Original Research Article

The Renoprotective effects of Liraglutide and Alfacalcidol against Cisplatin induced Nephrotoxicity in Mice

Abstract:

Cisplatin **(**CP) is an important platinum-based chemotherapy agent widely used to treat solid tumors. However, nephrotoxicity is the main limiting adverse effect in 25%–35% of patients treated with even a single dose of CP. This study aimed to evaluate the role of liraglutide (LIRA) and alfacalcidol (ALFA), each of them alone and in combination, in prevention of CP-induced nephrotoxicity. Forty male albino mice were divided into five groups including normal control group and groups CP, CP with LIRA, CP with ALFA, or CP with LIRA and ALFA. Nephrotoxicity was induced by single intraperitoneal injection of CP in a dose 12mg/kg. LIRA and/or ALFA treatment was started 5 days before induction of nephrotoxicity and continued till the end of experiment. Kidney function tests, oxidative stress and ferroptosis parameters were assessed. Histopathological and immunohistochemical examination were performed on kidney tissues. Both LIRA and ALFA monotherapyresulted in a significant improvement in kidney function tests, increased antioxidant levels, inhibition of ferroptosis and improved histopathological findings. LIRA showed more improvement compared to ALFA and their combination showed better results than each one alone. In conclusion, LIRA and ALFA either alone or in combination, represent a promising preventive modality for amelioration of CP-induced nephrotoxicity.

Keywords: Cisplatin; Nephrotoxicity, Liraglutide, Alfacalcidol, Ferroptosis

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1. Introduction

Cisplatin (CP) is a platinum-based chemotherapeutic agent widely used in the treatment of a variety of cancer conditions such as solid tumors, hematological malignancies, lymphoma, osteosarcoma, bladder, esophageal, gastric, pulmonary, testicular, ovarian, head and neck cancers.[1, 2] However, nephrotoxicity, which is dose, concentration, and time-dependent, is the main dose-limiting adverse effect in 25%–35% of patients, even in individuals administered a single dose of CP. [3] Additionally, 70% of individuals receiving chemotherapy containing CP showed signs of nephrotoxicity.[4] CP is predominantly eliminated by the kidneys via glomerular filtration and tubular excretion, causing a higher concentration of the drug within the kidneys compared to other organs. The accumulation of CP in the kidneys contributes to the increased risk of both acute and chronic nephrotoxicity.[5] The detailed pathophysiological mechanisms responsible for CP-induced nephrotoxicity are still not fully understood, resulting in insufficient progress in the development of effective preventive and therapeutic strategies.[6] Several studies have shown that the development of CP-induced nephrotoxicity may be associated with DNA damage, oxidative stress, inflammation, vascular dysfunction, and mitochondrial damage.[7] Recently, several studies have suggested that the administration of CP results in excessive lipid peroxidation, ferritinophagy-mediated free iron release, and a decline in the activity of glutathione peroxidase-4 (GPx4) [8]**.** These findings indicate a strong correlation between ferroptosis and CP-nephrotoxicity. Therefore, ferroptosis intervention may be an effective approach to alleviate CP-nephrotoxicity [9]**.**

Amifostine is the only FDA-approved drug to prevent CP-induced nephrotoxicity. However, despite its FDA approval, its cytoprotective effectiveness is not consistently established. Despite its approval, various reports indicate evidence of toxicity even with optimal doses, with ototoxicity being more commonly reported.[10] Although many studies have attempted to develop preventive methods or agents against CP-induced nephrotoxicity for many years, no treatment strategies are currently available for the prevention of CP-induced nephrotoxicity. The only preventive measure in use entails hydration therapy combined with the administration of diuretics like furosemide or mannitol.[11–13] Therefore, there is an urgent need to investigate new preventive safe and effective renoprotective drugs as supplementary therapy for patients undergoing CP treatment.[14]

Liraglutide (LIRA) is a Glucagon-like peptide-1 (GLP-1) receptor agonist, marketed as Saxenda® and Victoza®.[15] The FDA has approved its use as a supplementary therapy, along with diet and exercise, for management of type 2 diabetes mellitus (T2DM)[16] and for chronic weight management.[15] Numerous studies have highlighted the anti-ferroptotic, antioxidant and anti-inflammatory properties of LIRA, indicating that LIRA is a potential agent for the management of the disease states characterized by profound inflammation, oxidative stress, and ferroptosis.[17–20]

Alfacalcidol (ALFA) is a synthetic calcitriol analogue (1α-hydroxycholecalciferol). It is hydroxylated to calcitriol in the liver.[21] ALFA is mainly used as a therapeutic drug for osteoporosis, rickets, hypocalcemia, hypovitaminosis, chronic renal failure, and

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hypoparathyroidism in clinical practice.[22] ALFA administration showed antioxidant, anti-inflammatory and anti-ferroptotic activities.[3, 23, 24]

This study was designed to investigate the potential antioxidant, anti-inflammatory and anti-ferroptotic effects of LIRA and ALFA each alone and in combination in prophylaxis against CP-nephrotoxicity.

2. Materials and Methods:

2.1. Drugs and chemicals

CP 50mg/50ml was purchased from (Mylan S.A.S pharmaceutical company, France) in a concentration of 1mg/1ml, LIRA (Victoza pen) 6mg/ml was purchased from (Novo Nordisk A/S pharmaceutical company, Denmark), and ALFA (one alpha) ampules 2µg/ml was purchased from (LEO Pharm A/S pharmaceutical company, Ballarpur-Denmark). Phosphate buffer saline (PBS), potassium phosphate buffer and formalin 10% buffered solution were obtained from (Al-Gomhoria pharmaceutical company, Egypt). LIRA was diluted in saline to reach a final concentration of 50µg/ml and ALFA was diluted in saline to reach a final concentration of 0.05µg/ml. Consequently, the daily administration volume was adjusted to 0.1-0.2 ml/dose based on the weight of the animals. All chemicals used in the study were of analytical grade.

2.2. Experimental animals

The handling of animals and all experimental procedures were approved by the institutional Research Ethics Committee (REC), Faculty of Medicine, Tanta University, Egypt (Approval no. #34431/01/21). The study included 40 male swiss albino mice weighing 20-30 g. Mice were housed in animal laboratory room in wire mesh cages under constrict hygienic measures and had access to standard animal diet and water *ad libitum*. The animals were allowed for acclimatization for 2 weeks.

