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Nephroprotective potential of *Cynara scolymus* L. floral extract in cisplatin induced nephrotoxicity in rats

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Abstract
The study was aimed to determine the alterations in antioxidant, biochemical and histopathological parameters in cisplatin (cDDP) induced nephrotoxicity and its protection by treatment with hydro-alcoholic extract of *Cynara scolymus*. Acute nephrotoxicity was induced by cDDP (12 mg/kg) in wistar rats. Nephrotoxic rats were treated with quercetin (50 mg/kg) and hydro-alcoholic floral extract (150 and 300 mg/kg body weight) by oral gavage. Cisplatin treatment elevated (P<0.05) the levels of blood urea nitrogen, creatinine, uric acid, malondialdehyde but lowered (P<0.05) total plasma proteins, total thiols, blood glutathione levels and antioxidant enzymes as compared to the control. Pre and post treatment with plant extract at 150 and 300 mg/kg attenuated the altered levels of various enzymatic and oxidative parameters in blood and renal tissue in a dose dependent manner. The extract attenuated the degenerative and necrotic changes of proximal convoluted tubules induced by cDDP which initiates the good nephroprotective potential of *C. scolymus* extract.

Keywords: Antioxidant, nephroprotective, cisplatin, *Cynara scolymus*

Introduction
Kidney is a major target organ for drug induced toxic effects. Kidneys receive 25 per cent of cardiac output and as one of the major organs of excretion, are naturally exposed to a greater proportion of circulating drugs and chemicals. The use of nephrotoxic drugs frequently leads to acute kidney injury (AKI) which is associated with more and more morbidity and mortality. Nephrotoxicants exert toxic effects on the kidney by one or several mechanisms including altered systemic and local hemodynamics, direct toxic effects on renal cells, inflammation and crystal nephropathy. Acute tubular necrosis is the most common cause of AKI and follows ischemia or nephrotoxic injury to the tubules (Hoitsma *et al*., 1991) [19]. The epithelial cells of renal proximal convoluted tubules (PCT) are a major target for nephrotoxicants due to their roles in glomerular filtrate concentration and drug transport and metabolism. Several drugs *viz*. aminoglycosides, vancomycin, cisplatin and iodinated radiographic contrast agents are widely used despite known evidence of renal injury, PCT toxicity and other systemic toxicity (Uchino, 2006) [38].

A large proportion of medicinal compounds have been discovered with the help of ethno-botanical knowledge of their traditional uses. The rich knowledge base of countries like India and China in medicinal plants and health care has led to the keen interest by various pharmaceutical companies to use this knowledge as a resource for research and development programmes in the pursuit of discovering noble drugs (Krishnaraju *et al*., 2005) [25]. The use of traditional medicine and medicinal plants in most of the developing countries, as a basis for the maintenance of good health has been widely observed (UNESCO, 1996). Moreover, the increased use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs. Traditionally, many medicinal plants and its aerial parts including leaves have been used as an alternative remedy for treatment of various diseases.

Cynara scolymus belonging to family Asteraceae is commonly known by the name of 'Artichoke'. The plant has been reported to have anti-diabetic, hepato-protective, anti-inflammatory properties etc. This plant is well-known for its nutritional and curative properties due to some bioactive components that have antioxidant and antimicrobial activities. In addition, it provides protection against degenerative changes such as cancer. In folk medicine, *C. scolymus* has been widely used as astringent, blood cleanser, cardiotonic, detoxifier, digestive stimulant, diuretic, hypoglycemic and hypocholesterolemic as well as medicine

for liver complaints (Lattanzio *et al*., 2009) [26]. Artichoke leaf extracts have been reported to have hepato-protective, anti-carcinogenic anti-oxidative, anti-bacterial, anti-HIV, bile expelling activities as well as the ability to inhibit cholesterol biosynthesis, and LDL oxidation (Martino *et al*., 1999; Bundy *et al*., 2009; Lattanzio *et al*., 2009) [28, 7, 26]. These variable therapeutic actions of Artichoke cannot be attributed to a single component of the plant and it could be due to the presence of several bio-active components which generate synergistic pharmacological effects.

Cisplatin (cDDP) is widely and efficaciously used for chemotherapy to treat cancers (Einhorn, 2002) [11]. It is platinum based anti-cancerous agent used to treat effectively many carcinomas, sarcomas and lymphomas (Pianta *et al*., 2013) [34]. Cisplatin induced nephrotoxicity primarily occurs in kidney PCT (Karasawa and Steyger, 2015) [23]. Due to the accumulation of cDDP in kidneys, nephrotoxicity is the most common and consistent side effect of cDDP treatment (Oboh *et al*., 2013; Pianta *et al*., 2013) [32, 34]. Treatment with cDDP induces the inflammatory mechanism which leads to reduction in the antioxidant levels, leading to a failure of the antioxidant protection against free radicals damage generated by anti-neoplastic drugs. In turn cDDP disturbs the antioxidant/oxidant balance and its nephropathy is closely associated with an increase in lipid peroxidation (Schmetzer *et al*., 2012; Oboh *et al*., 2013) [37, 32]. Further, experimental and clinical studies have demonstrated that supplementation of natural antioxidants like curcumin (Antunes *et al*., 2001) [4], melatonin (Sener *et al*., 2000), vitamin C (Kadikoylu *et al*., 2004) [22], quercetin (Francescato *et al*., 2004; Behling *et al*., 2006, Verma *et al.,* 2017) [12, 5] etc protect drug induced renal damage in experimental models. Therefore, the present study was aimed to determine the nephroprotective potential of hydro-alcoholic floral extract of *Cynara scolymus* in cisplatin induced nephrotoxicity in wistar rats.

