determined using spectro star nano BMG lab tech. Reverse transcription is done using reverse transcription kit and biorad T100 thermal cycler. The 35 cycles of PCR were performed at 94°C for 30 s, 64°C for 60 s and 72°C for 60s, and final cycle of 72°C for 10min. Quantitative determination of gene expression of caspase-s is done using step one plus real time PCR with thermal profile (60ºc for 30 sec, 95ºc for 10 min, 95ºc for 15 sec, 60ºc for 90 sec) in the tissue culture and molecular biology center of Assiut University. Changes in gene expression were normalized relative to the mean critical threshold value of the GAPDH housekeeping gene. Fold change is calculated according to equation 2^(-ddCt) (**Idriss *et al.*, 2018**)

Statistical analysis:

Collected data were organized, tabulated, and analyzed by Prism software statistical computer package version 6 (GraphPad Software, San Diego, CA). Mean and standard deviation (SD) were calculated; one-way analysis of variance (ANOVA) was used to examine differences among the groups. Significance was set at $P < 0.05$

RESULTS

Biochemical parameters:

As shown in **Table (1)** exposure of rats to $KBrO_3$ induced a significant increase in BUN, creatinine and uric acid in plasma, however co-treatment with either CUR or BM-MSCs results in restoration of the previous changes. **Table (2)** showed that there was a significant difference in oxidative stress parmaters among the four studied groups. In

comparison with normal rats, $KBrO_3$ group exhibited the highest level of LPO and NO, and the lowest values of TAC, GSH, Vit C and E and the activities of SOD and CAT. However, CUR and BM-MSCs group showed close value to control group. Table (3) showed the fold of change in casapase-3 expression in the kidney tissue which indicates two fold increases in $KBrO_3$ and KBrO3 treated with BM-MSCs groups, however there a huge increase in the KBrO3 treated with CUR group in comparison with control group.

Histological observation:

Examination of kidney of control rat showed normal histological structure of glomeruli and renal tubules (A). Kidney of rat treated with $KBrO_3$ showed necrosis, sever congestion and atrophy of the glomerulan tuff with peri-glomerular fibrosis (B). However, examination of kidney from rats co-treated with BM-MSCs and CUR showed slight congestion of the renal blood vessels and dilatation of some renal tubules (C) and tubular nephrosis and severe congestion of the blood vessels (D), respectively (Fig 1). Apoptosis in kidney was confirmed by TUNEL staining (Fig 2). TUNEL-positive cells were not present in the kidney of controls (A); more apoptotic cells in the kidney of rat treated with $KBrO_3$ (B); few apoptotic cells in rat treated with $KBrO_3$ plus BM-MSC (C) and KBrO3 plus CUR (D).

Table (1): Kidney functions in plasma of control and treated rats

Parameter	Groups				
	Control	$KBrO_3$	$KBrO_3$+BM-MSc	BRO_3+CUR	P-Value
BUN (mg/dl)	19.90±1.32[a]	31.86±1.82[b]	22.05±1.89[a]	21.81±1.27[a]	0.001***
Creatinine (mg/dl)	1.49±0.12[a]	2.28±0.36[b]	1.02±0.08[a]	1.18±0.16[a]	0.001***
Uric acid (mg/dl)	1.51±0.16[a]	2.15±0.19[b]	1.23±0.12[a]	1.13±0.12[a]	0.001***

- P-values of a one-way ANOVA.

- Means in the same raw followed by the same letter are not significantly different based on Duncun test at 0.05 significance level. Symbols *, **, and *** represent a significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively.

Table (2): Oxidative stress markers in plasma of control and different treated groups

Parameter	Groups				
	Control	$KBrO_3$	BRO_3+BM-MSC	BRO_3+CUR	P-Value
LPO (nmoles/mg protein)	1.51±0.10[ab]	2.14±0.13[c]	1.32±0.14[a]	1.78±0.12[b]	0.001[***]
NO (µg/mgprotein)	75.10±6.60[a]	104.15±4.32[b]	63.78±9.19[a]	58.45±6.50[a]	0.001[***]
Vit. C (µg/mg protein)	15.47±0.99[b]	9.69±1.03[a]	13.35±1.59[b]	14.64±1.19[b]	0.017[*]
GSH (µg/mg protein)	2.05±0.20[b]	1.45±0.156[a]	2.35±0.137[b]	2.02±0.13[b]	0.022[*]
TAC (µg/mg protein)	0.84±0.05[b]	0.53±0.08[a]	0.66±0.08[a]	0.62±0.08[a]	0.035[*]
SOD (U/mg protein)	4.34 ±0.56	3.50 ±0.62	4.78 ±0.48	4.60±0.58	0.508
CAT (U/mg protein)	3.55±0.41[a]	2.55±0.19[a]	4.88±0.51[b]	5.53±0.52[b]	0.001[***]