2.3. Experimental design and treatment protocol

A schematic presentation of the experimental design is represented in Figure 1. Mice were divided randomly into 5 equal groups (8 mice for each). Group 1 (control group) received daily intraperitoneal (I.P) and subcutaneous (S.C) injections of normal saline for 10 days. Group 2 (untreated nephrotoxicity) received CP at a dose of 12mg/kg single I.P injection on day 6. Group 3 (LIRA group) received LIRA at dose of 200µg/kg/day I.P injection from first day till the end of experiment. Group 3 (LIRA group) received LIRA at dose of 200µg/kg/day I.P injection from first day till the end of experiment. Group 4 (ALFA group) received ALFA at dose of 0.2µg/kg/day subcutaneous injection from first day till the end of experiment. Group 5 (LIRA and ALFA group) received LIRA concomitantly with ALFA in the same dosage regimen and routes mentioned above. Nephrotoxicity was induced in groups 3, 4, and 5 using the same method as in group 2. 3 days after CP injection, the animals were kept in individual metabolic cages for 24-h urine collection to estimate creatinine clearance (Cr.Cl).At the end of the experiment, the mice were anesthetized with 3% sodium phenobarbital (30 mg/kg).[25] Blood was collected from each animal by intracardiac puncture and was allowed to clot for 10 minutes at room temperature (R.T) and then centrifuged for 20 minutes at speed of 2000 r.p.m to obtain serum for measuring

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serum creatinine (SCr) and blood urea nitrogen (BUN). Then, animals were dissected and both kidneys were removed, washed with PBS solution (pH 7.4) to remove any red blood cells and clots. One kidney was used for histopathological examination, Prussian blue staining and immunohistochemical stain to assess tumor necrosis factor alpha (TNFα). The other kidney was homogenized for determination of Malondialdehyde (MDA), Reduced glutathione (GSH), and Solute carrier family 7 member 11 (Cystine-glutamate transporter) (SLC7A11).

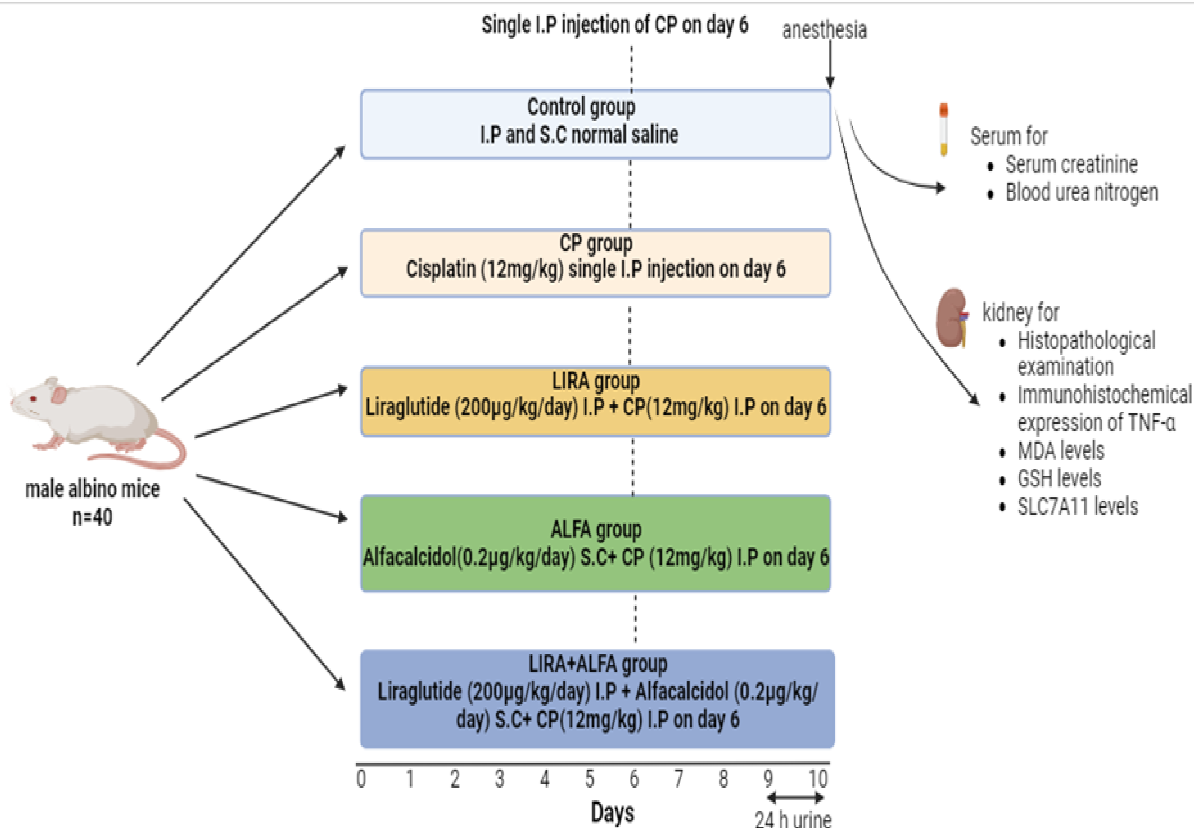

Figure 1.Summary of the experimental design

2.4. Biochemical analysis

2.4.1. Determination of serum creatinine (SCr), creatinine clearance (Cr.Cl) and blood urea nitrogen (BUN)

SCr (mg/dl), Cr.Cl (ml/min) and BUN (mg/dl) were measured spectrophotometrically using kits from Biodiagnostic Company, Egypt (Catalogue # CR 1251 and Catalogue # UR 2110, respectively). The SCr and Cr.Cl assay employed the spectrophotometric method developed by Bartles.[26]

2.4.2. Determination of malondialdehyde (MDA) and reduced glutathione (GSH) level in the kidney tissue

Kidney tissue MDA levels (nmol/g tissue) and GSH levels (mg/g tissue) were measured spectrophotometrically using kits from Biodiagnostic Company, Egypt (Catalogue # MD 2529

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and Catalogue # GR 2511, respectively). MDA levels were measured according to the method of Ohkawa.[28]While GSH levels were measured according to the method of Beutler.[29]

2.4.3. Determinationof tissue solute carrier family 7member11 (SLC7A11) level in the kidney tissue

Kidney tissue SLC7A11 was measured using ELISA kits obtained from Sun Red biotechnology, Shanghai, China, (Catalogue # 201-02-1684)following the manufacture`s protocol.

