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"Preclinical Evaluation of Daidzein on Cisplatin Induced Nephrotoxicity in Perimenopausal Mouse Model"

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ABSTRACT
Cisplatin is an effective on cancer like prostate cancer, bladder cancer, overian cancer, lung cancer that causes remarkable toxicity to the kidney, particularly to glomerular filter, by generating reactive oxygen species. From literature it is evident that women are more prone to Cisplatin induced nephrotoxicity, and the women >45 yrs of age are more prone to cisplatin induced nephrotoxicity. More specifically women of 45-55 age group (peri-menopausal age) are more prone for cisplatin induced nephrotoxicity. It has also become evident that inflammation provoked by injury to renal epithelial cells serves to amplify kidney injury and dysfunction.
Daidzein are responsible for the inhibition of inflammatory mediators and oxidative stress by scavenging reactive oxygen species (ROS). The objective of the present study is to determine the nephroprotective effect of Daidzein on cisplatin induced nephrotoxicity in perimenopausal mouse model.

Keyword: Cisplatin, Nephroprotective, Perimenopausal

I. INTRODUCTION

Acute kidney injury (AKI) is a clinical condition characterized by a rapid decline in renal function as well as the accumulation of waste products such as urea. (Ozkok and Edelstein, 2014). The kidneys are the most imperative organs of the human body. They are very essential for the maintenance of a variety of body fluids volume, regulations of fluid osmolality, balancing of acid-base concentrations, maintaining concentrations of various electrolytes, and removal or excretion of toxins from the body through the urine. Cisplatin is a platinum-based drug that has been widely using it as a chemotherapeutic agent to treat diseases such as bladder cancer, prostate cancer, cervix cancer, and lung cancer. Nonetheless, the cisplatin was associated with the various severe side effects such as nephrotoxicity, ototoxicity, neurotoxicity, and in some cases ocular toxicity (Zhao et al., 2020). It is also reported that CDDP-induced nephrotoxicity is gender-related.

Women, particularly perimenopausal women (45–55-year-old), had a higher chance of having CIN than men, according to our findings. Above graph showing the various stages including, pre-menopause, peri-menopause, and menopause but according to this literature cisplatin induced nephrotoxicity is more prone in perimenopausal woman. Organic cation transporters 2 (OCT2) are key mediators of cisplatin uptake and a cause of renal tubular cell death. They are found largely in the basolateral membrane of the renal proximal tubules. Proximal tubule injury, inflammation, oxidative stress, and vascular injury are all associated to the mechanisms. The perimenopause stage begins between the ages of 45 and 55, and lasts for one year after menopause. Estrogen levels are high and fluctuating during in the perimenopause stage, but they fall during the menopause period (Chen et al., 2017). Inflammation may have a major pathophysiological role in cisplatin-induced nephrotoxicity, according to recent studies. TNF-alpha, in particular, plays a key role in cisplatin-induced nephrotoxicity. TNF-alpha is known to cause further inflammatory reactions via stimulating the production of cytokines and/or chemokines including monocyte chemoattractant protein-1 (MCP-1) and intercellular adhesion molecule (ICAM)-1 (Ueki et al., 2013).

Diagnostic Criteria for Cisplatin Injury:

Cisplatin-induced renal injury is unlikely to have distinguishing diagnostic characteristics. Before there are changes in serum creatinine and glomerular filtration measured by creatinine collection, many patients have changes in glomerular filtration which could be identified by more sensitive tests such as inulin clearance. After cisplatin treatment, urinary excretion of proximal tubular injury markers such as-2 macroglobulin, N-acetyl-D-glycosaminidase, and 1-acid glycoprotein increases. The concentration of protein excreted in the urine has not changed much. Leukocytes, renal

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tubular epithelial cells, and granular casts are common findings on urinalysis. Two days after cisplatin exposure, a recent animal study found glucose, amino acids and tricarboxylic acid cycle metabolites in the urine. This altered metabolic profile could be used to detect early cisplatin-induced nephrotoxicity if it can be demonstrated in human studies (Yao et al., 2007).

Pathological Changes in Cisplatin Nephrotoxicity:

A routine kidney biopsy is not required for patients with cisplatin-mediated kidney injury unless the clinical situation necessitates a clarification that could change the patient's management. According to pathophysiology, most of the damage is expected to occur in the tubulo-interstitial compartment, with minimal glomerular changes, as evidenced by autopsy studies in both rats and humans (Tanaka et al., 1986). The tubules appeared to be gradually becoming involved, with a predominant proximal tubular component. Interstitial nephritis is not known to be caused by it (Cornelison and Reed, 1993).

Pharmacological Effects

Anticancer and anti-Breast Cancer activities:

Breast cancer is one of the most common malignant tumors in women which seriously threaten public health. Epidemiological studies have shown that the incidence of breast cancer in Asian women is lower than Western women due to the higher consumption of phytoestrogens. Thereby, the use of phytoestrogens may be a valid strategy in the prevention and treatment of breast cancer, via mechanisms including ER modulation and anti-angiogenesis. Due to phytoestrogens being a significant constituent of daidzein its anticancer activity in breast cancer has attracted wide public attention. Tumour necrosis factor-α (TNF-α), a type of endogenous cytokine, is able to affect tumorigenesis and dysregulation of TNF-α production contributes to cancer risk; Daidzein plays a vital role in the regulation of mammary tumor cell invasion induced by TNF-α. There are two distinct signaling pathways reported to elucidate the molecular basis of this, with one being the nuclear factor-kappa B (NF-κB) signaling pathway. In breast cancer cells MDA-MB-231, daidzein treatment suppressed TNF-α induced NF-κB and AP-1, followed by a reduction in the secretion of uPA from breast cancer cells, thus inhibiting the migration of breast cancer (Valachovicova et al., 2004). The other pathway is the Hedgehog (Hh) signaling pathway. Daidzein antagonized these effects via suppressing Gli1 activation and expression, thereby inhibiting migration and invasion of ER negative MCF10DCIS.com human breast cancer cells. The metabolites of daidzein in vivo exerted stronger activity at the same concentration. It was found that matrix metalloproteinase (MMP)-2 and MMP-9 also participated in breast cancer invasion. Daidzein inhibited the activity and expression of MMP-9 induced by TNF-α via Hh/Gli1 signaling pathway (Bao et al., 2014)..