- P-values of a one-way ANOVA.
- Means in the same raw followed by the same letter are not significantly different based on Duncun test at 0.05 significance level. Symbols *, **, and *** represent a significance at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively.

Table (3): Quantitative RT-PCR analysis of the fold change of Caspase-3 in kidney of control and different treated groups (each reading represent reading of pooled 3 samples)

Group	Caspase-3	GAPDH	DCT	Control/ DOCT	Fold of change $2^{\wedge}$-DDCT
Control	20.60	16.60	4.00	0.00	1.00
$KBrO_3$	21.41	18.40	3.01	-0.99	1.99
BM-MSCs	21.04	18.05	2.99	-1.01	2.02
CUR	19.60	21.89	-2.29	-6.29	78.25

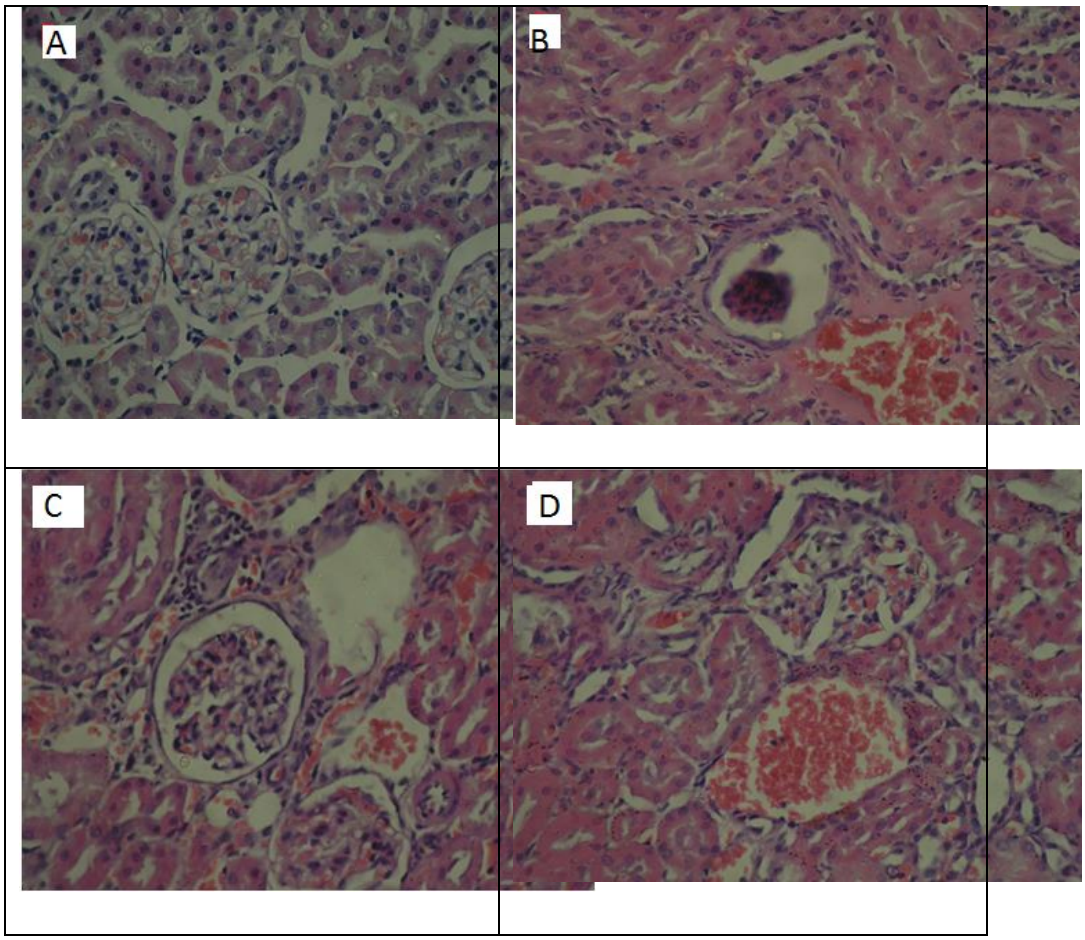

Fig (1): Showed the histological structure of kidney from control (A) and different treated groups with $KBrO_3$ (B); $KBrO_3$ and BM-MSCs (C); and KBrO3 and CUR (D). (H&E stain, magnification×400)

