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Nephroprotective Effect of Bergapten Against Cyclophosphamide-Mediated Renal Stress, Inflammation, and Fibrosis in Wistar Rats: Probable Role of NF-kB and TGF-β1 Signaling Molecules

Nehal Mohsin,* Mohammad Shabib Akhtar, Saad A Alkahtani, Ismail A Walbi, Yasir Alhazmi, Md. Niyaz Alam, and Alok Bhardwaj

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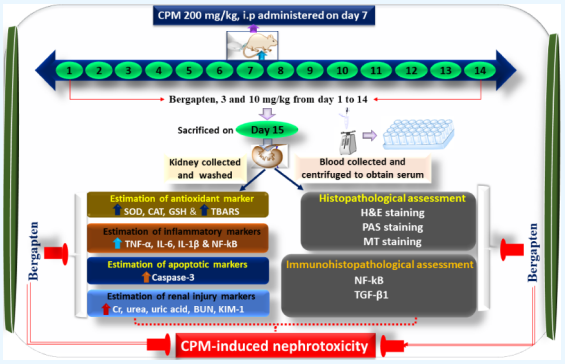
ABSTRACT: Cyclophosphamide (CPM) is a well-established antineoplastic drug with marked clinical outcomes in various types of cancers. Despite being a promising drug, its use is associated with significant renal toxicity and often limits its use, leading to compromised clinical outcomes. Therefore, this study explored the renal protective effect of bergapten (BGP), a natural bioactive compound that showed marked antioxidant, anti-inflammatory, anticancer, and neuroprotective effects. Till now, BGP has not been studied for its renal protective effect in an *in vivo* model. Animals were divided into control, toxic, BGP-3, BGP-10, and BGP *Per se*. The control group was treated with normal saline for 2 weeks. To the toxic group, CPM 200 mg/kg was given on day 7 as i.p. To BGP-3, 10, and *Per se*, BGP-3 and 10 mg/kg, ip was given 2 weeks with a single shot of CPM 200 day 7. To the *Per se* group, only BGP 10 mg/kg, ip was given from day 1 to day 14. After 14 days, animals were sacrificed, and kidneys were removed and studied for the markers of oxidative stress, inflammation, renal injury, renal fibrosis, and renal damage using biochemical, histopathological, and immunohistochemical studies. We found that BGP-10 effectively reversed the damage toward normal, whereas BGP-3 failed to exhibit a significant renal protective effect. We conclude that bergapten could be a potential renal protective drug, and hence, more detailed cellular molecular-based studies are needed to bring this drug from the bench to the bedside.



1. INTRODUCTION

Cyclophosphamide (CPM) is an explicitly used chemotherapeutic agent with significant clinical outcomes in various cancers, including Hodgkin's or non-Hodgkin's lymphoma.[1] CPM is a potential anticancer drug and immunosuppressant in nephrotic syndrome. As a well-established prodrug administered orally, CPM undergoes hepatic metabolism and, when acted by the metabolic enzyme CYP3A4, breaks into acrolein and phosphoramide mustard.[1] Phosphoramide mustard is an active metabolite with a significant anticancer effect.[2] Mechanistically, the phosphoramide guanine residue at *N*-7 leads to altered replication of DNA and, ultimately, cell death. Acrolein is considered a toxic metabolite and shows potential and deleterious toxic effects leading to multiorgan toxicity, including nephrotoxicity.[2] This is because when CPM is administered at the therapeutic dose, it exhibits anticancer and organ damage.[3] This property of CPM sometimes limits its clinical use and, when used, significantly hampers the clinical outcome and quality of life of patients. Among various toxicity manifested by CPM, renal toxicity is considered the most deleterious and potential one.[3] Since the kidney is one of the vital organs, any minor damage to this reflects altered homeostasis and hampers physiological function.

Therefore, it becomes important to understand the cellular and molecular mechanisms of CPM-induced nephrotoxicity. Based on the published evidence, it has been found that significant increase in oxidate stress in terms of increased reactive oxygen species (ROS) and reduced markers of antioxidant levels such as glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and increased level of malonaldehyde (MDA).[4] Evidence also showed the alteration in the expression level of nuclear factor erythroid 2−related factor 2 (Nrf2) and associate protein.[4−6] Additionally, recently published literature showed the increased expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB),

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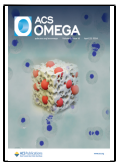

NLR family pyrin domain containing 3 (NLRP3), p38 mitogen-activated protein kinase (MAPK), and interleukins (ILs) leading renal inflammation.[7] Preclinical evidence also emphasizes the increased apoptosis where increased caspases, cytochrome C, dysfunctional mitochondria, and calcium overload were found.[4,5,7,8] In addition to increased oxidative stress, inflammation, and apoptosis, CPM administration is also reported to cause renal fibrosis and increased levels of transforming growth factor beta 1 (TGF-β1).[6]

Hence, a multifactorial approach exists for the management of CPM-induced nephrotoxicity. In line with this, it was found that natural products have been explicitly investigated for their role in various disease conditions, including as an adjuvant with chemotherapeutic agents.[4] These natural products possess significant antioxidant, anti-inflammatory, and apoptotic properties. Additionally, most of the natural products are considered safe to use, as these are used in traditional systems of medicine.[4] Bergapten (BGP) is one such natural product that has significant pharmacological activity in terms of being a potent antioxidant, anti-inflammatory, and antiapoptotic agent, anticancer, neuroprotective, antiosteoporotic, and colon protective potency.[9,10] However, BGP has not been studied for renal protective potency in any *in vivo* model of renal toxicity.