2.4.4. Histological examination of H&E and Prussian blue stained sections

One kidney was immediately fixed in 10% neutral buffered formalin, processed, and embedded in paraffin using standard techniques. Subsequently, tissue sections with a thickness of five microns were cut using a microtome. These sections were stained with hematoxylin and eosin (H&E) as well as Prussian blue stain and examined by light microscope for assessment of the histopathological changes and detection of iron deposits. Tissue injury was scored by the percentage of damaged renal tubules (0, no damage; 1, <25%; 2, 25−50%; 3, 50−75%; and4, >75%.).[30]

2.4.5. Immunohistochemical detection oftissue tumor necrosis factor alpha (TNFα)

IHC expression of TNFα was done by rabbit anti-TNFα polyclonal antibody (Catalogue # AMC3012; Biosource, Solingen, Germany). According to the percentage of TNFα-positive cells, immunostaining results were semiquantitatively scored as following: negative (-) = 0 %, 1 = 1–10 % positive staining/mm², 2 = 11–25 % positive staining/mm², 3 = 26–50 % positive staining/mm², 4 = 51–75 % positive staining/mm², and 5 = ≥76 % positive staining/mm².[31]

2.4.6. Statistical analysis

All obtained data from biochemical assays and morphometrics of this study were tabulated and statistically analyzed using Graph Pad Prism software 8.0.1 (GraphPad Software, San Diego, CA 92108). Shapiro-Wilk test for normality was performed. The parametric data were analyzed using one-way ANOVA, followed by post-hoc Tukey's multiple comparison tests. The parametric values wereexpressed as mean ±SEM. The non-parametric data were analyzed using Kruskal-Wallis test, followed by Mann-Whitney U test to test the difference between groups. The non-parametric values were expressed as median (minimum-maximum). The significance was considered at values of $p < 0.05$.

3. Results

3.1. Effect of Liraglutide and Alfacalcidol on BUN,SCr, and Cr.Cllevels

SCr, BUN, and Cr.Cl were assessed to evaluate the nephrotoxicity induced by CP and the potential protective effects of LIRA and ALFA. CP-treated group demonstrated a significant increase in SCr(Figure (2a)) and BUN (Figure 2(b)), along with a significant decrease in Cr.Cl levels(Figure 2(c)) compared to the normal control group. However, both the LIRA and ALFA groups showed a significant decrease in SCr and BUN, as well as a significant increase in Cr.Cl levels when compared to CP untreated group. The combination group showed more

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pronounced reduction in SCr and BUN, along with more pronounced elevation in Cr.Cl levels in comparison to both the LIRA treated group and ALFA treated group.

3.2. Effect of Liraglutide and Alfacalcidol on kidney tissue oxidative stress markers (MDA and GSH)

Assessment of oxidative stress was conducted by measuring the kidney tissue levels of MDA and GSH in the various study groups. The CP group demonstrated a significant increase in kidney tissue levels of MDA (Figure 3(a)), indicating higher oxidative stress, along with a significantdecrease in kidney tissue levels of GSH (Figure 3(b)) compared to the normal control group. However, both the LIRA and ALFA groups showed a significant decrease in in kidney tissue levels of MDA, as well as a significant increase in in kidney tissue levels of GSH when compared to CP untreated group. The combination group showed more pronounced reduction in kidney tissue levels of MDA, along with more pronounced elevation in kidney tissue levels of GSH when compared to both the LIRA treated group and ALFA treated group.

3.3. Effect of Liraglutide and Alfacalcidol on kidney tissue ferroptosis (SLC7A11)

Assessment of ferroptosis was conducted by measuring the kidney tissue levels of SLC7A11 in the various study groups. The CP group demonstrated a significant decrease in kidney tissue levels of SLC7A11 (Figure 4), indicating induction of ferroptosis, compared to the normal control group. However, both the LIRA and ALFA groups showed a significant increase in in kidney tissue levels of SLC7A11 when compared to CP untreated group. The combination group showed more pronounced elevation in kidney tissue levels of SLC7A11 when compared to both the LIRA treated group and ALFA treated group.

3.4. Effect of Liraglutide and Alfacalcidol on immunohistochemical expression of TNF-α

TNF-α was used as an indication of inflammation. Analysis of the scores of IHC expression of TNF-α in the different studied groups is shown in (Figure 5a).Negative TNF-α expression (score 0) in kidney tissue was observed in the control group (Figure 5b). Kidney tissue of the group treated with CP alone showed marked diffuse TNF-α expression (score 5) (Figure 5c),indicating occurrence of inflammation, in comparison to normal control group. LIRA and ALFA treated groups showed moderate diffuse TNF-α expression in kidney tissue (score 2 and 3, respectively) (Figure 5d) (Figure 5e). The combination group showed weak TNF-α expression in kidney tissue (score 1) (Figure 5f). This result implicates the superior effectiveness of combination treatment in preventing inflammation.

3.5. Histopathological examination of the kidney tissues stained with H&E and Prussian blue stain

(Figure 6(a)) represents histopathological scoring of the kidney tissues stained with H&Ein different experimental groups.Normal glomeruli and normal tubules lined with columnar epithelium with regular lumens were observed in the normal control group (no damage, score 0) (Figure 6(b)). CP untreated group showed vacuolization of tubular cells, tubular dilatation and sloughing of tubular cells in the lumen, eosinophilic casts within tubular lumens, infiltration by inflammatory cells mainly lymphocytes and plasma cells, loss of brush border and tubular

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dilatation, and glomerular atrophy (damage score 4) (Figure 6(c)).However, administration of LIRA or ALFA resulted in a significant reduction of tissue damage (damage score 2 ,3 respectively) (Figure 6(d,e)). The combination group showed near normal architecture of glomeruli and tubules (damage score 1) (Figure 6(f)).
Figure 7 represents the examination of Prussian blue stained kidney tissue in different studied groups to detect iron deposition. Normal control group showed no iron deposition (Figure 7(a)). Sever iron deposition was observed in CP untreated group indicating stimulation of ferroptosis (Figure 7(b)). LIRA or ALFA group showed marked reduction of iron deposition compared to CP untreated group (Figure 7(c,d)). The combination group showed more pronounced reduction in iron deposition when compared to both the LIRA treated group and ALFA treated group (Figure 7(e)).

3.6.Correlation between histopathological score and different parameters assessed in cisplatin untreated group

There was a significant positive correlation between histopathological score and BUN, SCr, and tissue MDA.Additionally, a significant negative correlation was identified between the histopathological score andCr.Cl, tissue GSH, and tissue SLC7A11. On the other hand, there was a non-significantcorrelation betweenhistopathological score and IHC score of TNF-α(Table.1).

4. Discussion

CP is an important platinum-based anticancer drug commonly used in the treatment of head, neck, ovarian, and testicular cancers.[2] However, nephrotoxicity, which is dose, concentration, and time-dependent, is the main dose-limiting adverse effect in 25%–35% of patients treated with even a single dose of CP. Unfortunately, less nephrotoxic CP analogues are less potent anticancer drugs; therefore, CP cannot be completely replaced at the moment.[32]Amifostine is the only FDA-approved drug to prevent CP-induced nephrotoxicity. Despite optimal dosage administration, various reports highlight instances of toxicity.[10] No treatment strategies are currently available for the prevention of CP-induced nephrotoxicity; only hydration therapy with the administration of diuretics.[13] In the scope of these facts about CP, there is a drastic need to investigate new preventive safe and effective renoprotective drugs.