Material and Methods

Collection and Preparation of Extract

The floral part of the plant *Cynara scolymus* was used. The flowers of the selected plant were collected from Pulwama region and Floriculture Park and were identified by Taxonomists, University of Kashmir. After proper identification and deposition of the voucher sample, sufficient fresh floral parts of the plant were collected in polythene bags and transported to laboratory at R.S. Pura, Jammu. In the laboratory, floral parts of *Cynara scolymus* were cleaned with moist cloth and were air dried in shade with temperature not exceeding 40°C for 2-3 weeks prior to extraction process. Dried parts were pre-crushed and later pulverized into fine powder using electric grinder. The dry powder was collected in polythene zip bags and stored in cool dry place. Powdered floral parts were subjected to hydro-alcoholic extraction. These powdered floral parts were weighed and were placed in thimble which was placed in the flask of soxhlet distillation apparatus and the extraction was done with 50% hydro-alcoholic solution. Extractions were done by maintaining hot plate temperature (70-80°C). The final drying was done in a rotatory evaporator. The dried extract was scrapped off and transferred to a glass container and stored in refrigerator under desiccation. The extract (0.1%) was freshly prepared in distilled water for oral administration in *in-vivo* studies in experimental animals.

Nephrotoxicity Induction in Experimental Animals

Healthy wistar rats of either sex weighing 150-200 g obtained from Indian Institute of Integrative Medicine (CSIR lab), Jammu. The animals were provided standard pelleted ration and clean drinking water *ad libitum* and standard management conditions were provided to all the animals. The experiment was conducted on seven groups of rats with six rats in each group. Normal untreated rats (Group I) served as normal control and received only distilled water. Group II received a single intra-peritoneal dose of cisplatin (cDDP) @ 12 mg/kg BW. Group III and IV received hydro-alcoholic extract of two doses *viz*. 150 and 300 mg/kg BW orally. In Group V and VI, the extracts were given @150 and 300 mg/kg BW 1h prior and 24h and 48h after cDDP administration. In Group VII, single intra-peritoneal dose of quercetin @ 50 mg/kg BW was given, 6h before cDDP administration. The dose of plant extract was determined on the basis of the reported toxic dose and other pharmacological activities (Khattab *et al*., 2016; Najim *et al*., 2018) [24, 31]. The experimental protocol was dully approved by Institutional Animal Ethics Committee (IAEC) vide proposal no 7/IAEC-17/2017.

Collection and Processing of Samples

After 72h of cDDP administration, blood samples were collected from retro-orbital fossa in a sterilized tube containing heparin. Animals were sacrificed and renal tissue (1g) was collected in ice cold phosphate buffer solution (10 ml) (0.5 M, pH 7.4). The blood samples were centrifuged at 3000 rpm for 10 minutes and plasma was collected in glass vials and stored at -4°C for the estimation of biochemical parameters and oxidative stress parameters on same day. Tissue sample was homogenized using Teflon coated homogenizer at 1000 rpm for 5-7 minutes and 10% tissue homogenate was prepared.

Assaying of Antioxidant Parameters

Total thiols (TTH) level was determined in plasma and renal tissue as per the methods described by Prakash *et al*., 2009 [35]. In brief, reaction mixture contained 900µl of EDTA (ethylene diamine tetra acetic acid) (2mM in 0.2 M Na_2HPO_4), 20µl of DTNB (5-5'- dithiobis, 2-nitrobenzoic acid) (10mM in 0.2 M Na_2HPO_4) and 100µl of fresh plasma or tissue homogenate. The reaction mixture was incubated at room temperature for 5 minutes and the absorbance was read at 412nm in UV visible spectrophotometer. A reagent blank without sample and sample blank without DTNB were prepared in the same manner. Concentration of total thiols (mM) was determined

using standard (Motchink *et al*., 1994) [30]. Similarly, malondialdehyde (MDA) levels in erythrocyte lysate or tissue homogenate were determined. 1ml of 10% trichloroacetic acid was added in erythrocyte lysate or tissue homogenate. After vortexing the mixture was centrifuged at 3000 rpm for 10 minutes. To 1 ml of supernatant, 1ml of 0.67% thiobarbituric acid was added and was kept in boiling water bath for 10 minutes. Then it was cooled and diluted with 1 ml of distilled water. The blank was prepared by adding all the reagents except 1 ml of sample substituted with equal volume of distilled water. The absorbance was read at 535nm in UV-visible spectrophotometer. The amount of lipid peroxidation was expressed as nmole of MDA formed ml/h in blood and in renal tissue nmole MDA formed g in tissues/h. The other antioxidant enzymatic activities *viz* Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GP_X), glutathione-S-transferase (GST) and glutathione reductase (GR) were determined in blood and renal tissue using standard methods (Aebi *et al*., 1983; Marklund *et al*., 1974;

Hafeman *et al*., 1974; Habig *et al*., 1974 and Carlberg *et al*., 1985) [1, 27, 16, 8]. The level of reduced blood glutathione was determined as per the standard method of Beutler (Beutler, 1975).

Assaying of Blood Biochemical Parameters

Biochemical parameters like blood urea nitrogen (BUN), creatinine (CR), uric acid (UA), total plasma proteins (TP), albumin (ALB) were determined in different groups by standard kits (Transasia Bio-Medicals Ltd, India) using Chemistry Analyzer (CHEM-7, ERBA, Mannheim).

Histopathological studies

The histopathological studies of the renal tissues were carried out according to standard method (Drury and Wallington, 1980) [10]. Briefly, a small piece of tissue was immediately fixed in 10% formalin. These formalin fixed tissues were embedded in paraffin sectioned, stained with hematoxylin and eosin (H & E) and examined under a light microscope for histopathological assessment.

Statistical Analysis

The antioxidant and biochemical parameters were depicted as mean± standard error. The results were subjected to One- way analysis of variance (ANOVA) using completely randomized design (CRD) with statistical significance at $P<0.05$ being tested using the Duncan Multiple Range Test.