Hence, this is the first report of renal protective potency of BGP against CPM-induced nephrotoxic manifestations. Therefore, looking into previously published work and its potential pharmacological properties, we herein first time explored the nephroprotective effect of BGP against CPM-induced nephrotoxic manifestations where markers of oxidative stress (SOD, CAT, GSH, MDA), markers of inflammation (IL-1β, TNF-α, IL-6, and NF-kB), fibrosis (TGF-β1, Masson's Trichrome staining) along with histological and immunohistochemical studies using H and E, and periodic acid-Schiff (PAS) staining were performed.

2. MATERIALS AND METHODS

Renal toxicity in Swiss Albino Wistar rats was induced using cyclophosphamide, which was obtained from Sigma-Aldrich. For the treatment, BGP was also obtained from Sigma-Aldrich USA. Inflammatory markers were estimated using ELISA kits, and immunohistochemistry antibodies were obtained from Santa Cruz Biotechnology. The rest of the chemicals and drugs were analytical grade.

2.1. *In Vivo* Study. In the *in vivo* study, Wistar rats (180–200 g) were used. Rats used in the study were obtained from the central animal house facility of Lloyd Institute of Management and Technology, having IAEC Approval No. 1206/PO/Re/S/08/CPCSEA/08/2023/10/14. All the animals kept in the animal house were acclimatized for 7 days before the study's initiation and kept at standard humidity, temperature, and diet.

2.2. Dosing Schedule. Bergapten was administered from day 1 to day 14 at 3 mg/kg and 10 mg/kg, with a single dose of cyclophosphamide 200 mg/kg, i.p on day 7, as shown in Figure 1.[6,11] For the induction of renal toxicity, a single dose of cyclophosphamide was administered on day 7, i.p. On day 15, i.e., after 24 h of last dosing, animals were sacrificed, and both the kidneys were removed and washed with normal saline.[6] A section of renal tissue was stored in formalin solution (10%) for histopathological and immunohistochemical analysis, and the rest of the part was stored for various biochemical estimations.[6]

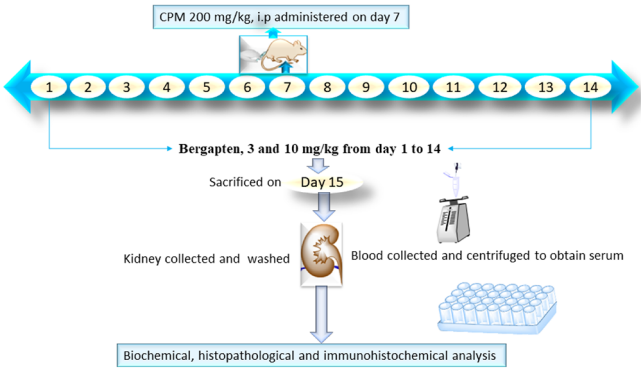

Figure 1. Showing the treatment regimen of the present study.

2.3. Markers of Oxidative Stress Assessment (Superoxide Dismutase Activity, Catalase Activity, Glutathione Level, and Lipid Peroxidation). A section of renal tissue was collected and washed to assess oxidative and lipid peroxidation markers, and a homogenate was prepared. SOD activity was evaluated by using the method given in Marklund and Marklund.[12] Catalase activity was estimated using Claiborne's method.[13] Similarly, Glutathione level estimation was conducted using Sedlak and Lindsay's (1968) method,[15] and MDA/lipid peroxidation was estimated by using Ohkawa et al.'s method.[14,15]

2.4. Renal Injury Markers Estimation. Markers of renal injury were estimated in serum using an autoanalyzer and according to the manufacturer's instructions.[16]

2.5. Inflammatory Marker's Estimation. The estimation of inflammatory and apoptotic markers, such as TNF-α, IL-1β, Il-6, and caspase-3, as well as KIM-1, was estimated using the commercially available ELISA kits. A section of renal tissue was collected and rinsed, and homogenate was prepared. The test sample or tissue homogenate was transferred to the ELISA plate wells, and various reagents were added to the wells per the manufacturer's instruction. At the end, absorbance was recorded.[16]