SCr and BUN levels serve as established markers for assessing renal function, and elevated levels of these markers serve as indirect indicators of kidney damage.[33]Cr.Cl is the volume of blood plasma cleared of creatinine per unit time. It is a rapid and cost-effective method for the measurement of renal function.[34]

The results of the present study showed significant increase in SCr and BUN, and a significant decrease in Cr.Cl in CP group when compared to control group. This was in accordance withIbrahim et al., (2023)[35]who reported thatCP caused significant reduction in Cr.Cl and significant increase in SCr and BUN.

Both LIRA and ALFA exhibited significant protective effects against CP-nephrotoxicity, as evidenced by improvements in renal function markers (SCr, BUN and Cr.Cl)

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Treatment with LIRA prior to CP maintained the function and morphology of kidney via decreasing CP renal uptake by significant inhibition of organic cation transporter-2 (OCT2), in addition, LIRA modulate oxidant-antioxidant balance and this reflected on the levels of urea and creatinine as well as the histopathological changes induced by CP.[36] The data obtained from our work, administration of LIRA showed significant decrease in SCr and BUN levels, and significant increase in Cr.Cl when compared to CP group. This comes in the same line with **Xu et al., (2023)**[37]who reported that LIRA prevented an increase in SCr and BUN levels induced by CP.

In the present study, ALFA treated group showed significant decrease in SCr and BUN levels, and significant increase in Cr.Cl when compared to CP group. This was in agreement with another study, which reported decrease in SCr and BUN levels after administration of ALFA**.**[3]**Abd Elsamie Mohamed Khalil et al., (2023)**[38]reported that ALFA showed significant level of protection and restoration of normal renal structure in nephrotoxicity induced by CP.

Oxidative stress is a pivotal mechanism of CP-nephrotoxicity**.**[39] Numerous processes and mechanisms lead to CP nephrotoxicity and contribute to its complexity. However, the generation of toxic reactive oxygen species(ROS) remains a significant causative agent.[40]CP-induced ROS production, which could be a result of neutrophil activation and mitochondrial dysfunction, which can damage cellular macromolecules, including membrane lipids via peroxidation.[41] CP causes oxidative stress by directly damaging mtDNA, which impairs appropriate expression of mitochondrial enzymes forming the respiratory chain, and thus induces oxidative stress.[39] The decrease in GSH level and antioxidant enzymes and increase in MDA due to oxidative stress are seen as the main reason for the toxicity caused by CP.[42]

The study's findings demonstrated a reduction in renal tissue GSH levels and an elevation in MDA levels within the CP group, indicating the presence of oxidative stress. However, the administration of both LIRA and ALFA effectively reversed these effects, indicating their antioxidative characteristics.**Elkhoely, (2023)**[20]found that pre-treatment with LIRA resulted in a notable decrease of MDA level, as well as a significant enhancement of GSH level as relevant to diseased mice. Similar study revealed that LIRA treatment reduced MDA levels, increased GPx4 and GSH expression.[43]**Abd Elmaksoud et al., (2020)**[23]reported that ALFA administration revealed decrease in SCr, urea, renal tissue MDA, on the other hand, significant increase in renal tissue GPx activity was observed. Also, vitamin D has demonstrated protection against gentamicin induced renal damage. The mechanism of this protection is through the anti-oxidant effect of vitamin D.[44]

Ferroptosis is a new type of programmed cell death mediatedthroughiron-dependent lipid peroxidation.[45] Ferroptosis is mainly induced and promoted by lipid peroxidation due to GSH depletion, decreased activity of GPx4, and the ferrous iron-catalyzing Fenton reaction.[46] Ferroptosis is closely linked to the nephrotoxicity of CP as it could induce mitochondria oxidative stress through disrupting antioxidation molecular and enzymes such as GSH and GPx4, leading to the accumulation of excessive ROS, which activates iron-dependent renal tubular cells ferroptosis**.**[47] Iron metabolism and lipid peroxidation signaling are thought to be central mediators of ferroptosis. Hydrogen peroxide (H_2O_2) can react with ferrous ions and produce

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hydroxyl radicals with strong oxidizing properties, this reaction is called Fenton reaction.[48]Excessive iron can lead to the production of ROS that mediate ferroptosis through Fenton-like chemistry.[49] Impairment of the system Xc^{-} dependent antioxidant defense system contributes to oxidative damage and thus triggers ferroptosis.[50] System Xc^{-} is a heterodimer composed of two subunits, SLC7A11 and SLC3A2. Cystine and glutamate are exchanged in and out of the cell by system Xc- at a ratio of 1:11. The cystine that is taken up is reduced to cysteine in cells, which is involved in the synthesis of GSH.[51] A reduction of SLC7A11 disrupts the metabolism of GSH, however, overexpression of SLC7A11 may increase the synthesis of GSH to alleviating the stress-induced injury caused by lipid ROS, improving the ability of cells against ferroptosis [52]**.** Therefore, SLC7A11 is an essential subunit of system Xc- involved in the protection of cells from oxidative injury and lipid peroxide-induced ferroptosis.[53]

In our study, there was significant decrease in kidney tissue levels of SLC7A11 in CP group when compared to control group, indicating the potential involvement of ferroptosis in CP-nephrotoxicity. This was in accordance with **Meng et al., (2021)**[54]who reported that The expressions of ferroptosis related proteins SLC7A11 and GPx4 in CP group were significantly decreased when compared to control group, indicating that ferroptosis in kidney tissue was enhanced by CP.

Our study revealed that SLC7A11 decreased in the CP group and increased in the LIRA and ALFA groups. Similarly, GSH levels decreased in the CP group and increased in the LIRA and ALFA groups. This is consistent with **W. Lin et al. (2020)**[52]**,** who suggested that the reduction of SLC7A11 disrupts the metabolism of GSH; however, overexpression of SLC7A11 may increase the synthesis of GSH, improving the ability of cells against ferroptosis. Therefore, our findings support the concept that alterations in SLC7A11 affect GSH metabolism. Additionally, based on these findings, LIRA and ALFA improve cellular resistance against ferroptosis.

CP has also been found to have an inflammatory effect on the kidneys. TNFα, interleukin 1 beta (IL-1β), and NF-κB play central role in the inflammatory response triggered by CP.[55]

In the present study, CP was found to induce inflammation in the kidneys, as evidenced by increased TNFα expression. However, both LIRA and ALFA demonstrated anti-inflammatory effects, as indicated by a significant decrease in TNFα expression. **Abdel Moneim et al., (2019)**[3]reported that CP caused significant down regulation of VDR and suggested that VDR down regulation plays a role in the pathogenesis of CP induced nephrotoxicity by enhancing the inflammatory response because the suppression of VDR was associated with activation of NF-κBand TNF-α, which are the key players in CP induced inflammation and cell death. **Elkhoely, (2023)**[20] reported that pre-treatment with LIRA caused marked decrease in inflammatory markers including TNF-α. Also, **Abdel Moneim et al., (2019)**[3]reported decrease in TNF-α after administration of ALFA. This could be related to the antioxidant property of LIRA and ALFA in scavenging ROS and there for suppressing the inflammatory cytokines production as supported by our study results.