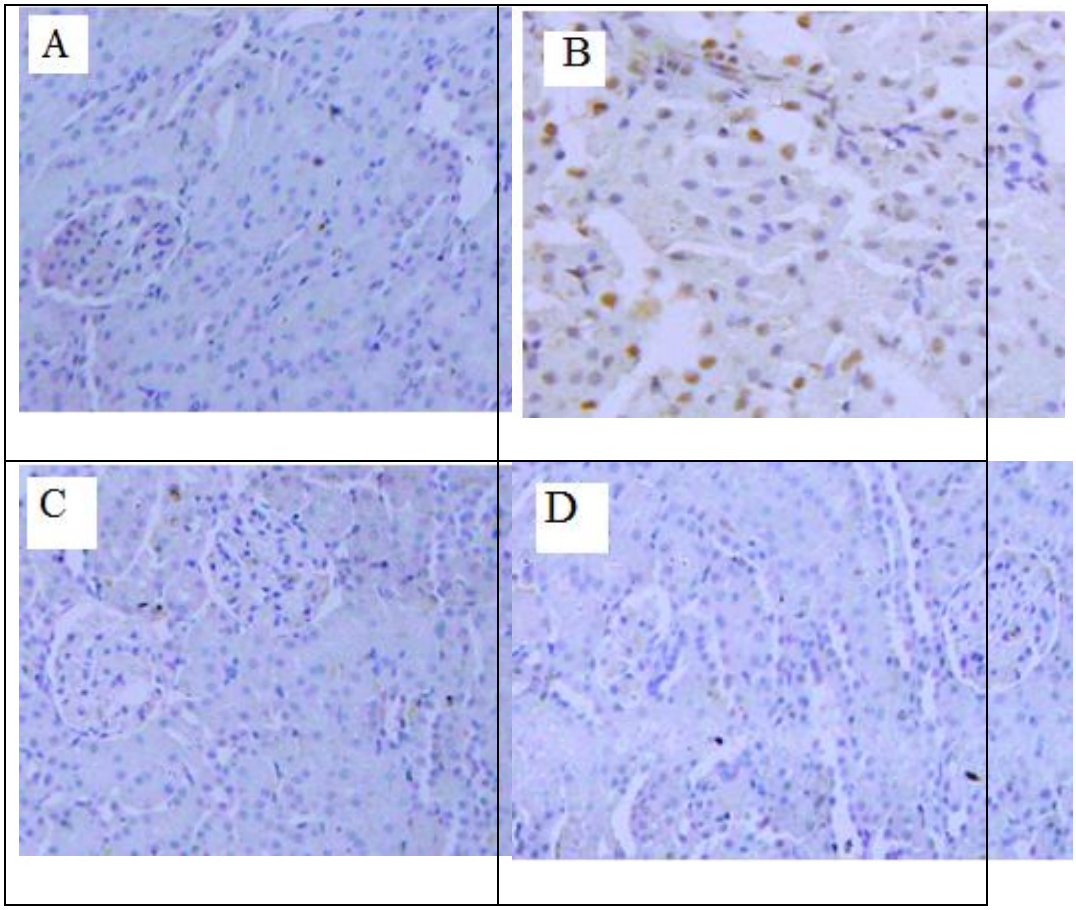

Fig (3): Showed apoptotic cells in the kidney of control rats (A); more apoptotic cells in the kidney of rat treated with KBrO3 (B); few apoptotic cells in rat treated with KBrO3 plus BM-MSC (C) and KBrO3 plus CUR (D). (Tunnel assay, magnification×100)