II. DRUG PROFILE

DAIDZEIN

Chemical Structure

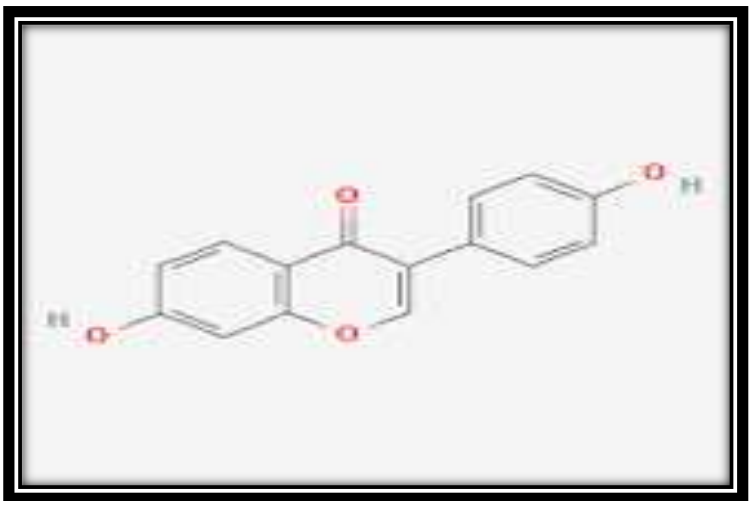

Figure 1: Structure of Daidzein

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IUPAC Name: (4′, 7-dihydroxyisoflavone)
Molecular formula: C15H10O4
Molecular weight: 254.23 g/mol
Melting point: 315 to 323 °C (599 to 613 °F;
Category:Antineoplastic.

Background

Daidzein (4′, 7-dihydroxyisoflavone) whose chemical structure is shown in Figure 1 is a naturally occurring isoflavonoid phytoestrogen belonging to the non-steroidal estrogens and is mainly derived from the leguminous plants such as soybean and mung bean. It is also the major bioactive ingredient in traditional Chinese medicine Gegen which is used frequently in the treatment of fever, acute dysentery, Diarrhea, diabetes, cardiac dysfunctions, liver injury etc. The chemical structure of daidzein is like mammalian estrogens and it exerts a dual-directional function by replacing/interfering with estrogen and the estrogen-receptor (ER) complex. Therefore, daidzein exerts protective effects against some diseases which are linked to the regulation of estrogen such as breast cancer, osteoporosis, diabetes, cardiovascular diseases it also has several other biological activities independent of the ER such as anti-inflammation, anticancer, inhibition of oxidative damage, protection of skin and the nerves. These beneficial effects are mainly due to regulation of the immune response scavenging of oxygen free radicals, inhibition of proliferation and so on. However, when daidzein is presented in the bound form "daidzein," it becomes inactive and some metabolites of daidzein also display a similar pattern. The safety of phytoestrogens is rather controversial as these may exert some negative effects on human health. In addition, the general absorption of daidzein is poor and many studies have been conducted to improve the bioavailability of daidzein.

III. MATERIAL AND METHODS

StudyAnimal:

Healthy female Swiss-albino mice 22-35 gm body weight were used for Cisplatin induced Nephrotoxicity in peri-menopausal mouse model.

Drugs:

Diadzein 5gPurity-98%, Cisplatin (Cizcan).

Chemicals:

5-5 -dithiol bis (2-nitrobenzoic acid) and Thiobarbituric acid (Loba Chemicals Pvt. Ltd.), Sodium chloride, Phosphoric acid, n-butanol, nitro blue tetrazolium, N-N- dimethyl acetamide (Sigma), hydroxylamine, glacial acetic acid.

Parameter evaluation:

Physical parameter

Body weight:

The body weight of mice was recorded daily throughout the experimentation phase using the effect of treatment intervention on body weight, percent change in body weight was evaluated, using basa.

Feed and water consumption:

The mice were housed as 4 mice/cage and the pre-weighed 20g of pelleted food were provided to each cage.

Urine Collection:

There was plan to collect urine to assess the urinary creatinine, BUN and albumin. However, due to Cisplatin administration animals were very dull

Blood collection and serum separation:

Mice was first anesthetize by anesthesia then gently scruffed to made to eye bulge..

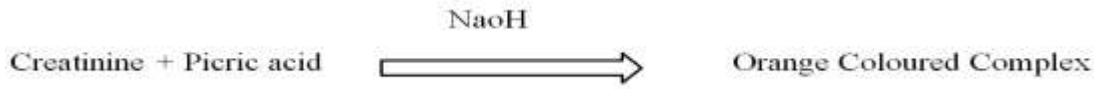
Sacrification of animal:

At the end of the treatment protocol (Treatment Day 8) all animals were sacrificed by cervical dislocation. The abdominal cavity was opened to collect the Kidneys.

Estimation of Serum parameter:

Creatinine Estimation:

Principle: In alkaline medium, creatinine reacts with picric acid to form an orange coloured complex and the rate of change in this absorbance is measured at 505 nm. By predetermined time interval.