Concerning the histopathological examination of CP untreated group, H&E staining showed vacuolization of tubular cells, tubular dilatation and sloughing of tubular cells in the lumen, eosinophilic casts within tubular lumens, infiltration by inflammatory cells mainly lymphocytes

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and plasma cells, loss of brush border, and glomerular atrophy (damage score 4). These changes were in the same line with **Ali et al., (2020)**[2]who found that CP-alone group showed marked renal tubular necrosis with desquamated epithelial cells, cystic dilatation of multiple tubules, hyaline casts and marked basophilia. Prussian blue staining of CP group revealed sever iron deposition.

The results of the present study showed significant decrease in histopathological score in LIRA and ALFA groups when compared to CP group. This further supporting their renoprotective effects. Prussian blue staining of LIRA and ALFA groups revealed mild and moderate iron deposition respectively.

To our best knowledge, the present study is one of the leading studies that assessed the effects of ALFA on nephrotoxicity.

When the LIRA group and ALFA group were compared to each other,They showed non-significant differences in BUN, SCr, Cr.Cl and histopathological score.However, LIRA treated group showed significant increase in GSH and SLC7A11 and significant decrease in MDA and IHC TNF-α score in kidney tissue as compared to ALFA group.

To our best knowledge, this is the first study to investigate the combination of LIRA and ALFA in CP induced nephrotoxicity and compare it with the monotherapy by LIRA or ALFA each alone. The combination of LIRA and ALFA exhibited superior protective effects compared to monotherapy, indicating the potential synergistic advantages of these agents in mitigating CP-induced nephrotoxicity.

5.Conclusion

LIRA and ALFA are promising candidates for the prevention of CP nephrotoxicity by antioxidant, anti-inflammatory, and anti-ferroptotic mediated mechanisms. Their combination is superior to monotherapy by each drug alone. Further research is vitally needed to explore the exact molecular mechanisms that may underlie these effects and to test the possibility of clinical application of the findings of the present study.

6.References

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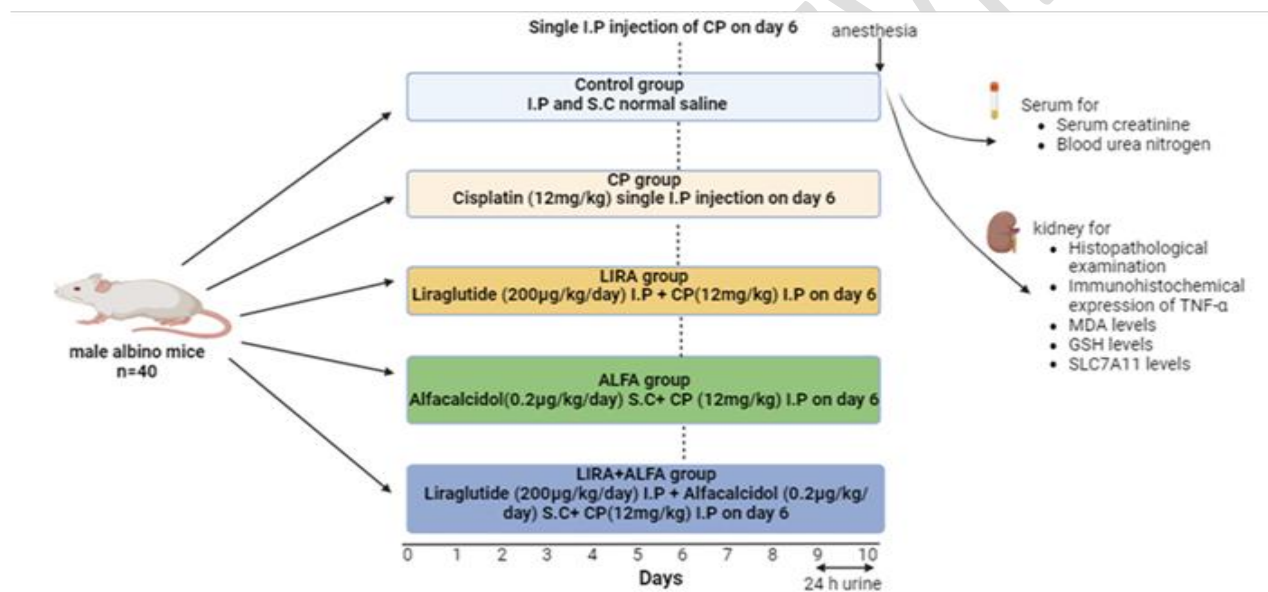

Figure 1. Summary of the experimental design.

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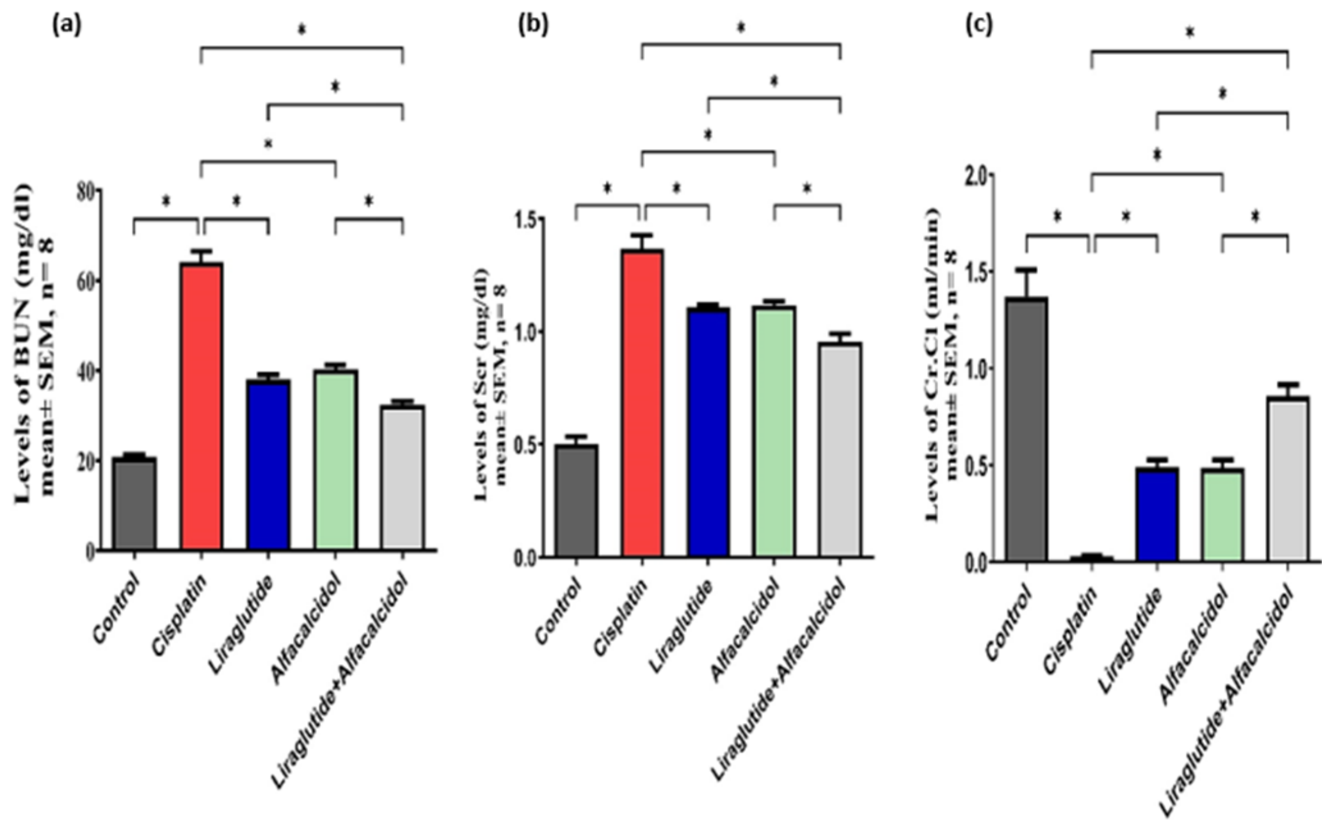