2.6. Histopathology and Immunohistopathological Analysis. For the histopathological study, mainly H and E, PAS, MT, and the immunohistochemical analysis, a section of renal tissue was collected and stored in 10% formalin for 48 h. After 48 h, tissue was embedded in the wax, and a thin section (4 μm) was cut using a microtome. For H and E staining, hematoxylin and eosin were used and further processed as per the protocol.[16] For the PAS staining, the grossed section was cut from the paraffin block, deparaffinized and stained with Schiff reagent, and further counterstained with Mayer's hematoxylin.[17] Similarly, prepared deparaffinized slides were stained with Weigert's iron hematoxylin solution and Biebrich scarlet-acid fuchsin stain for MT staining and processed as per the protocol.[8]

Immunohistochemistry for NF-kB and TGF-β1 was performed using commercially available antibodies. Grossed tissue was cut into a thin section and stained with diluted primary antibodies (1:800 for NF-kB and 1:500 for TGF-β1), followed by the application of secondary antibodies and counterstained with Mayer's hematoxylin dye followed by the further processing as per the protocol.[8] The prepared slides were used for imaging and interpretation using a Motic compound microscope and ImageJ software.

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3. STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad software. One-way ANOVA-Tucky's multiple comparison test was used for data analysis. Data were expressed as the mean ± SEM.

4. RESULTS

4.1. Protective Effect of Bergapten on CPM-Induced Oxidative Stress in Kidney. CPM exhibited significant oxidative in the kidney compared to the control group ($p < 0.001$). When the renal protective effect of BGP-3 was compared with CPM 200, it was seen that BGP-10 effectively reversed the CPM-induced oxidative stress ($p < 0.001$ for TBARS, CAT, SOD, and GSH), respectively, as represented in Figure 2. BGP-3 failed to exhibit any protective effect against CPM-induced derailed level and activity of CAT, SOD, and GSH ($p > 0.05$) but showed mild reduction in the level of a marker of lipid peroxidation, i.e., MDA ($p < 0.01$), as sown in Figure 2.

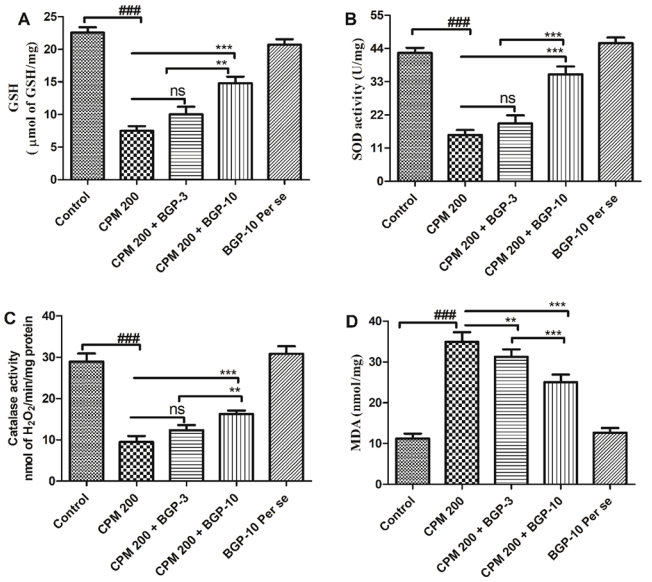

Figure 2. Represent the effect of BGP-3 and BGP-10 against CPM-induced oxidative stress. One-way ANOVA-Tucky's test was used for data analysis. As seen in A−D, the control and BGP-10 *Per se* groups exhibit almost comparable effects and signify no oxidative stress and lipid peroxidation. CPM 200 causes significant oxidative stress and increased lipid peroxidation. Treatment with BGP-10 significantly reduced the activity and level of markers of oxidative stress and lipid peroxidation. In contrast, treatment with BGP-3 failed to exhibit any effect against derailed oxidative stress markers but mildly reduced lipid peroxidation.

4.2. Protective Effect of Bergapten on CPM-Induced Inflammation in Kidney. CPM exhibited significant inflammation in the kidney when compared to the control group ($p < 0.001$). When the renal protective effect of BGP-3 was compared with CPM 200, it was seen that BGP-3 failed to exhibit any protective effect against CPM-induced inflammatory markers such as Il-6, TNF-α ($p > 0.05$) except for IL-1β ($p < 0.05$) and also failed to exhibit any significant antiapoptotic effect ($p > 0.05$). On the contrary, BGP-10 effectively reversed the CPM-induced renal inflammations ($p < 0.01$ for IL-6 and IL-1β whereas $p < 0.001$ for TNF-α) and for renal apoptosis ($p < 0.001$), respectively, as represented in Figure 3.