Procedure:

Firstly, stock was prepared by adding 100 µl of serum in 190 µl of DW for all group of sample total was 200 µl stock solution prepared from that stock solution withdraw 10 µl for each stock of different animal sample

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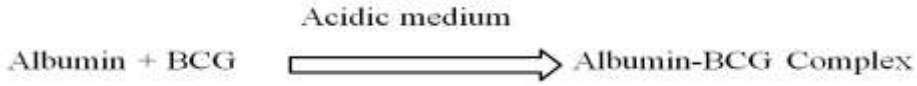
Albumin Estimation:

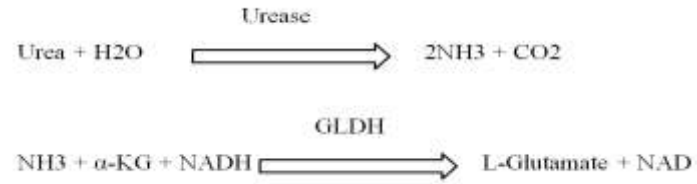


Procedure:

The protocol was set on a microplate spectrophotometer with GEN5 software along with the pathlength correction. 200 µl of reagent blank with 2 µl of DW, for std 200 µl reagent-1 with 2 µl std

Blood Urea Nitrogen:

Principle: The enzyme methodology employed in this reagent is based on the reaction that Talke and Schubert first described.



1. Urea is hydrolysed in the presence of water and Urease to produce ammonia and carbon dioxide.
2. In the presence of Glutamate Dehydrogenase (GLDH) and reduced Nicotinamide Adenine Dinucleotide (NADH), ammonia combines with α-ketoglutarate (α-KG) to produce L-Glutamate.
3. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm as NADH is converted to NAD.

Alkaline Phosphatase:

Principle: The estimation of ALP method used as IEFC recommendation, which utilizes 4-nitrophenyl phosphate as the substrate. In this method following reaction is catalysed by ALP present in the sample.

Preparation of Renal Homogenate:

Immediately after the sacrifice, the right kidney from each mouse was dissected and rinsed with isotonic saline and weighed.

Estimation of total protein content:

Biuret method:

Principle:

The peptide bonds of protein react with copper II ions in alkaline solution to form a blue-violet ion complex, (the so-called biuret reaction), each copper ion complexing with 5 or 6 peptide bonds.

Estimation of lipid peroxidation:

During prostaglandin biosynthesis in cells. MDA reacts with amino groups on proteins and other biomolecules to form a variety of adducts, including adducts with DNA bases that are mutagenic and possibly carcinogenic.

Estimation of reduced glutathione:

Glutathione (GSH) is a powerful antioxidant found in plants, animals, fungi, and bacteria that protects vital cellular components from reactive oxygen species like free radicals, peroxides, lipid peroxides, and heavy metals.

Estimation of superoxide dismutase:

Principle:

The inhibition of nitro blue tetrazolium (NBT) reduction was the basis for the SOD activity assay. Superoxide anions are produced when riboflavin is illuminated in the presence of O2 and an electron donor like methionine, and this has been used as the basis of SOD assays.

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IV. RESULTAND DISCUSSIONS

Physical parameter:

Body weight:

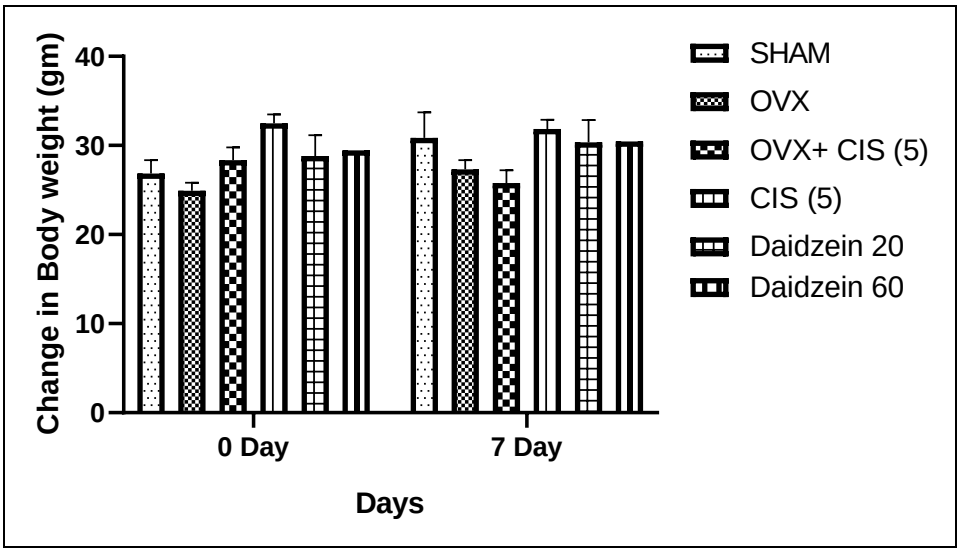
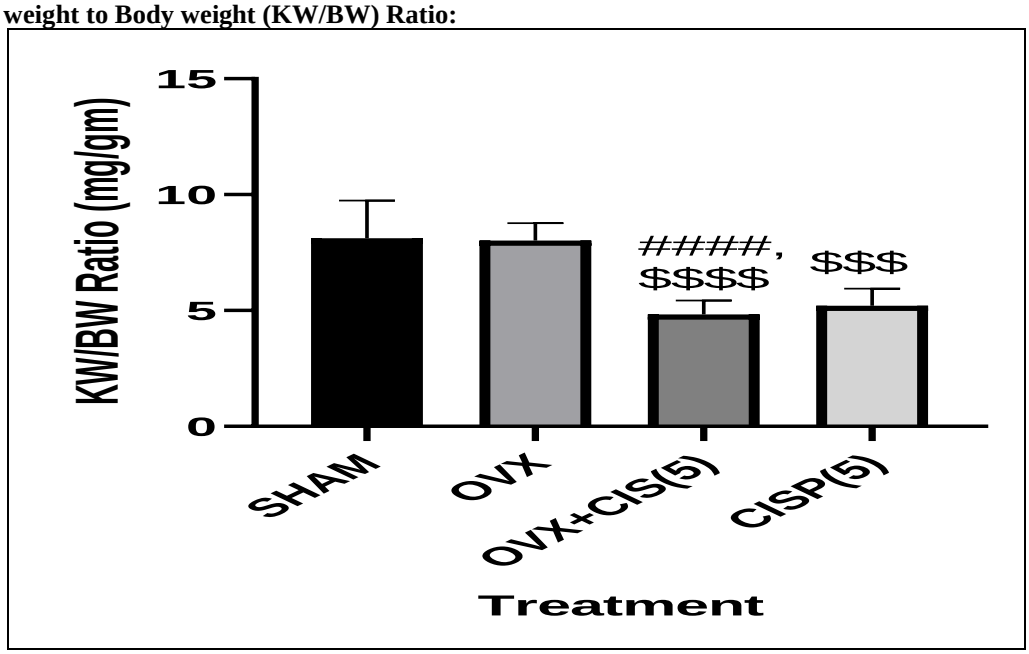