Figure 2. Effect of LIRA and ALFA on BUN, SCr and Cr.Cl and levels, (a) BUN, (b) SCr, (c) Cr.Cl.They were measured spectophotometrically. Data are presented as means ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by post-hoc Tukey's multiple comparison test.

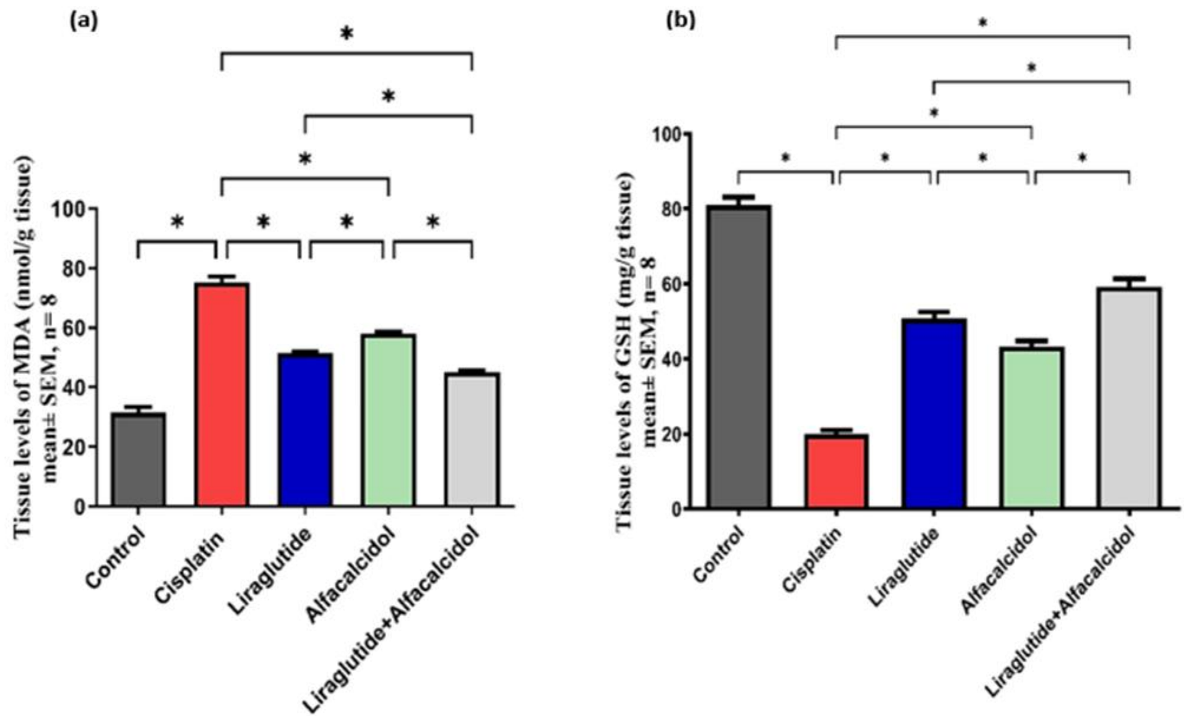

Figure 3. Effect of Liraglutide and Alfacalcidol on kidney tissue oxidative stress markers (MDAand GSH), (a) MDA, (b) GSH. MDA & GSH were measured spectophotometrically.Data are presented as means ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by post-hoc Tukey's multiple comparison test

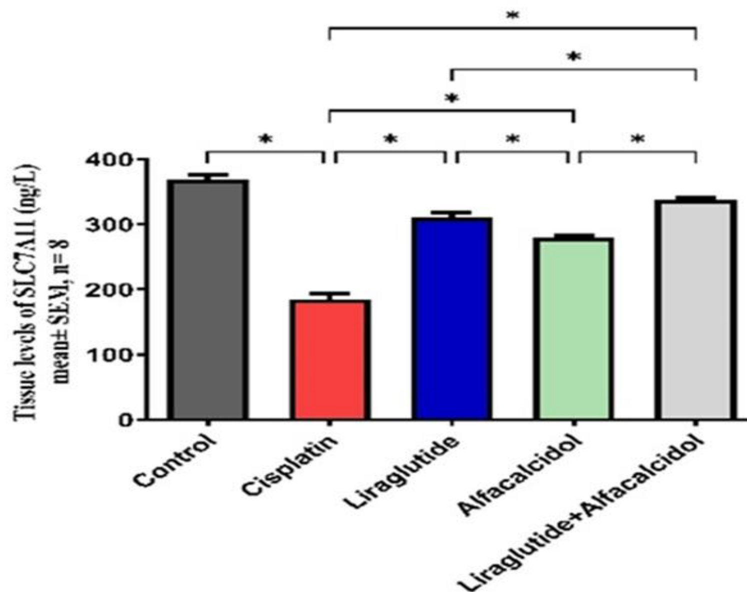

Figure 4. Effect of Liraglutide and Alfacalcidol on kidney tissue ferroptosis (SLC7A11). SLC7A11 was measured by ELISA. Data are presented as means ± SEM (n = 8). Data were analyzed by one-way ANOVA followed by post-hoc Tukey's multiple comparison test.