DISCUSSION

Potassium bromate ($KBrO_3$) is widely used as improving food additive for bread making**,** but it has been forbidden in various countries due to its hazardous effects **(Oloyede and Sunmonu, 2009).** In the present study KBrO3 induced significant elevation of plasma BUN, creatinine and uric acid. In this aspect, elevated level of creatinine in plasma confirming previous reports that $KBrO_3$ ingestion causes acute kidney damage of male Wistar and Sprague-Dawley rats (**Bao *et al*., 2008; Ahmad *et al*., 2012; Ali *et al*., 2018)** because creatinine is a

common marker of renal dysfunction, and BUN is an important parameter in the clinical evaluation of renal impairment. It is known that a major mechanism of KBrO3- induced nephrotoxicity is by the production of ROS, which initiates lipid peroxidation and decreases the antioxidants (**Nishioka *et al.*, 2006; Spassova *et al.*, 2015**). Generally, elevation of BUN levels suggests that exposure to xenobiotics induces oxidative stress and affects antioxidant systems **(Feng *et al.*, 2012).** Co-treatment of intoxicated rat by either CUR or BM-MSCs results in a restoration of kidney function and the parameters of oxidative stress in plasma. It is known that CUR ameliorates renal structural damage and improved proteinuria and creatinine **(Ghosh *et al*., 2009)**. Moreover, uric acid is an important antioxidant in plasma because it can react directly with free radicals **(Alvarez-Lario and Macarron-Vicente, 2010)**.

In the present study, $KBrO_3$ induced a significant increase in LPO and NO and significant decreases in TAC, Vit C and E and the activity of SOD and CAT in comparison with control group. Similarily, KBrO3-induced renal oxidative stress and hyperproliferative response in Wistar rats (**Khan *et al.*, 2004**) and indirectly induced DNA modification by oxygen radicals that is involved in its carcinogenesis (**Ballmaier and Epe, 1995)**. According to the present study, KBrO3 group exhibited the highest ratio in the level of LPO and NO in comparison with normal rats. However, CUR group showed more value than the BM-MSCs group. These results were in agreement with that of **Moghaddam *et al.* (2015)** who reported that the protective effect of CUR was related to its ability to adjust the imbalance of antioxidant enzymes and reduced LPO levels in rat. The biomembrane-protective effect of CUR against peroxidative damage was mainly linked to its ROS scavenging ability **(Farzaei *et al.*, 2018)** and its ability to reduce NO levels **(Alp *et al.*, 2012)** through down-regulation of nitric oxide synthase **(Černý *et al.*, 2011).** Also, CUR induced the synthesis of reduced glutathione (**Zheng *et al.*, (2007)** leading to a marked decrease in LPO products **(Reyes-Gordillo *et al.*, 2007; Fu *et al.*, 2008).**

In the present study, kidney of rats exposed to $KBrO_3$ showed necrosis, severe congestion and atrophy of the glomerular tuff with peri-glomerular fibrosis. In this aspect, renal dysfunction induced by $KBrO_3$ in experimental animals characterized by tubular damage, loss of brush border, tubular necrosis, tubular dilatation, tubular cell swelling and glomerular injuries (**Khan *et al.*, 2010**; **Khan *et al.*, 2012). Aboryag *et al.* (2017)** suggested that glomerular atrophy with widening of Bowman's space, epithelial shedding in tubular structures, pyknotic nuclei, desquamated cells, are indicators of apoptosis. This suggestion was confirmed in the present study by Tunnel assay which shows higher number of apoptotic cells in kidney of $KBrO_3$ treated rats. It is known that the pathophysiology of acute kidney injury involves dysregulation of oxidative stress, necrosis, autophagy and apoptosis. Apoptosis is primarily mediated by cysteine-aspartic proteases (caspases), produced as inactive proteins that must be dimerized, cleaved, or both, to be activated resulting in DNA fragmentation, nuclear condensation, and cell death (**Holditch *et al.*, 2019**). Also, this observation was confirmed by the increase two fold of caspases-3 expression in kidney of $KBrO_3$ treated rats compared to control rats.