Fig No. 1 Body weight

Data was represented as mean± SEM and evaluated using two-way analysis of variance (ANOVA). The p value <0.05 is considered as statistically significant. OVX-Ovariectomy, CIS- Cisplatin, Daidzein.

Kidney weight to Body weight (KW/BW) Ratio:



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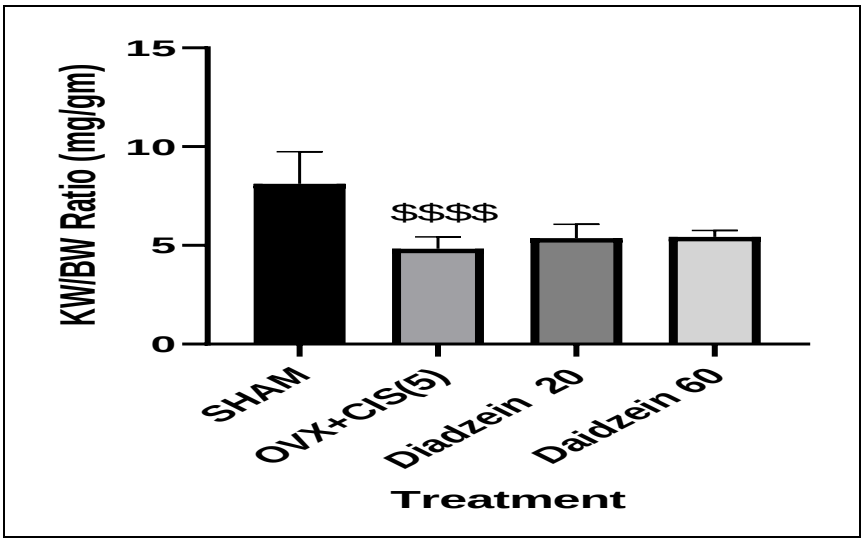
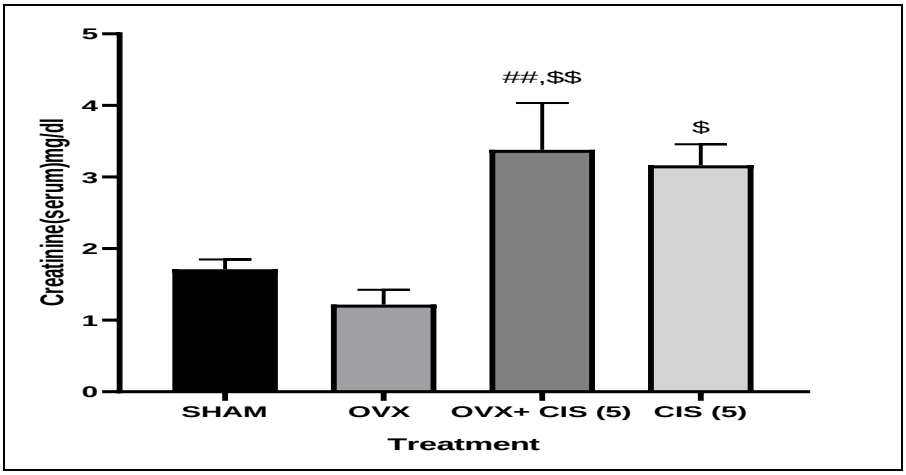


Fig No. 2 Effect of cisplatin KW/BW ratio in cisplatin treated perimenopausal mouse model:

Data represented as mean ± SEM (n=6) and evaluated using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test; The p value <0.05 is considered as statistically significant. ##p<0.01 is compared with normal group; *p<0.05, is compared with disease control group. The values in parenthesis are doses in mg/kg. CIS- Cisplatin, Daidzein.