Results and Discussion

Cisplatin induced nephrotoxicity is mainly occur due to release of platinum containing intermediate compounds during its metabolism once cisplatin enters the cells through organic cation transporter 2 (OCT2) and copper transporter. These intermediates compounds are highly active and causes injury to nuclear and mitochondrial DNA, activation of cell apoptosis, stimulation of inflammatory responses and necrosis (Fumie *et al*., 2000; Pabla and Dong, 2008 and Ronald *et al*., 2010) [13, 33, 36]. Due to the accumulation of cDDP in kidneys, nephrotoxicity is the most common and consistent side effect of cDDP treatment (Oboh *et al*., 2013; Pianta *et al*., 2013) [32, 34]. Alterations in the plasma BUN, CR, UA, TP and ALB levels after administration of hydroalcoholic floral extract of *Cynara scolymus* in cDDP induced acute nephrotoxicity are depicted in table 1. Single intra-peritoneal cDDP administration increased ($P<0.05$) the levels of BUN, CR and UA after 72h exposure indicating renal impairment. Increased level of BUN and CR are seen due to increased level of protein catabolism in mammalian body and also from either increased breakdown of tissue or dietary protein or impaired excretion. Treatment with quercetin, a potent free radical scavenger and a metal chelator in cDDP induced nephrotoxicity decreased the alterations in BUN and CR. Involvement of free radicals in the pathogenesis of cDDP induced nephrotoxicity have been reported in different experimental models (Chirino *et al*., 2008; Jariyawat *et al*., 2009) [20]. Administrations of floral extract at both the doses in cDDP exposed rats, lowered ($P<0.05$) the BUN and CR levels. At higher dose, the BUN and CR levels were restored to normal levels and these values were not significantly different from normal control group. The study also observed that treatment with cDDP lowered total plasma proteins and albumin levels as compared to control group. The reduction may be due to decreased synthesis or increased metabolism of plasma proteins like hemoglobin and signaling proteins (Ali *et al*., 2006) [3]. Treatment with extract at both the doses increased the levels of TP and ALB but the values were not significantly different from the normal control group. Administration of cDDP increased the levels of UA ($P<0.05$) as compared to control but the administration of floral extract of *Cynara scolymus* in cDDP administered rats at both the doses decreased ($P<0.05$) the levels and at higher dose, the UA levels were restored to normal but the values were not significantly different from the normal control groups. Uric acid is a well known low molecular weight water soluble plasma antioxidant and its concentration in plasma is almost 10 fold higher than other antioxidants such as vitamin C or vitamin E. Increased level of UA contributes to plasma antioxidant potential, thus introducing possible confounding factor in the measurement of total antioxidant capacity (Maxwell *et al*., 1993) [29].

Table 1: Effect of *Cynara scolymus* floral extract on renal biomarkers in the plasma of cDDP induced nephrotoxic rats

Groups	BUN	CR	UA	TP	ALB
Normal control	42.19^{a}±3.76	0.70^{a}±0.03	3.53^{a}±0.29	7.11^{c}±0.39	3.04^{a}±0.29
Cisplatin @12mg/kg	169.32^{c}±24.52	2.02^{b}±0.25	8.44^{d}±0.22	5.70^{a}±0.25	2.76^{a}±0.13
Extract @150mg/kg	38.33^{a}±2.71	0.82^{a}±0.14	3.43^{a}±0.50	6.18^{abc}±0.21	2.95^{a}±0.37
Extract @300mg/kg	47.56^{a}±2.69	0.80^{a}±0.05	3.73^{ab}±0.36	6.78^{bc}±0.28	3.25^{a}±0.43
Extract @150mg/kg + Cisplatin @12mg/kg	79.96^{b}±7.38	0.92^{a}±0.07	6.62^{c}±0.27	6.04^{ab}±0.32	2.87^{a}±0.18
Extract @300mg/kg + Cisplatin @12mg/kg	68.63^{ab}±4.68	0.79^{a}±0.05	4.25^{ab}±0.22	6.64^{abc}±0.34	3.11^{a}±0.22
Quercetin @50mg/kg + Cisplatin @12mg/kg	93.61^{b}±7.47	1.08^{a}±0.25	4.58^{b}±0.24	5.94^{ab}±0.33	2.88^{a}±0.21

Values are given as mean ± SE of 6 animals unless otherwise stated

Values having different superscripts (a, b, c, d, e) in a column are statistically different from one another at 5 % level of significance

Values of BUN (blood urea nitrogen), CR (creatinine), TP (total proteins), albumin (ALB) and uric acid (UA) are expressed in mg/dl.

Antioxidant system of renal tissue

Alterations in the levels of non-enzymatic parameters *viz*. TTH and MDA in renal tissue are shown in table 2 where as activities of CAT, SOD, GP_X, GST and GR in renal tissue of different groups are presented in table 3. Treatment with cDDP decreased ($P<0.05$) the levels of TTH, CAT, SOD, GP_X and GST where as increased the levels of MDA but non-significant decreased change was observed in levels of GR. Administrations of *C. scolymus* floral extract raised the levels of TTH, SOD, CAT, GP_X and GR and these values were significantly similar to the normal control group. Higher dose (300 mg/kg BW) was more effective in normalizing the altered levels. MDA levels were decreased by the administration of floral extract of *Cynara scolymus* and high dose was more effective in normalizing the increased levels. However, significantly ($P<0.05$) decreased levels of high dose of extract (300 mg/kg BW) in MDA and TTH were observed when compared with cDDP group alone. Further, significantly ($P<0.05$) increased levels of CAT, SOD (higher dose), GP_X and GST were observed in treatments with extract