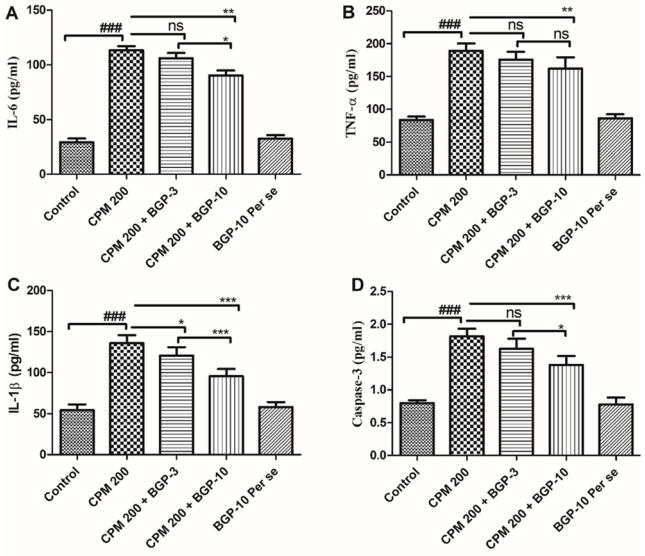

Figure 3. Represent the effect of BGP-3 and BGP-10 against CPM-induced inflammation in the kidney. One-way ANOVA-Tucky's test was used for data analysis. As seen in A−D, the control and BGP-10 *Per se* groups exhibit almost comparable effects and signify no renal inflammation and renal apoptosis. CPM 200 causes significant renal inflammation and apoptosis, as IL-6, TNF-α, IL-1β, and caspase-3 levels increased compared to the control group. Treatment with BGP-10 significantly reduced renal inflammation and apoptosis, as IL-6, TNF-α, IL-1β, and caspase-3 levels were reduced considerably compared to the CPM 200 group. Treatment with BGP-3 failed to exhibit any effect against the derailed marker renal inflammation but mildly reduced the level of caspase 3.

4.3. Protective Effect of Bergapten on CPM-Induced Increased Level of NF-kB in Kidney. CPM exhibited significant inflammation in the kidney as the increased NF-kB level was found compared to the control group ($p < 0.001$). When the anti-inflammatory effect or renal protective effect of BGP-3 was compared with CPM 200, it was seen that BGP-3 failed to exhibit any protective effect against CPM-induced inflammation, as BGP-3 failed to significantly reduce or reverse the level of NF-kB ($p > 0.05$). On the contrary, BGP-10 effectively reduced the level of NF-kB ($p < 0.001$) and exhibited anti-inflammatory and renal protective effects, as represented in Figure 4.

4.4. Protective Effect of Bergapten on CPM-Induced Derailed Renal Injury Markers. CPM treatment exhibited significant renal toxicity in terms of derailed serum levels of urea, BUN, uric acid, and Cr, and increased expression of KIM-1 was found when compared to the control group ($p > 0.001$). When the renal protective effect of BGP-3 was compared with CPM 200, it was seen that BGP-3 failed to exhibit any protective effect against CPM-induced altered levels of renal injury markers such as BUN, urea, and expression of KIM-1 ($p > 0.05$) but showed mild renal protective effect in terms of reduction in serum level of Cr ($p < 0.01$) and uric acid ($p < 0.05$). On the contrary, BGP-10 effectively reduced the derailed level of renal injury markers such as urea, BUN, uric acid, Cr, and expression of KIM-1 ($p < 0.001$). It exhibited a renal protective effect, as represented in Figure 5.

4.5. Protective Effect of Bergapten on CPM-Induced Histopathological Aberrations. In the current study, we observed normal histopathological attributes of renal tissue, as evident from the normal appearance of renal corpuscles,

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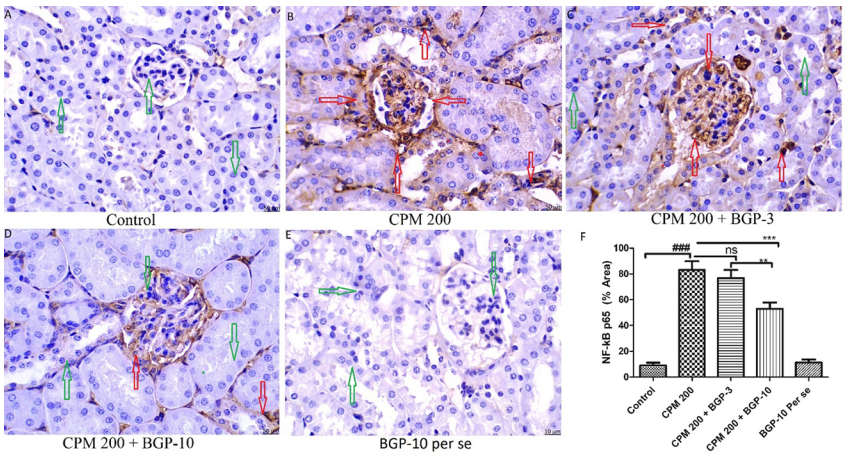

Figure 4. Effect of BGP-3 and BGP-10 against CPM-induced increased levels of NF-kB kidney is estimated by immunohistochemical analysis. One-way ANOVA-Tucky's test was used for data analysis. As seen in 4A−F, the control and BGP-10 *Per se* groups exhibit negative expression for NF-kB (green arrow), whereas significantly increased NF-kB expression was seen in the CPM 200 group (red arrow). When animals were treated with BGP-3 and BGP-10, BGP-10 effectively and significantly reduced the NF-kB expression, whereas BGP-3 did not exhibit any significant effect (scale bar 50 μm; 400×).