Serum Creatinine Estimation:



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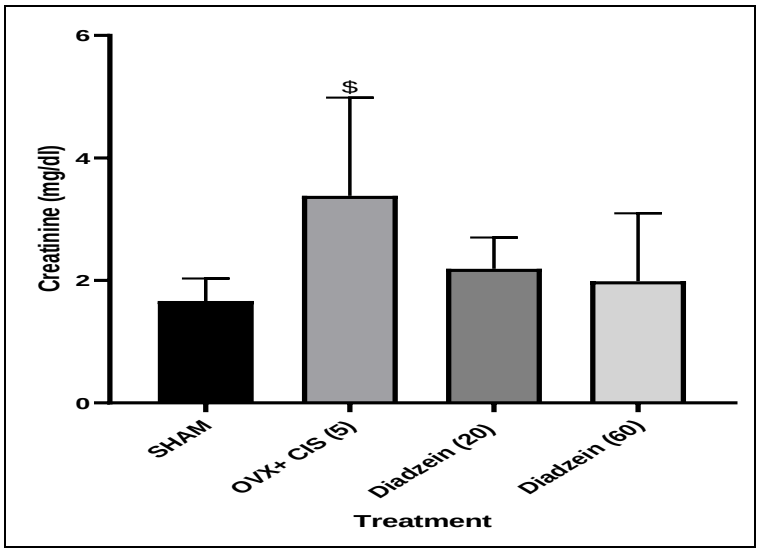
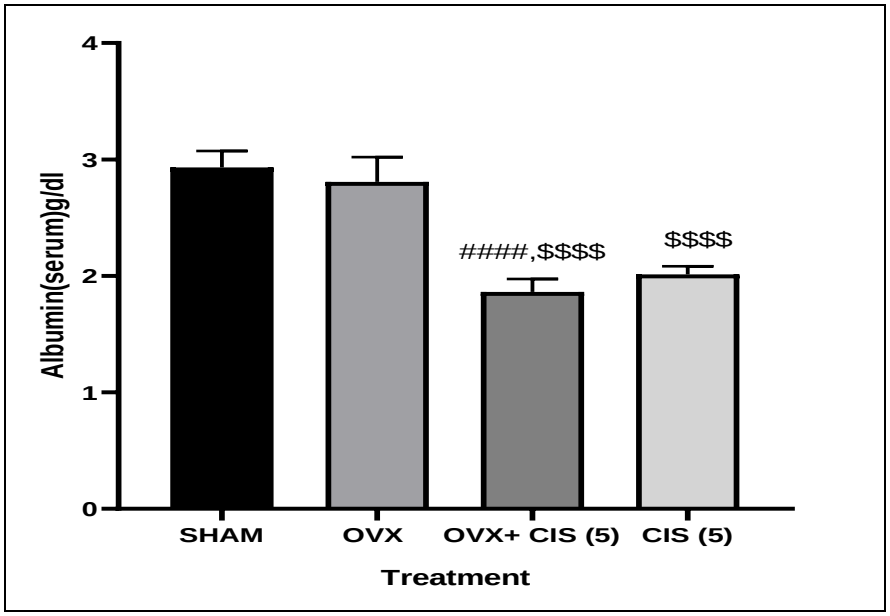


Fig No. 3,4 Effect of cisplatin on serum creatinine in cisplatin treated perimenopausal mouse model:

Data represented as mean ± SEM (n=6) and evaluated using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test; The p value <0.05 is considered as statistically significant. ##p<0.01 is compared with normal group; *p<0.05, is compared with disease control group. The values in parenthesis are doses in mg/kg. CIS- Cisplatin, Daidzein

Serum Albumin Estimation:



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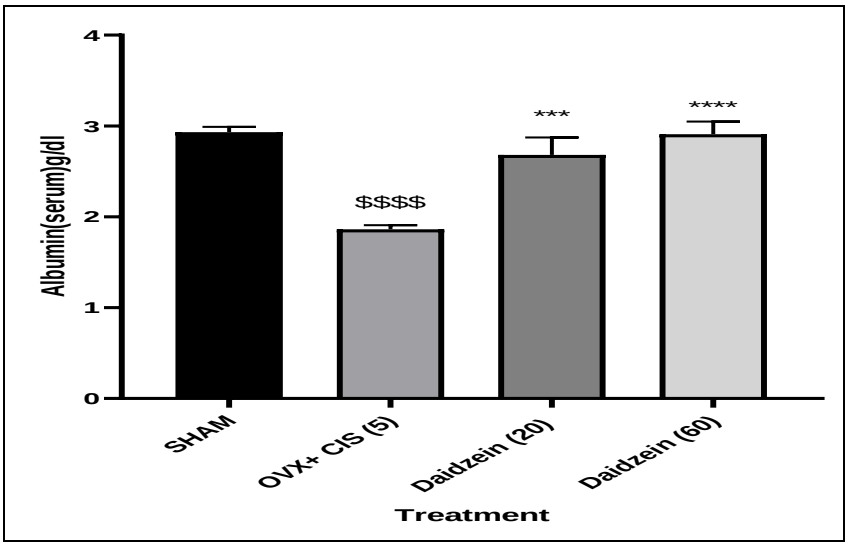
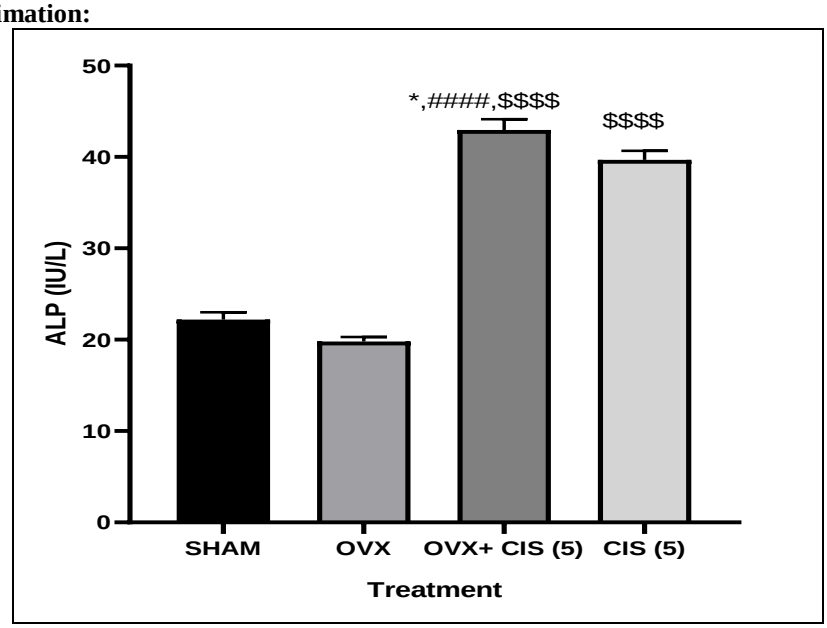


Fig no. 5, 6 Effect of cisplatin on serum albumin in cisplatin treated perimenopausal mouse model:

Data represented as mean ± SEM (n=6) and evaluated using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test; The p value <0.05 is considered as statistically significant. ##p<0.01 is compared with normal group; *p<0.05, is compared with disease control group. The values in parenthesis are doses in mg/kg. CIS- Cisplatin, Daidzein.