in cDDP exposed rats. Treatment with quercetin in cDDP administered rats restored the levels of CAT, SOD, TTH, MDA, GP_X, GST and GR. The extract of *Cynara scolymus* is a rich source of polyphenols, flavanoids, tannins, carotenoids, etc which are having ability to scavenge free radicals like superoxide, hydroxyl and other free radicals (Lattanzio *et al*., 2009) [26]. The increased lipid peroxidation may be resultant of excessive generation of free radicals or reduced free radicals scavenging capacity of tissues. Chiefly, the hydroxyl radical and to a lesser extent superoxide anion leads to peroxidation of membrane lipids thereby causing production of malondialdehyde (MDA) and 4-hydroxyalkenals. These substances directly induce renal tissue damage with generation of pro-inflammatory cytokines, activation of spindle cells and fibrinogenesis (Galal *et al*., 2012) [14] thus lead to membrane damage, protein damage, enzyme dysfunction and damage to DNA or RNA (Afroz *et al*., 2014) [2]. In the present study, activities of SOD and CAT were reduced in cDDP treated rats which may be due to increased production of superoxide and peroxide radicals due to impaired mitochondrial respiratory chain reaction by cDDP. Studies also suggested that cDDP exposure decreased the activity of Cu-Zn SOD while enhancing Mn-SOD suggesting role of mitochondria in excessive production of ROS/ free radicals in cytosolic fraction (Uriu *et al*., 2005) [40]. Reductions in SOD and CAT activities in rat kidney were observed suggesting that cDDP induced nephrotoxicity resulted from excessive generation of ROS/ free radicals. It is assumed that superoxide generated by cDDP is converted by SOD to H_2O_2, resulting in the reduced SOD activity. Catalase is a heme- containing enzyme that results in the dismutation of hydrogen peroxide into water and oxygen. The enzyme is found in all aerobic eukaryotes and is important in the removal of hydrogen peroxide generated in peroxisomes (microbodies) by oxidases which are involved in β- oxidation of fatty acids, the glyoxylate cycle (photo-respiration) and purine catabolism. Stress conditions in which there is a large free radical generation results in depletion of Catalase activity (Hertwig and Feirabend, 1992) [18]. Blood GSH acts as co-factor for GST and GP_X. GSTs are a major group of enzymes that constitute 10 per cent of the cytosolic protein in some mammalian organs. GST catalyses the conjugation of reduced glutathione via sulfhydryl group to electrophilic centres on a wide variety of substances. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids. This catalytic activity of combined glutathione with electrophiles helps in excretion of toxicant from the cells and protects the tissue from oxidative stress (Hayes and Paiford, 1995) [17]. Glutathione peroxidase is a selenium containing enzyme which reduces hydrogen peroxide forming GSSG and thereby serves as alternative means of detoxifying activated oxygen. Thus, the reduced activities of GP_X and GST may be due to declined level of GSH, required for metabolism of free radicals.

Table 2: Effect of *Cynara scolymus* floral extract on Non-enzymatic Antioxidant parameters in the renal tissue of cDDP induced nephrotoxic rats.

Groups	TTH	MDA
Normal control	3.29^{cd}±0.20	26.97^{ab}±3.96
Cisplatin@12mg/kg	1.92^{a}±0.09	42.88^{c}±4.34
Extract@150mg/kg	3.25^{cd}±0.28	21.13^{a}±2.28
Extract@300mg/kg	3.45^{d}±0.11	24.54^{a}±2.42
Extract@150mg/kg+ Cisplatin @12mg/kg	2.57^{b}±0.09	37.12^{bc}±4.15
Extract@300mg/kg+ Cisplatin @12mg/kg	3.38^{cd}±0.18	28.70^{ab}±2.97
Quercetin@50mg/kg+ Cisplatin@12mg/kg	2.93^{bc}±0.07	31.10^{ab}±3.19

Values are given as mean ± SE of 6 animals unless otherwise stated
Values having different superscripts (a, b, c, d & e) in a column are statistically different from one another at 5 % level of significance
Values of TTH (total thiols) are expressed in Mm
Values of MDA (malondialdehyde) level are expressed in nmoles MDA produced / g of tissue/ hr.

Table 3: Effect of *Cynara scolymus* floral extract on enzymatic antioxidant parameters in the renal tissue of cDDP induced nephrotoxic rats.

Groups	CAT	SOD	GP_X	GST	GR
Normal control	3086.57^{b}±172.66	340.27^{b}±36.25	41.94^{b}±5.01	8.28^{b}±0.91	35.45^{a}±5.84
Cisplatin@12mg/kg	1201.12^{a}±210.80	233.93^{a}±21.29	25.14^{a}±3.74	4.20^{a}±0.36	26.11^{a}±3.36
Extract@150mg/kg	2969.78^{b}±399.24	347.19^{b}±40.05	40.71^{b}±5.49	8.38^{b}±0.95	32.93^{a}±4.28
Extract@300mg/kg	3092.17^{b}±126.14	341.05^{b}±20.89	43.29^{b}±5.83	8.48^{b}±1.41	33.74^{a}±3.31
Extract@150mg/kg + Cisplatin@ 12mg/kg	2863.06^{b}±272.70	279.73^{ab}±26.04	33.14^{ab}±2.92	5.73^{ab}±0.52	33.48^{a}±5.46
Extract@300 mg/kg + Cisplatin @12mg/kg	2965.68^{b}±315.88	347.73^{b}±36.81	39.38^{ab}±4.68	7.45^{ab}±0.53	34.8^{a}±4.49
Quercetin@50mg/kg + Cisplatin@12 mg/kg	3186.59^{b}±180.95	316.02^{ab}±31.63	35.93^{ab}±4.76	7.08^{ab}±0.80	29.74^{a}±4.39

Values of CAT (catalase) are expressed in mol H_2O_2 decomposed /min/g tissue
Values of SOD (Superoxide dismutase) and GPx (glutathione peroxidase) are expressed in Unit/g of tissue
Values of GST (glutathione S transferase) are expressed in µmol of CDNB conjugate formed/ min/g of tissue
Values of GR (glutathione reductase) are expressed in µmol of NADPH/min