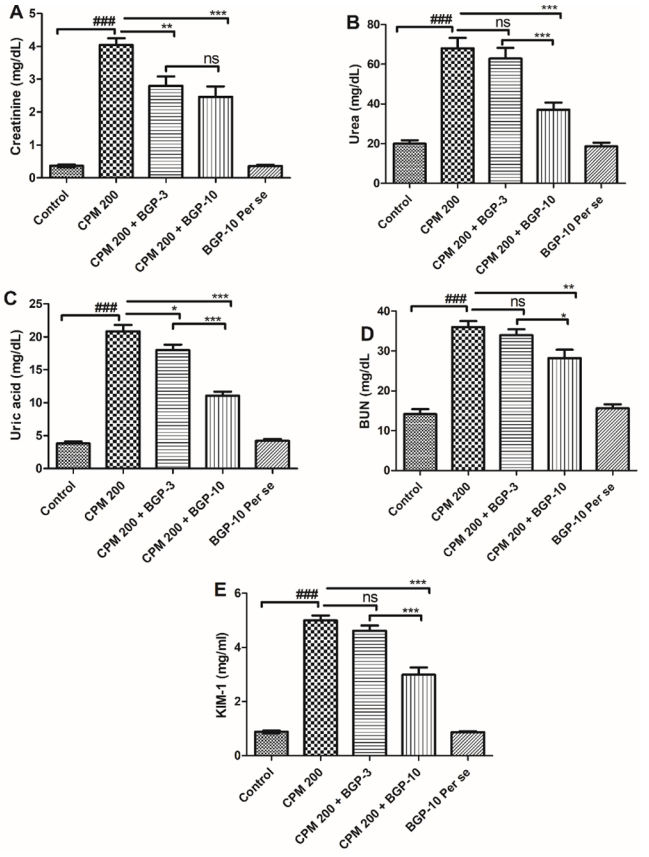

Figure 5. Represent the effect of BGP-3 and BGP-10 against CPM-induced derailed serum renal injury markers and the level of KIM-1 in the kidney. One-way ANOVA-Tucky's test was used for data analysis. As seen in A−D, the control and BGP-10 *Per se* groups exhibit almost comparable effects and signify no deleterious effect on renal function or markers of renal injury. CPM 200 causes deleterious effects on renal function or markers of renal injury. Treatment with BGP-10 significantly reduced the derailed renal injury markers and brought them closer to normal than that of the CPM 200 group. Treatment with BGP-3, however, failed to exhibit any renal protective effect against a derailed marker renal injury except in reducing the serum creatinine level.

glomerulus, and Bowman's space; along with this, no evidence of pyknosis, cellular disintegration, vacuolation, fatty changes, no damaged podocytes, parietal layer or mesangium, PCT or DCT was found. Significant damage to renal tissue was seen in the CPM-treated group, with evident damage to renal corpuscles, glomerulas, and Bowman's space. Significant pyknosis, cellular disintegration, vacuolation, fatty changes, damaged podocytes, parietal layer, or mesangium, PCT, or DCT were found. When the animals were exposed to BGP-3, no significant reversal of histologically damaged attributes was seen ($p > 0.05$), whereas BGP-10 effectively reversed the damaged attributes toward normal ($p > 0.01$), as shown in Figure 6.

4.6. Protective Effect of Bergapten on CPM-Induced Histopathological Damage (PAS Staining). CPM administered group showed thickened glomerular and basement membrane of the mesenchymal layer and deposition of glycogen in the interstitials. Exposure to drug BGP-3 failed to exhibit any reversal toward normal ($p > 0.05$), whereas BGP-10 effectively reduced the glycogen deposition toward normal ($p < 0.001$), as shown in Figure 7.

4.7. Protective Effect of Bergapten on CPM-Induced Fibrotic Changes (MT Staining) and TGF-β1 Expression. The CPM-administered group showed increased collagen deposition in the glomerulus and interstitials compared to the control group ($p < 0.001$). CPM administration also causes increased expression of TGF-β1 when compared to the control group ($p < 0.001$). Treatment with BGP-3 did not show a significant reduction in collagen-rich area and expression level of TGF-β1 ($p > 0.05$). On the contrary, BGP-10 effectively reduced the collagen-rich area ($p < 0.01$) and expression level of TGF-β1 ($p < 0.001$), as shown in Figure 8.