Serum ALP Estimation:



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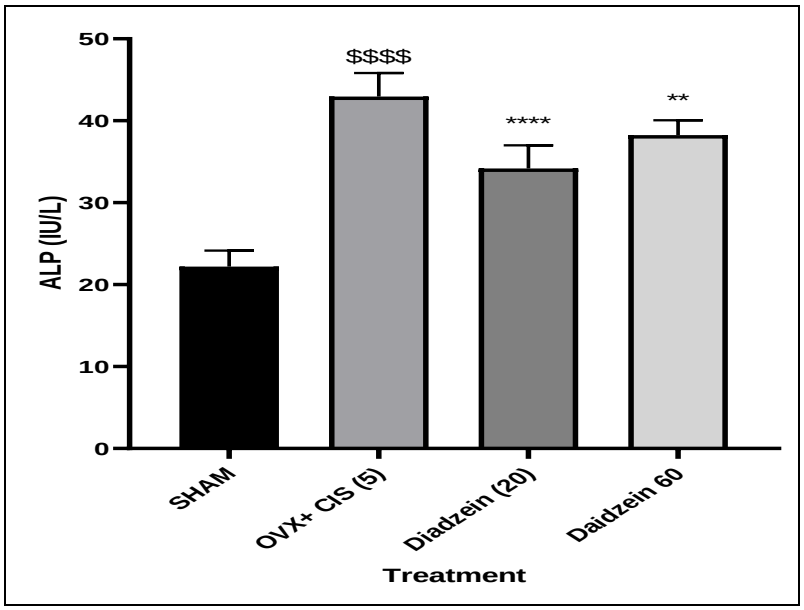
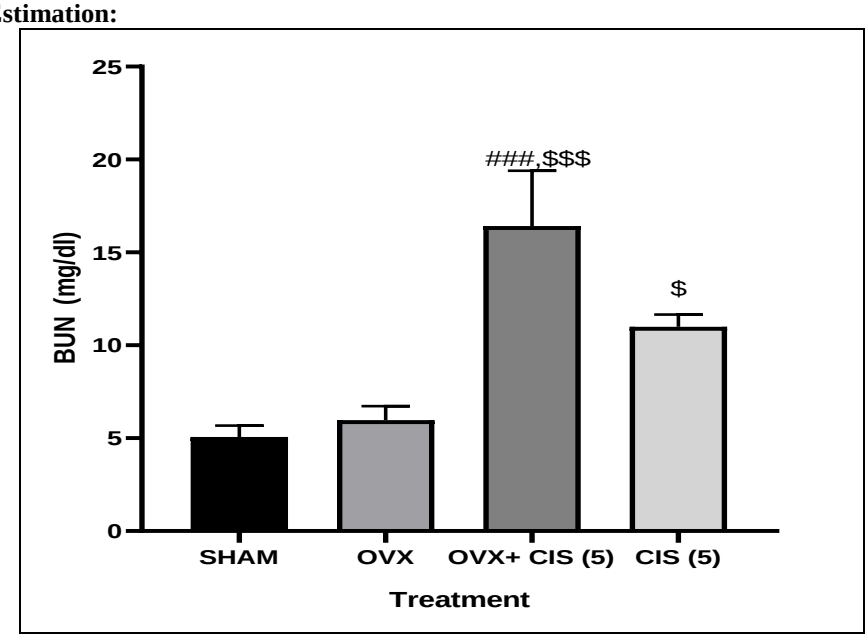


Fig No.7, 8 Effect of cisplatin on serum ALP in cisplatin treated perimenopausal

Mouse model:

Data represented as mean ± SEM (n=6) and evaluated using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test; The p value <0.05 is considered as statistically significant. ##p<0.01 is compared with normal group; *p<0.05, is compared with disease control group. The values in parenthesis are doses in mg/kg.CIS- Cisplatin, Daidzein

Serum BUN Estimation:



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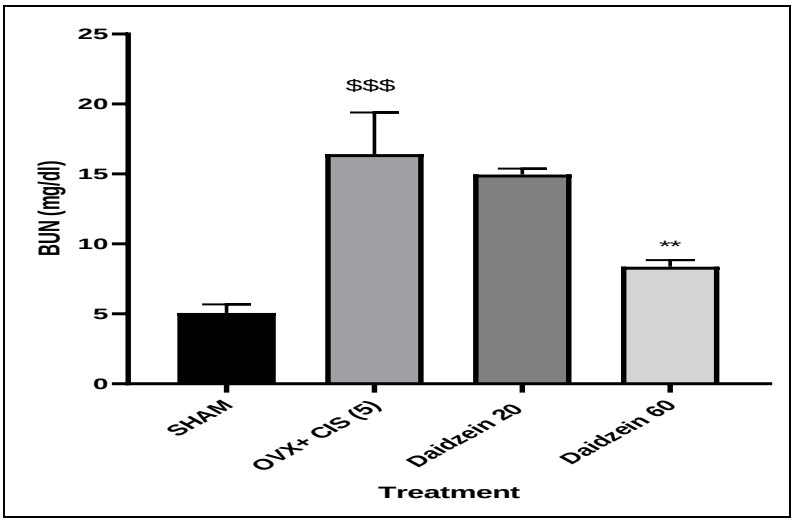
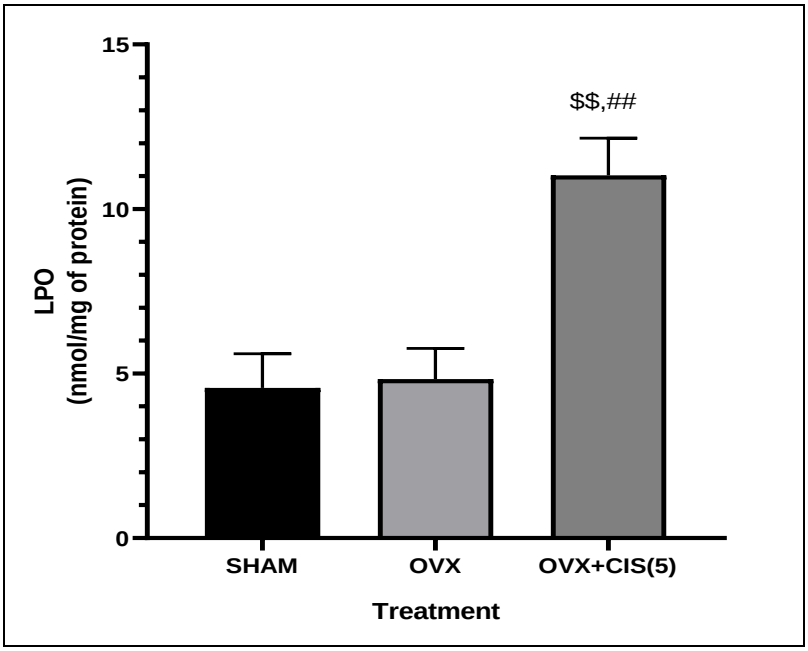


Fig No. 9, 10 Effect of cisplatin on serum BUN in cisplatin treated perimenopausal

Mouse model:

Data represented as mean ± SEM (n=6) and evaluated using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test; The p value <0.05 is considered asstatistically significant. ##p<0.01 is compared with normal group; *p<0.05, is compared with disease control group. The values in parenthesis are doses in mg/kg.CIS- Cisplatin, Daidzein

Lipid Peroxidation estimation:



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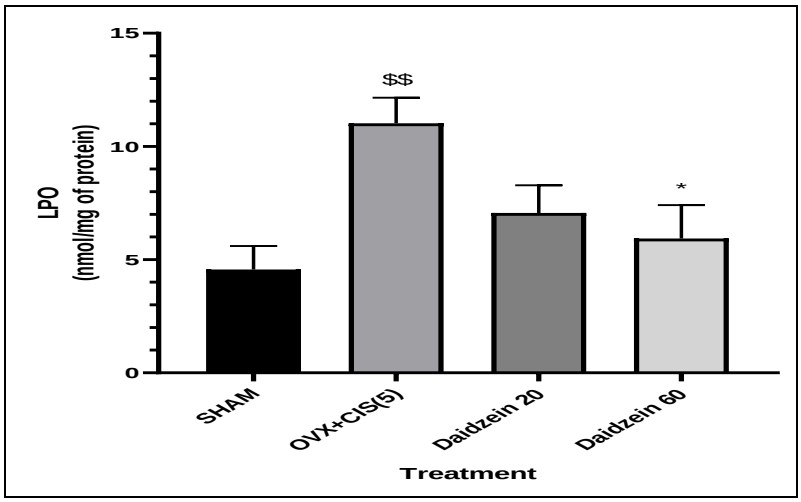
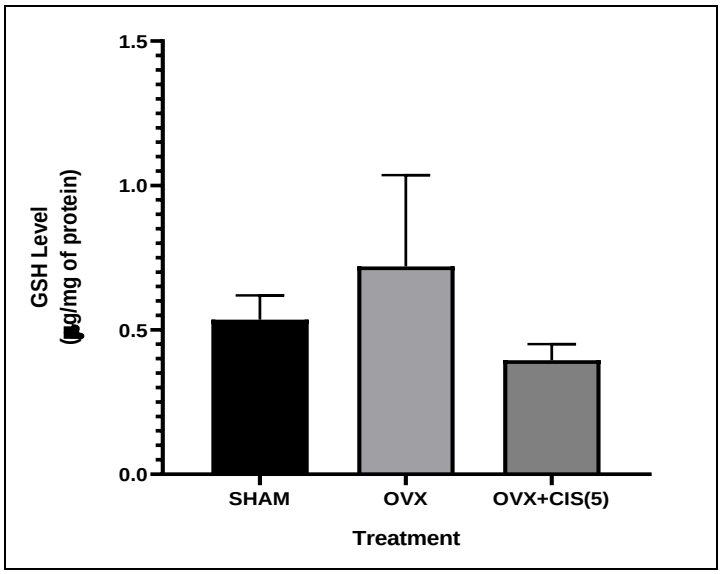


Fig No. 11, 12 Effect of cisplatin on lipid peroxidation in mouse model of perimenopausal mice model:

Data represented as mean ± SEM (n=6) and evaluated using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test; The p value <0.05 is considered as statistically significant. ##p<0.01 is compared with normal group; *p<0.05, is compared with disease control group. The values in parenthesis are doses in mg/kg.CIS- Cisplatin, Daidzein.