Antioxidant system of Blood

Alterations in the level of TTH, GSH and MDA are presented in Fig 1. cDDP treatment in rats decreased (P<0.05) the levels of GSH, CAT, GST and GP_X and increased (P<0.05) the levels of MDA but non-significant change was observed in SOD, TTH and GR. Pre and post administration of floral extract in cDDP exposed rats increased (P<0.05) the levels of GSH, higher dose was more effective but it did not differ significantly from the normal control group. Total thiols of plasma comprise of non protein thiols (predominantly GSH) and protein thiols (Protein-SH) and are the primary site for the attack of free radicals. Blood GSH has multiple functions in

living organisms. It acts as a carrier of an active thiol group in the form of a cysteine residues and also acts as an antioxidant either directly by interacting with ROS/RNS or by acting as an essential cofactor for GST and GP_X. Similarly, the levels of TTH, GP_X and GST were increased after administration of *C. scolymus* floral extract but these were not significantly different from normal control group. The MDA levels were decreased after extract administration but the values were not significantly different from normal control group. High dose was more effective in normalizing the levels. But the extract failed to normalize the CAT levels. Treatment with quercetin with cDDP restored the levels of GSH, MDA, TTH, GP_X, SOD and GR. Fig.2 depicts the alterations in the activities of SOD, GST and GP_X on administration of extract and the activities were decreased in cDDP administered rats.

Phytochemicals have potent direct anti-oxidant potential by scavenging free radicals (Volko *et al*., 2007) [41] and indirectly by boosting the antioxidant defense of host (Verma *et al*., 2015) [42]. Most of the pharmacological activities of plant *viz* anti-diabetic, nephroprotective, hepatoprotective etc reside in these phytochemicals, which have been proven in various experimental and clinical studies (Wang *et al*., 2003; Colak *et al*., 2016; Khattab *et al*., 2016 and Najim *et al*., 2018) [45, 9, 24, 31]. In the present study, nephroprotective potential of *C. scolymus* may be due to presence of total phenols, flavonoids, tannins, β-carotene in plant extract (Lattanzio *et al*., 2009) [26]. These ingredients protect cellular oxidative damage on erythrocyte membrane, probably because of high scavenging potential of ROS/ free radicals. Various experimental studies have also suggested that supplementation of natural dietary products endowed with high antioxidant potential have protective effects against cDDP induced nephrotoxicity in experimental animals (Chirino *et al*., 2008; Joy *et al*., 2008 and Verma *et al*., 2016) [21]. Further in clinical trials, supplementation of dietary antioxidants has shown the protection against cDDP induced acute nephrotoxicity.