5. DISCUSSION

Cyclophosphamide-induced nephrotoxicity is well-documented in clinical and preclinical settings. Among various reported mechanisms of CPM-induced nephrotoxicity, oxidative stress is one of the most extensively studied. In normal physiological conditions, ROS or RNS is regularly produced as the body is exposed to various intoxicants. Compared to other organs, the kidney is affected mostly.[7] Li et al. have reported

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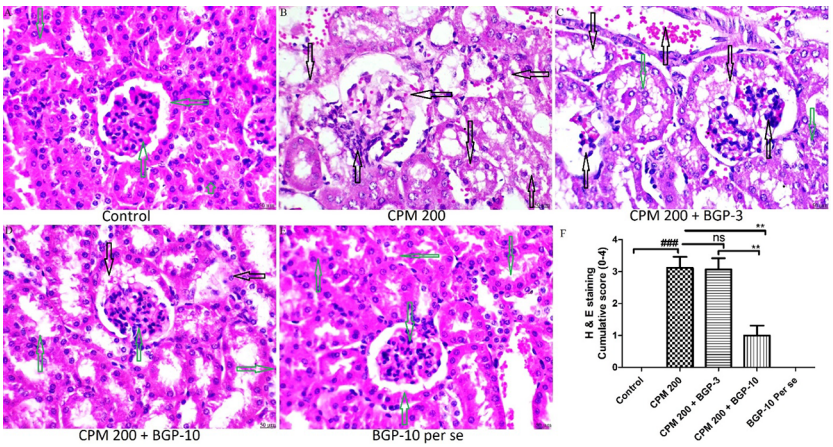

Figure 6. Represent the effect of BGP-3 and BGP-10 against CPM-induced histopathological damage in kidney tissue. One-way ANOVA-Tucky's test was used for data analysis. As seen in H and E staining, A−F, the control and BGP-10 *Per se* groups exhibit normal structural orientation of renal tissue (green arrow). In the CPM 200 treated group, marked histopathological damage such as renal fibrosis, cellular disintegration, pyknosis, damaged glomerulus, damaged PCT, and DCT, along with vacuolation, is seen (black arrow). When animals were treated with BGP-3 and BGP-10, BGP-10 effectively and significantly reduced the histopathological damage (green arrow), whereas BGP-3 did not exhibit any significant effect. (Scale bar 50 μm; 400×).

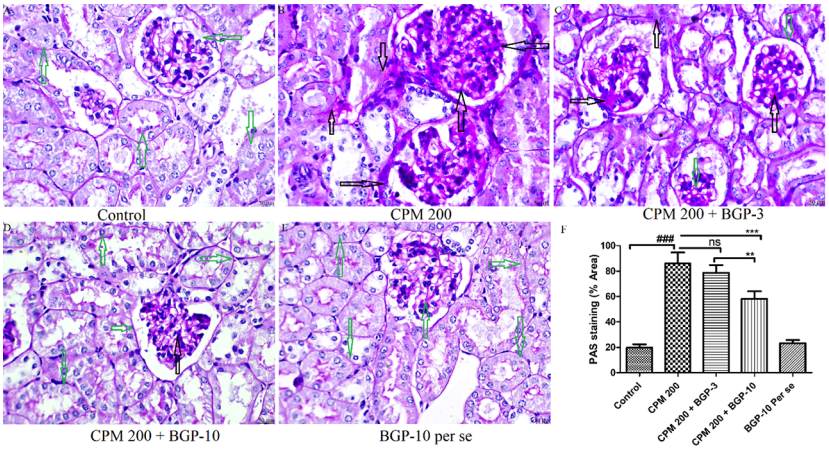

Figure 7. Represent the effect of BGP-3 and BGP-10 against CPM-induced glycogen deposition in kidney tissue. One-way ANOVA-Tucky's test was used for data analysis. As seen in A−F, the control and BGP-10 *Per se* group showed minimal glycogen deposition (green arrow), whereas significantly increased glycogen deposition was seen in the CPM 200 group (black arrow). When animals were treated with BGP-3 and BGP-10, BGP-10 effectively and significantly reduced the glycogen deposition, whereas BGP-3 did not exhibit any significant effect (scale bar 50 μm; 400×).

that CPM induces significant oxidative stress and ultimately results in renal injury.[18] Considering this fact, we also evaluated bergapten's antioxidant and reno-protective potency at 3 and 10 mg/kg, i.p., against CPM-induced oxidative stress. It was found that CPM administration effectively reduced the activity of SOD, CAT, and GSH and elevated the level of MDA and, hence, showed marked oxidative stress. BGP-10 showed a promising antioxidant effect where the activity of SOD, GSH, and CAT was increased and the MDA level was reduced to normal. BGP-3, however, failed to reduce the marker of oxidative stress compared to CPM 200, as shown in Figure 2. Findings of our study correlate with the previously published study where BGP exhibited significant antioxidant activity in various preclinical models.[19,20]