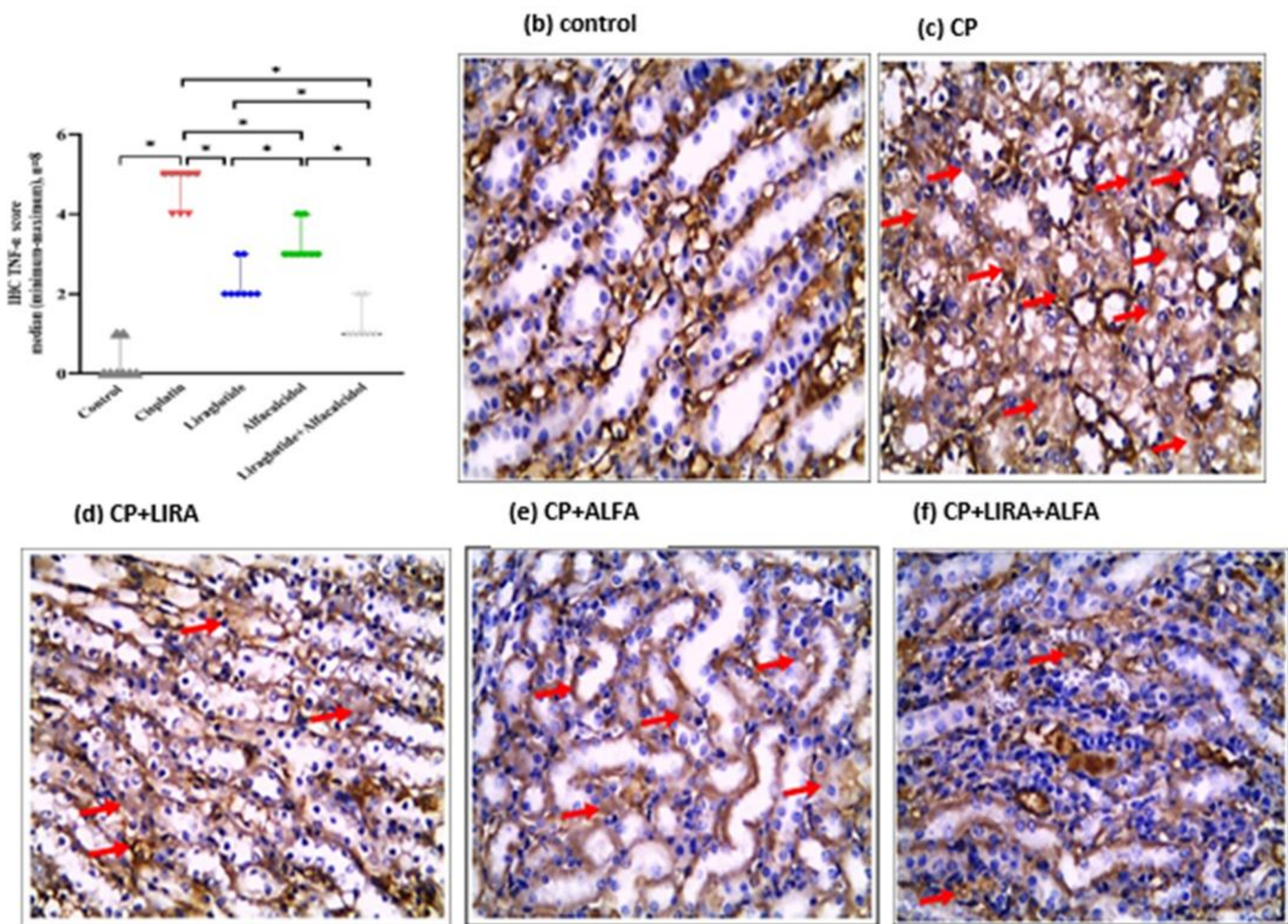

Figure 5. Effect of Liraglutide and Alfacalcidol on immunohistochemical expression of TNF-α (X400). (a) the scores of IHC expression of TNF-α in the different studied groups, Data are presented as median (minimum-maximum) (n = 8). Data were analyzed by Kruskal-Wallis test followed by Mann-Whitney comparison test. (b) control group:negative cytoplasmic TNF-α expression in tubular cells (score 0); (c) CP group:strong diffuse cytoplasmic TNF alpha expression in > 75% of tubular cells (score 5); (d) LIRA group:moderate diffuse cytoplasmic TNF alpha expression in tubular cells (score 2); (e) ALFA group: moderate diffuse cytoplasmic TNF alpha expression in tubular cells (score 3); (f) LIRA+ALFA group:weak cytoplasmic TNF alpha expression in ≤10 % of tubular cells (score 1). Abbreviations; CP: Cisplatin, LIRA: Liraglutide, ALFA: Alfacalcidol.