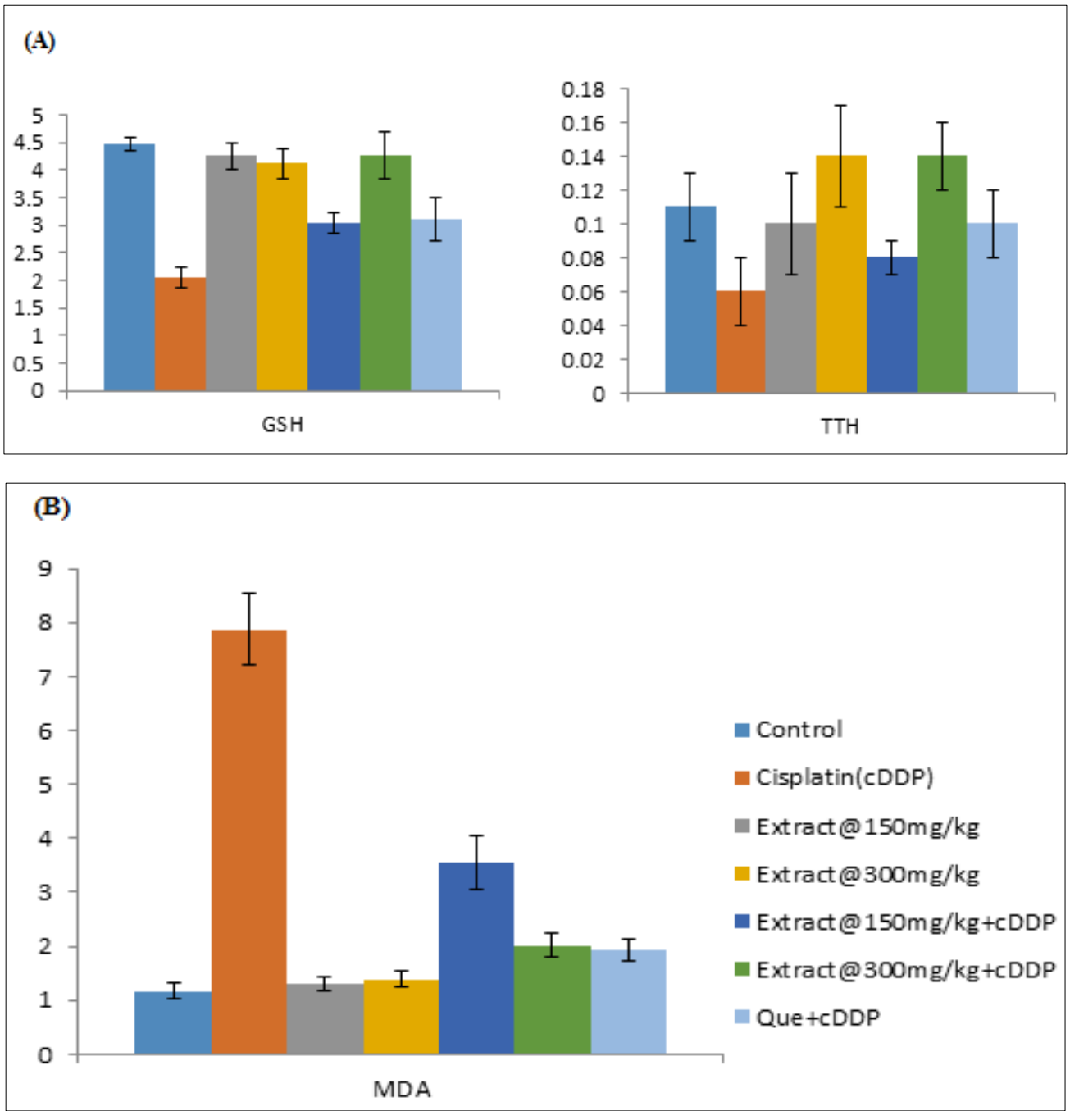


Fig 1: Effect of *C. scolymus* floral extract on (A) blood Glutathione (GSH) and total thiol (TTH) (B) malondialdehyde (MDA) in blood of cDDP induced nephrotoxic rats.

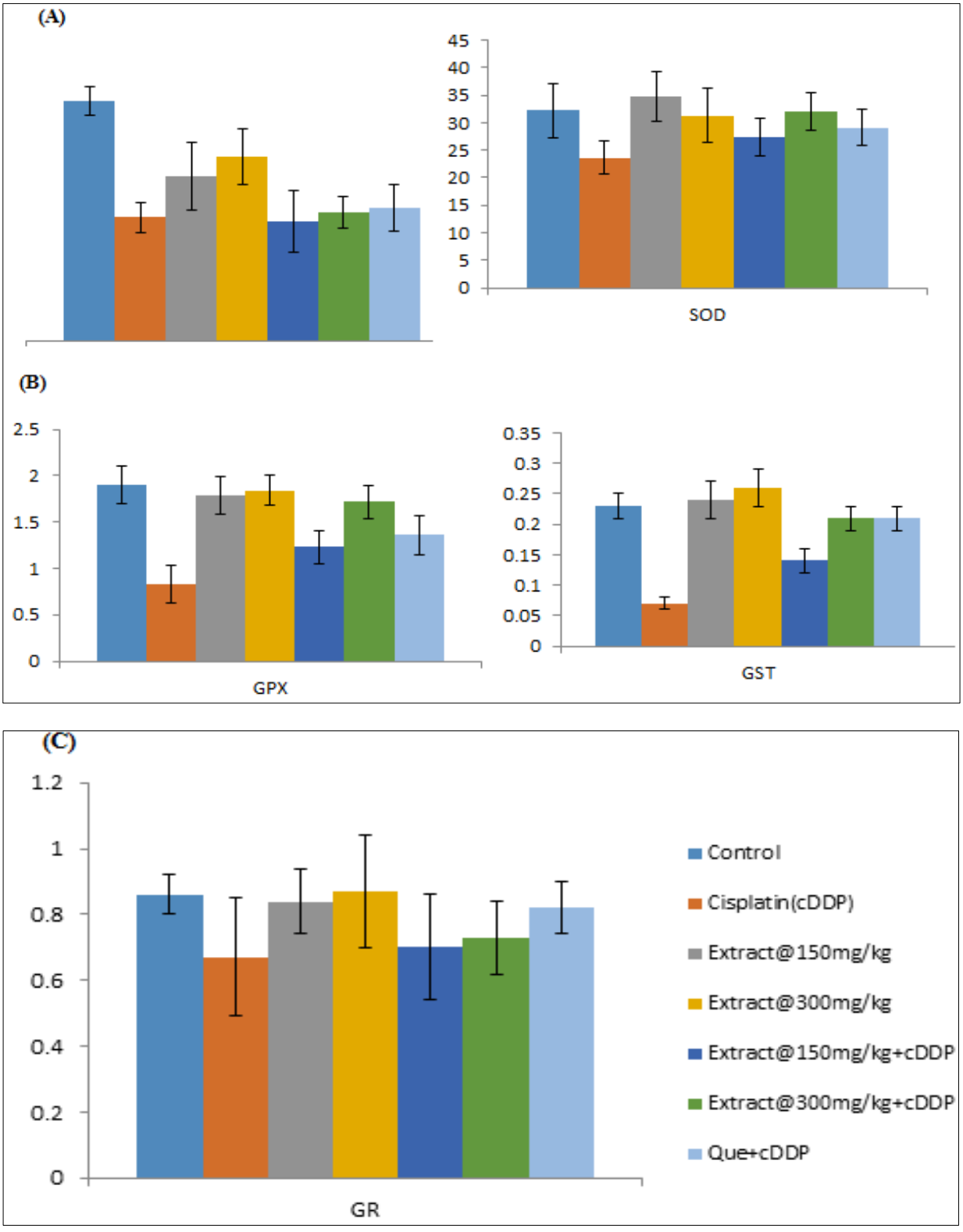
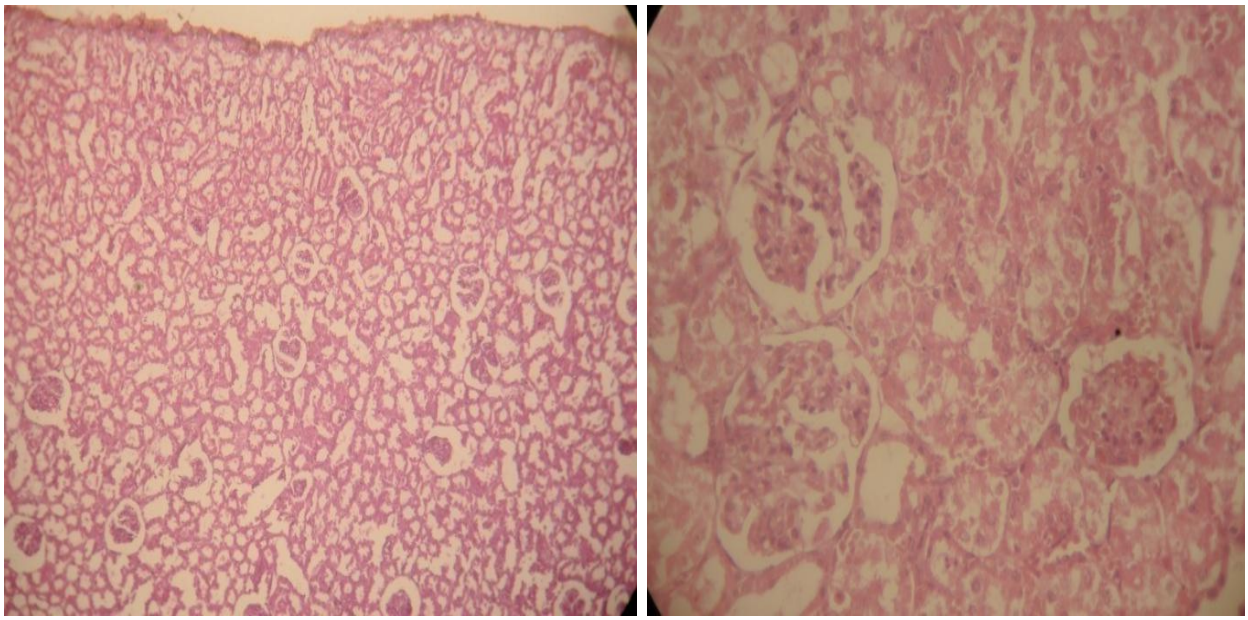