CPM-mediated renal toxicity is not only a manifestation of oxidative stress, but induced inflammation also plays a pivotal role in its toxicological attributes.[16] Among various mediators of inflammation caused by CPM, NF-kB is one of the extensively explored regulators of renal inflammation.[18] In the previously published studies, CPM has also been reported to increase the level of NF-kB and manifest renal toxicities in various preclinical studies.[9,17] Considering this fact, we also evaluated bergapten's anti-inflammatory and reno-protective potency at 3 and 10 mg/kg, i.p., against CPM-induced renal inflammation. It was found that CPM administration effectively elevated the levels of IL-6, IL-1β, TNF-α, and NF-kB and reduced the level of IL-10 and, hence, showed marked renal inflammation. BGP-10 showed a promising anti-inflammatory effect where the level of IL-6, IL-1β, TNF-α, and NF-kB gets reduced. BGP-3, however, failed to reduce the marker of renal inflammation compared to CPM 200, except for the level of TNF-α, as shown in Figures 3 and 4, respectively. The findings of our study correlate with the previously published study where BGP exhibited significant anti-inflammatory activity in various preclinical models.[21,,23]

It also became necessary to highlight that being important mediators and mechanisms, such as oxidative stress and inflammation, these are not renal-specific markers. Henceforth, it became permissive to estimate and evaluate the level of renal-specific injury markers such as serum creatinine (Cr),

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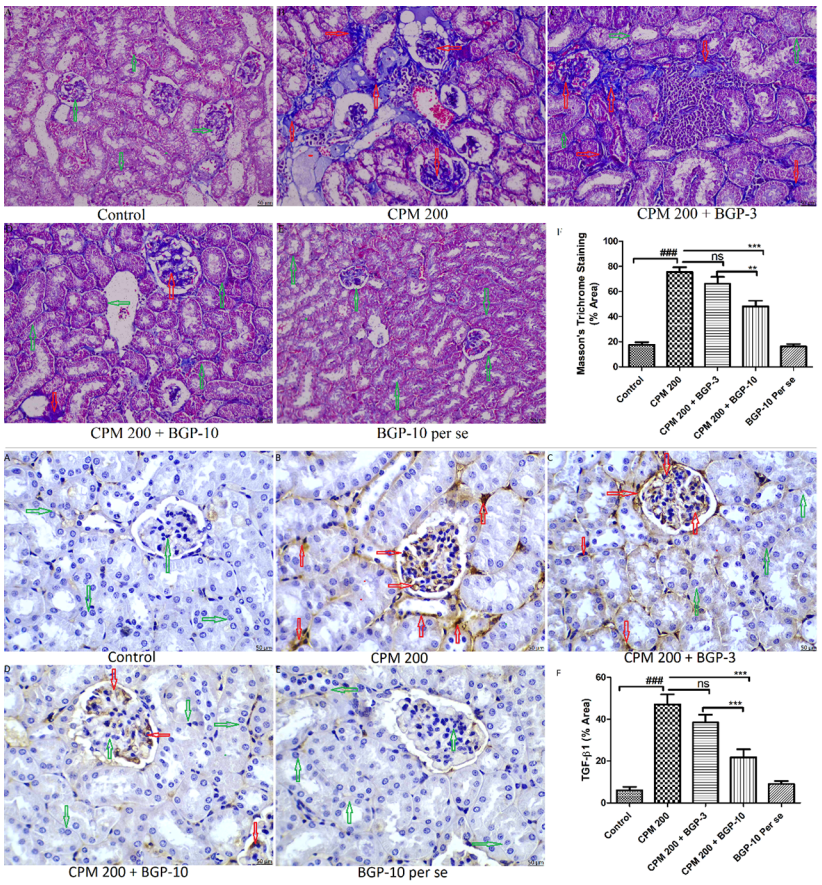

Figure 8. Represent the effect of BGP-3 and BGP-10 against CPM-induced fibrosis (MT staining) and expression of TGF-β1 in kidney tissue. A one-way ANOVA- test was used for data analysis. The upper panel (A−F) represents the MT staining, whereas the lower panel (A−F) exhibits the immunohistochemistry of TGF-β1. The control and BGP-10 *Per se* groups show minimal collagen deposition and TGF-β1 expression (green arrow). CPM 200 treatment caused significantly increased collagen deposition and expression of the TGF-β1 (red arrow) group. When animals were treated with BGP-3 and BGP-10, BGP-10 effectively and significantly reduced the collagen deposition and expression of TGF-β1, whereas BGP-3 did not exhibit any significant antifibrotic effect. (Scale bar 50 μm; 400×).

uric acid (UA), blood urea nitrogen (BUN), and various other electrolytes.[24,25] Uric acid, in normal physiology, undergoes renal excretion, and when its serum level increases beyond a certain level, it signifies compromised renal function and renal toxicity. Like uric acid, creatinine, and blood urea nitrogen also indicate the extent of healthy renal activity, and their derailed level signifies damaged renal function.[25] Kidney injury molecule-1 (KIM-1) is another important and sensitive renal injury marker and has been extensively studied in various preclinical and clinical models.[26,27] Considering this fact, we also evaluated the serum levels of renal injury markers and KIM-1. It was found that CPM administration effectively elevated the urea, uric acid, creatinine, blood urea nitrogen, and KIM-1 levels and validated the compromised renal function. BGP-10 showed a promising renal protective effect, where the urea, uric acid, creatinine, blood urea nitrogen, and KIM-1 levels were reduced significantly. BGP-3, however, failed to reduce the marker of renal injury compared to CPM 200, except for the level of uric acid, as shown in Figure 5.