Glutathione Estimation:



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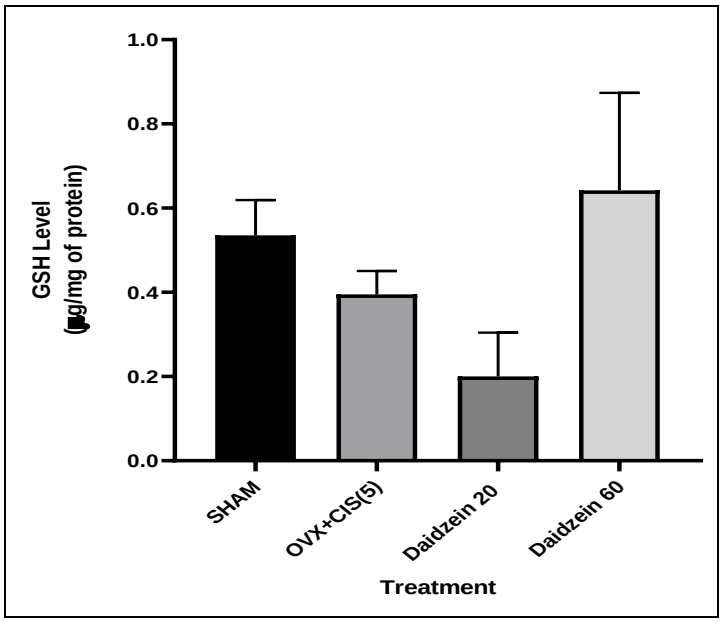
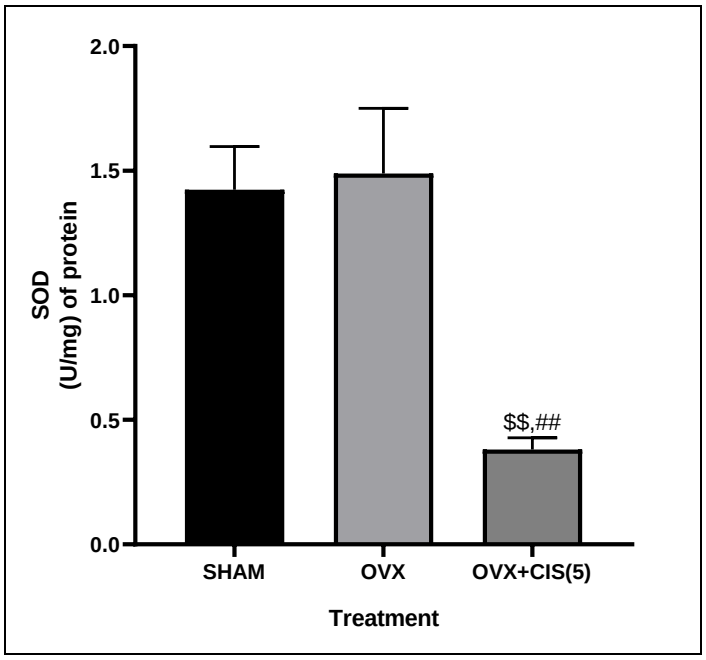


Fig No.13, 14 Effect of cisplatin on glutathione in cisplatin treated perimenopausal mouse model:

Data represented as mean ± SEM (n=6) and evaluated using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test; the p value <0.05 is considered as statistically significant. ##p<0.01 is compared with normal group; *p<0.05, is compared with disease control group. The values in parenthesis are doses in mg/kg.CIS- Cisplatin, Daidzein

Superoxide Dismutase Estimation:



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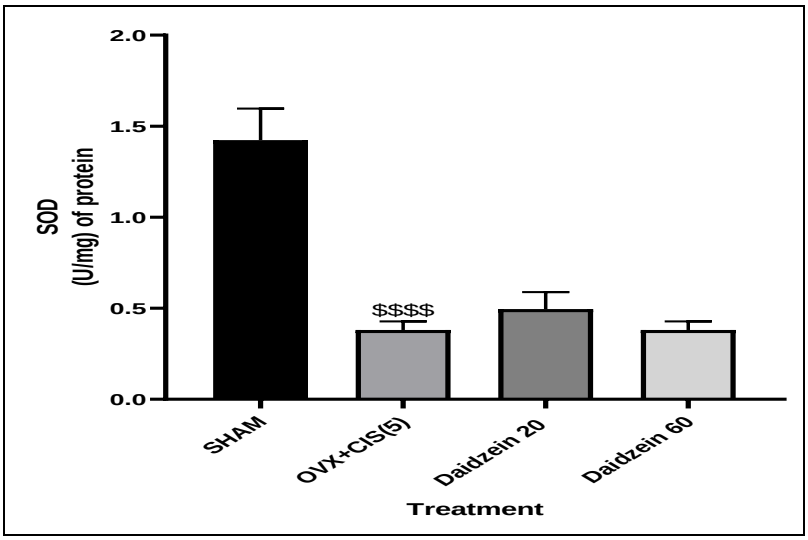

Fig No. 15, 16 Effect of cisplatin on superoxide dismutase in cisplatin treatedperimenopausal mouse model:

Data represented as mean ± SEM (n=6) and evaluated using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test; The p value <0.05 is considered as statistically significant. ##p<0.01 is compared with normal group; *p<0.05, is compared with disease control group. The values in parenthesis are doses in mg/kg.CIS- Cisplatin, Daidzein

V. SUMMARY AND CONCLUSION

While the Diadzein treatment with low and high-dose (20–60 mg/kg) was possessed the significant protective effects against the cisplatin-induced renal damage, more importantly it also increased survival and delayed mortality in cisplatin treated perimenopausal mice model. The observed results of the current research work clearly exhibited the clinical potential of Diadzein as cotreatment with cisplatin for cancer treatment in perimenopausal women to reduce cisplatin induced nephrotoxicity.

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