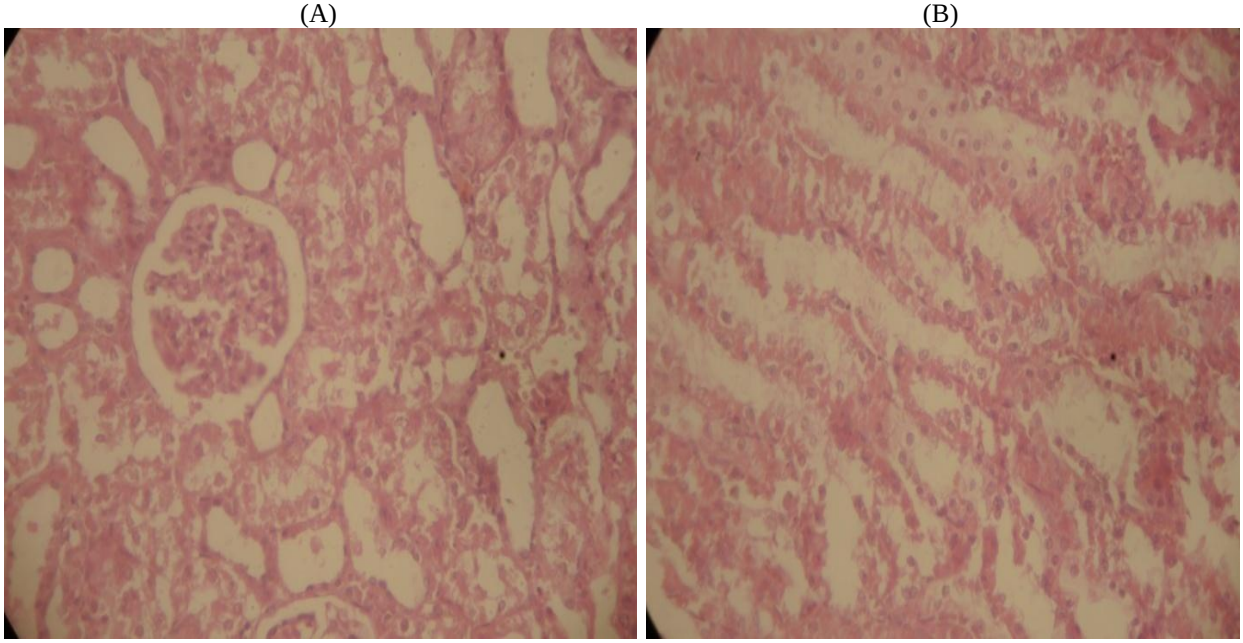
Fig 2: Effect of *C. scolymus* floral extract on activities of (A) Catalase (CAT). Superoxide dismutase (SOD) and (B) Glutathione peroxidase (GPX), Glutathione-s-transferase (GST) and (C) Glutathione reductase (GR) in blood of cDDP induced nephrotoxic rats.

Histopathological changes in kidney

Fig 3 depicts the histopathological changes in renal tissue of cDDP induced nephrotoxic rats. Kidney of the normal control animals did not show any significant histopathological change. Intra-peritoneal administration of cDDP in wistar rats induced congestion in capillaries with marked increase in Bowman's space. Degeneration of some proximal convoluted tubular epithelium with loss of nuclei and detachment from basement membrane, dilatation of distal convoluted tubules with degeneration of collecting tubular epithelium of the renal medulla was also observed by cDDP administration. The normal animals which were provided exclusively extract @ 150 and 300 mg/kg BW showed normal renal architecture. Pre and post administration of extract @ 150 and 300 mg/kg BW in cDDP administered rats showed congestion of the glomerulus with increase in Bowman's space and mild proximal convoluted tubular epithelial degeneration. The nephrotoxic animals administered with standard antioxidant (quercetin) showed that glomerular space is nearly normal. The blood vessels appear congested with degeneration and vacuolation of proximal tubular epithelium. There is complete lysis of some cells and dilatation of distal convoluted tubule.