The hallmark of any pathological condition is the intact integrity or structural integrity of organs, such as the liver or kidney. CPM directly induces increased renal oxidative stress, apoptosis, or inflammation, resulting in histopathological aberrations.[28] In this study, we also explored the effect of CPM on renal histopathological damage. We find marked cellular disintegration, pyknosis, fibrotic changes, damaged glomerulus, Bowman's capsule, PCT, DCT, basement membrane, mesangial cells, etc., as reported by various earlier published studies.[7,28−] BGP-10 showed a promising renal protective effect and reversed the damaged histopathological attributes toward normal, whereas BGP-3 failed to reduce the histopathological damage toward normal, as compared to CPM 200, as shown in Figure 6. Apart from H and E staining, PAS staining is also routinely used to evaluate the extent of renal damage, upon exposure to various renal toxic stimulus or chemicals. In this study, we also explored the effect of CPM on renal histopathological damage via PAS staining. We find increased glycogen deposition in the glomerular and interstitial areas, as reported in the various previously published reports.[8,19,35] BGP-10 showed reduced glycogen deposition toward normal, whereas BGP-3 failed to reduce glycogen deposition toward normal, as compared to CPM 200, as shown in Figure 7.

One of the essential attributes of CPM-induced nephrotoxicity is renal fibrosis, which is a direct consequence of CPM as well as the indirect effect of renal oxidative stress, inflammation, and apoptosis.[8,38] Transforming growth factor-β1 (TGF-β1) is one of the extensively studied profibrotic markers. TGF-β1 becomes active when exposed to oxidative stress or inflammatory cytokines and binds to the respective receptors.[39] Activated TGF-β1 modulates the NF-kB pathway and potentiates mesangial cells' collagen production and

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proliferation, leading to the deposition of matrix and renal fibrosis.[39,40] Considering these facts, we also studied the effect of CPM on renal fibrosis. We found that CPM exposure significantly increased the collagen deposition area, as evidenced by MT staining. CPM administration further increased the level of TGF-β1 and hence validated the extent of renal fibrosis, which was in the various previously published reports.[41,42] BGP-10 showed promising renal protective and antifibrotic effects where collagen deposition area, TGF-β1, and Smad3 levels reduced significantly. BGP-3, however, failed to reduce the marker of renal fibrosis as compared to CPM 200, as shown in Figure 8

6. CONCLUSION

The finding of this study, for the first time, showed the renal protective effect of bergapten in a preclinical model. In other words, this is the first report of the renal protective effect of bergapten. We found marked oxidative stress, inflammation, histopathological damage, and fibrosis when the animals were exposed to cyclophosphamide at 200 mg/kg. mg/kg, ip effectively reduced the MDA, IL-6, and IL-1β levels. TNF-αBergapten at the dose of 10 mg/kg, ip effectively reduced the MDA, IL-6, IL-1β, TNF-α, NF-kB, and TGF-β1 levels. Bergapten at the dose of 10 mg/kg also reduced the serum markers of renal injury such as uric acid, urea, creatinine, blood urea nitrogen, collagen deposition area, and glycogen deposition and reversed the histopathological aberrations toward normal. We also found increased activity of antioxidant enzymes such as SOD, CAT, and GSH. However, bergapten failed to exhibit a significant renal protective effect at the dose of 3 mg/kg. We further conclude that bergapten could be a potential renal protective drug, and hence, more detailed cellular and molecular-based studies are needed to bring this drug from bench to bedside.

AUTHOR INFORMATION

Corresponding Author

Nehal Mohsin − *Department of Clinical Pharmacy, College of Pharmacy, Najran University, Najran 1644, Kingdom of Saudi Arabia;* orcid.org/0009-0007-0308-9249; Phone: 00966175428888; Email: nehalmohsin@gmail.com

Authors

Mohammad Shabib Akhtar − *Department of Clinical Pharmacy, College of Pharmacy, Najran University, Najran 1644, Kingdom of Saudi Arabia*

Saad A Alkahtani − *Department of Clinical Pharmacy, College of Pharmacy, Najran University, Najran 1644, Kingdom of Saudi Arabia*

Ismail A Walbi − *Department of Clinical Pharmacy, College of Pharmacy, Najran University, Najran 1644, Kingdom of Saudi Arabia*

Yasir Alhazmi − *Department of Clinical Pharmacy, College of Pharmacy, Najran University, Najran 1644, Kingdom of Saudi Arabia*

Md. Niyaz Alam − *Ram-Esh Institute of Vocational & Technical Education, Greater Noida, Uttar Pradesh 201306, India*

Alok Bhardwaj − *Lloyd Institute of Management & Technology, Greater Noida, Uttar Pradesh 201306, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c00124

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