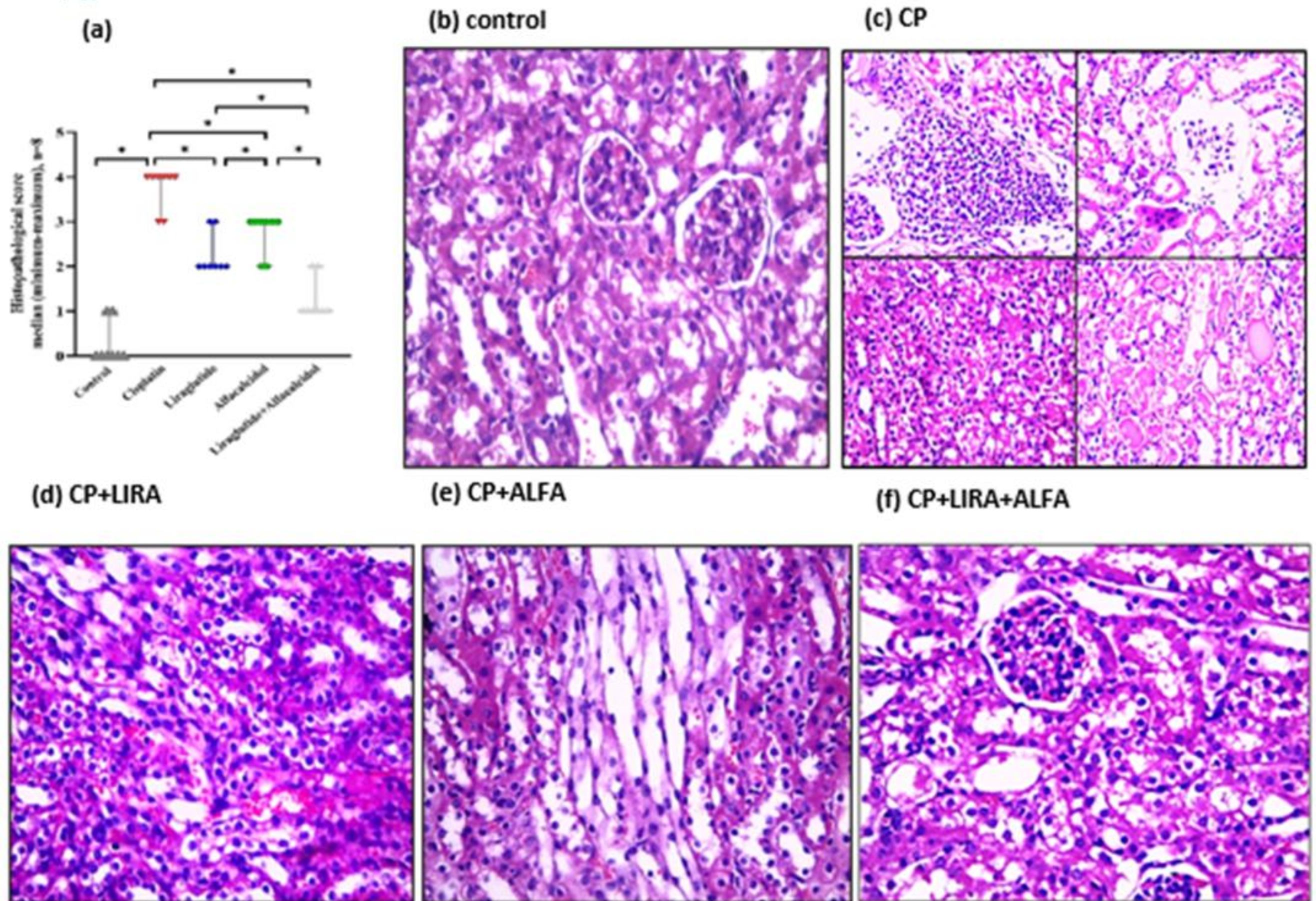

Figure 6. Histopathological examination of the kidney tissues stained with H&E (X400). (a) the histopathological score in different studied groups, Data are presented as median (minimum-maximum) (n=8). Data were analyzed by Kruskal-Wallis test followed by Mann-Whitney comparison test. (b) control group: normal glomeruli and normal tubules (score 0); (c) CP group: vacuolization of tubular cells, tubular dilatation and sloughing of tubular cells in the lumen, eosinophilic casts within tubular lumens, infiltration by inflammatory cells mainly lymphocytes and plasma cells, loss of brush border and tubular dilatation, and glomerular atrophy (damage score 4); (d) LIRA group: tubular cells vacuolization, eosinophilic appearance and loss of brush border (damage score 2); (e) ALFA group: tubular cells vacuolization, eosinophilic appearance, and loss of brush border and dilatation (damage score 3); (f) LIRA+ALFA: near normal architecture of glomeruli and tubular cells with slight cell vacuolization and tubular lumen dilatation (damage score 1).

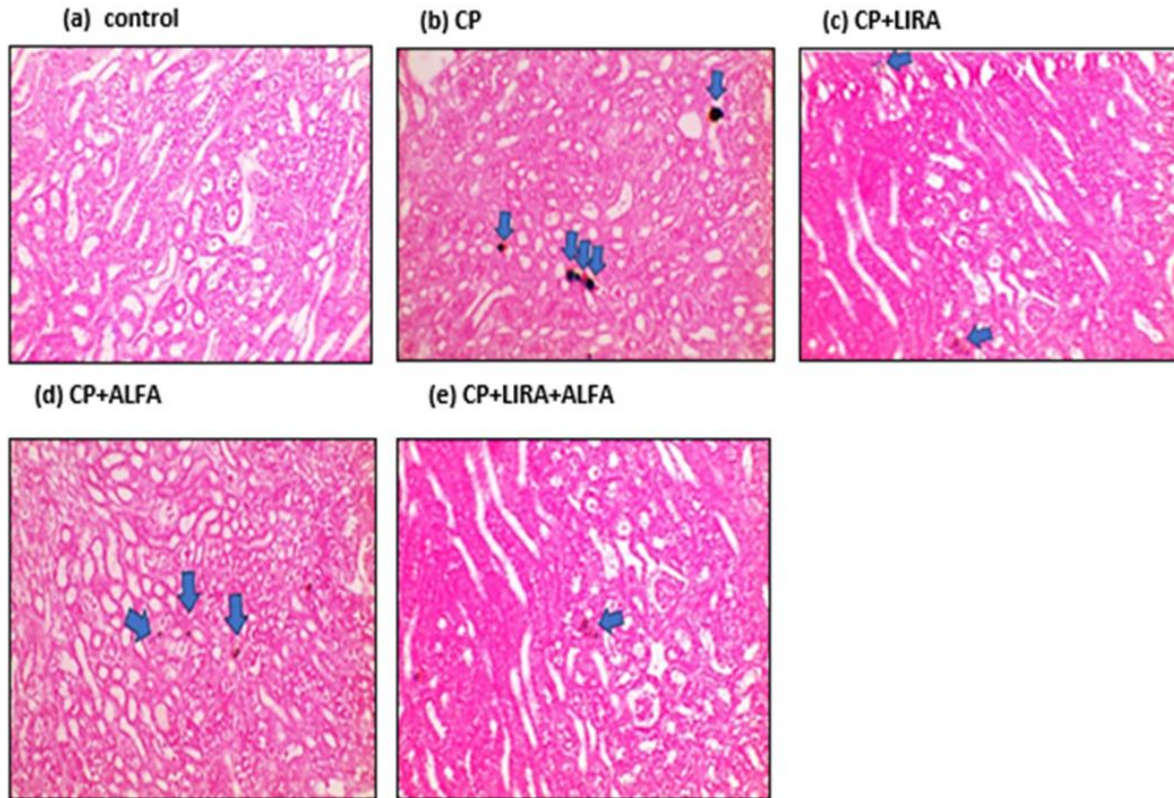

Figure 7. Examination of the kidney tissues stained with Prussian blue stain (X200).(a) control group: No iron deposition; (b) CP group: sever iron deposition; (c) LIRA group: mild iron deposition; (d) ALFA group: moderate iron deposition; (e) LIRA+ALFA group: minimal iron deposition.

Spearman's correlation coefficient (r)		
Parameters	histopathological score	
	r value	P value
BUN	0.850	P <0.05
SCr	0.850	P <0.05
Cr.Cl	-0.796	P <0.05
Tissue MDA	0.845	P <0.05
Tissue GSH	-0.850	P <0.05
Tissue SLC7A11	-0.845	P <0.05
IHC score of TNF-α.	0.745	P: NS

Table. 1 Correlation between histopathological score and different parameters assessed in cisplatin untreated group