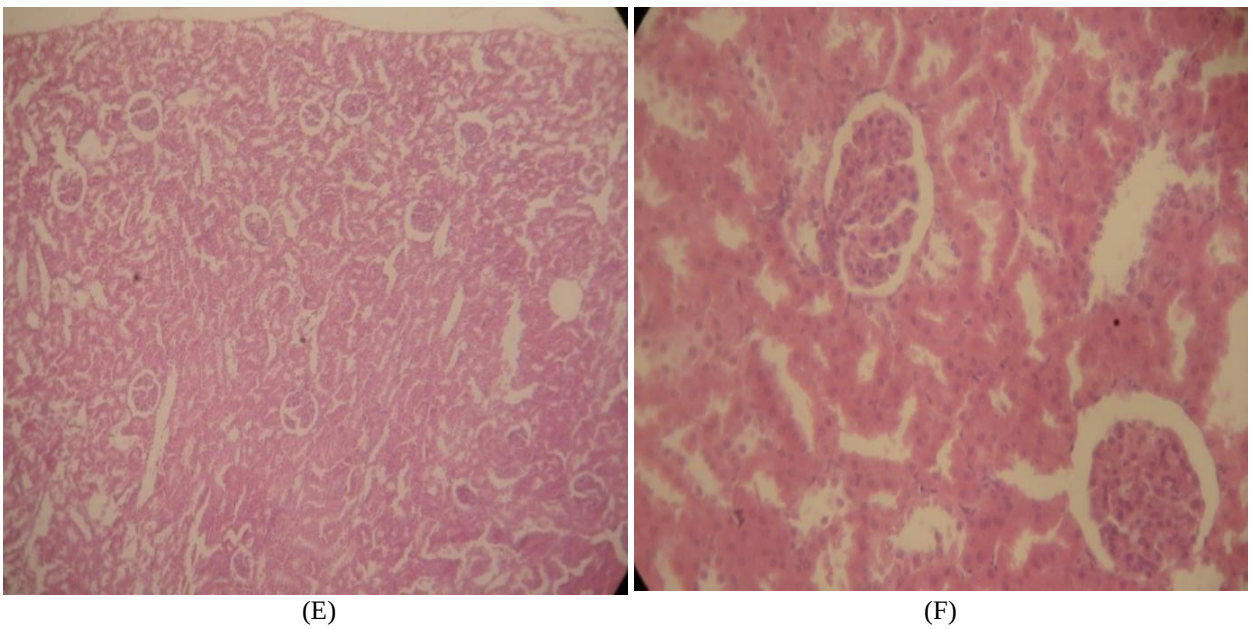


(A) (B)

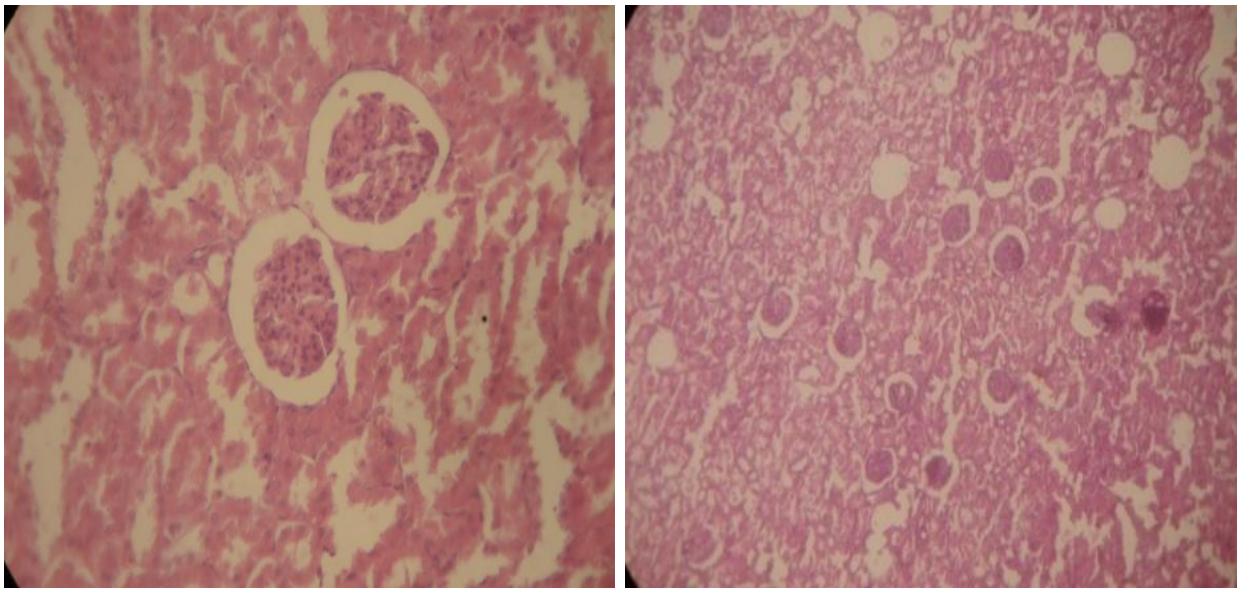


(C) (D)

Fig 3: Histomicrograph of H & E stained sections of the formalin fixed kidney: normal kidney (A), alteration in renal Histomicrograph on cisplatin treated rats (B, C, D).



(E) (F)



(G) (H)

Fig 4: Histomicrograph of H & E stained sections of the formalin fixed kidney on treatment of extract @ 150 & 300 mg/kg BW (E, F) alone and along with cisplatin (G and H) in wistar rats.

Conclusion

Observations of the study suggests that reduced TTH, GSH and anti-oxidant enzymes and increased MDA levels in blood and renal tissue indicated reduction in antioxidant defense system on cDDP administration leading to free radicals induced acute renal damage as indicated in microscopic observation in wistar rats. Administration with hydro-alcoholic floral *C. scolymus* extract minimized the cDDP induced renal damage as indicated by reduced MDA levels by restoring the disturbance in antioxidant system (increased TTH, GSH, CAT, SOD, GP_X, GST and GR) of blood and renal tissue during cDDP treatment in animals.

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