In the present study, co-treatment with CUR or BM-MSCs significantly ameliorated histpathological changes and apoptosis in kidney by KBrO3 but up-regulated the capases expression. However, **Soetikno *et al.* (2019)** found that CUR has nephroprotective properties against cisplatin-induced kidney damage in rats due to its antioxidants and anti-apoptosis profiles. Moreover, studies have demonstrated that the administration of MSCs could reverse kidney injury through paracrine mechanisms rather than by MSC transdifferentiation (**Zarjou *et al.*, 2011; He *et al.*, 2012**). Autophagy is essential to the homeostasis and physiological function of podocytes in the kidney (**Bray *et al.*, 2012**). Notably, autophagy induction as a self-protection mechanism has been demonstrated in renal tubular cells in ex-perimental models of acute kidney injury caused by nephrotoxicants such as cisplatin (**Inoue *et al.*, 2010**). **Dang *et al.* (2014)** reported that autophagy play an important role in MSC-promoted tissue regeneration. BM-MSCs have been recognized

as an important component of the hematopoietic stem cell niche release of cytokines and growth factors **(Morigi and Benigni, 20013; Bianco *et al*., 2013)** able to stimulate renal regeneration and limit tubular injury and apoptosis in cisplatin treated mice **(Bi *et al.*, 2007).** In addition, it can exert renoprotective effects via paracrine production of prosurvival, mitogenic, anti-inflammatory, and vasculotropic factors **(Togel *et al*., 2005)**. In conclusion CUR or BM-MSCs showed protective effects against $KBrO_3$ induced nephrotoxicity in rats with priority of CUR due to its antioxidant properties.

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كفاءة الوقاية بخلايا نخاع العظم الجذعية والكركم ضد التسمم الكلوي ببرومات البوتاسيوم في الجرذان

يستخدم برومات البوتاسيوم في العديد من البلدان في صناعة مستحضرات التجميل والمواد الغذائية. في هذا البحث، تم دراسة التأثيرات الضارة لبرومات البوتاسيوم علي إناث الجرذان والتأثير الوقائي المحتمل لكل من الكركم والخلايا الجذعية المعزولة من نخاع العظم ضد هذه الأثار. تمت الدراسة علي اثنان وثلاون جرذ تم تفسيمهم إلي اربع مجموعات بكل مجموعو ثمانية. الأولي تركت كمجكوعة ضابطة، الثانية جرعت ببرومات البوتاسيوم بمفدار ١٠٠ مليدرام لكل كيلوجرام من وؤن الجسم في مياه الشرب لمدة أربعة أسابيع، الثالثة جرعت ببرومات البوتاسوم بنفس جرعة وفترة المجموعة الثانية مع العلاج بالكركم بجرعة ١٠٠ مليجرام لكل كيلوجرام من ورن الحسم مرتين أسبوعيا لمدة اربع أسابيع، بينما المجموعة الرابعة جرعت ببرومات البوتاسوم بنفس جرعة وفترة المجموعة الثانية مع العلاج بخلاي العظم الجزعية بمفدار ١٠ مرة واحدة أسبوعيا لمدة أربع أسابيع. بعد أربع اسابيع تم تخدير الجرذان وذبحها لتجميع الدم وفصل البلازما والكلي. تم قياس وظائف الكلي ودلالات الإجهاد الأكسيدي في البلازما، بينما تم عمل قطاعات هستولوجية في الكلي لدراسة التغيرات الباثولوجية وموت الخلايا المبرمج، كما تم قياس التعبير الجيني للكاسبيس في نسيج الكلي باستخدام تفاعل البلمرة المتسلسل. أشارت النتائج إلى أن العلاج بـالبرومات تسبب في سمية كلوية كما اتضح من الخلل في دلالات وظيفة الكلي بالبلازما والتغيرات الباثولوجية وزيادة معدل موت الحلايا المبرمج والذي ارتبط بزيادة التعبير الجيني للكاسبيس بمقدار الضعف وزيادة الشوارد الحرة مثل الأكسدة الفوقية للدهون واكسيد النيتريك ونقص في مضادات الأكسدة مثل الجلوتثيون وفيتامين ج و ه والقدرة الكلية لمضادات الأكسدة ونشاط كل من السوبر اكسيد ديسميوتيز والكتاليز عن مثيلتها في المجموعة الضابطة. بينما أدت معالجة الجرذان بأي من الكركم وخلايا العظم الجزعية إلي تحسن في وظائف الكلي ودلالات الإجهاد التأكسدي والتغيرات الباثولوجية وموت الخلايا المبرمج ولكن مع زيادة كبيرة في التعبير الجيني للكاسبيس في كلي الجرذان المعالجة بالكركم. وخلصت النتائج إلي قدرة أي من الكركم و خلايا العظم الجزعية في الحماية من سمية برومات البوتاسيوم بالجرعة والفترة المستخدمة مع الأفضلية للكركم بسبب تأثيره المضاد للأكسدة .