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Cisplatin induced nephrotoxicity and its amelioration with eugenol in wistar rats

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Abstract
This study investigated cisplatin-induced nephrotoxicity and eugenol's potential ameliorative effects in 40 Wistar rats (20 male, 20 female) randomly assigned to four groups. Group I (control) received distilled water and saline; Group II received eugenol (10 mg/kg, oral, 28 days); Group III received cisplatin (6.5 mg/kg, intraperitoneal, on day 21); Group IV received both. Groups III and IV exhibited reduced food intake, lethargy, oliguria, diarrhea, and significant ($P<0.05$) reductions in body weight, RBC, Hb, HCT, MCV, platelets and total protein, with elevated BUN, creatinine and uric acid. Organ weight changes included decreased liver, spleen, testes/ovaries and thymus, with increased adrenal (males) and kidney (females) weights. Histopathology revealed renal tubular damage, sperm and lymphoid depletion in Groups III and IV. Immunohistochemistry showed increased CYP2E1 expression in Group IV. Eugenol did not attenuate cisplatin-induced clinical, hematological, biochemical, or histopathological changes.

Keywords: Cisplatin, nephrotoxicity, eugenol, histopathology, CYP2E1

1. Introduction
Cancer poses a significant public health burden in India, driven by lifestyle factors, a prolonged latent phase, and the need for specialized treatment infrastructure. Environmental carcinogens physical (e.g., ultraviolet and ionizing radiation), chemical (e.g., asbestos, tobacco smoke components, aflatoxin, arsenic), and biological (e.g., viral, bacterial, or parasitic infections) account for 70-90% of cancer cases, interacting with genetic predispositions to initiate oncogenesis [1]. In 2020, global cancer incidence reached 19.29 million cases, with 9.9 million deaths. India, with a population of 1.39 billion, reported 1.32 million cases, predominantly breast (14.3%), cervical (12.1%), lip-oral cavity (7.6%), lung (6.9%), and colorectal (6.3%) cancers [2].
Chemotherapy and radiotherapy are primary cancer treatments, targeting rapidly proliferating malignant cells. However, chemotherapeutic agents like cisplatin lack specificity, damaging healthy cells and causing adverse effects [3]. Cisplatin, a platinum-based drug developed in 1845 by Michele Peyrone and recognized for its antineoplastic properties in 1965 by Dr. Barnett Rosenberg, is used in approximately 50% of cancer patients, generating $2 billion in global sales [4]. Effective against solid tumors (e.g., testicular, ovarian, bladder, lung, cervical, head and neck, gastric), cisplatin induces cytotoxicity via DNA adducts, forming mono-, inter, and intrastrand cross-links that arrest the cell cycle at S, G1, or G2-M phases, triggering apoptosis [5]. Its efficacy is limited by dose-dependent toxicities (nephrotoxicity, ototoxicity, hepatotoxicity, gastrointestinal toxicity, neurotoxicity) and drug resistance.
Nephrotoxicity, affecting 5-40% of patients after a single cisplatin dose, is a major dose-limiting toxicity [6]. The kidneys, critical for cisplatin excretion via tubular secretion and glomerular filtration, accumulate the drug at fivefold higher concentrations in proximal tubular epithelial cells compared to plasma [7]. Biotransformation into toxic cysteinyl glycine conjugates and thiols causes proximal tubular damage, impairing water and sodium reabsorption, followed by distal tubular dysfunction, reduced renal blood flow, and glomerular

filtration. This results in elevated urinary excretion of proteins, enzymes, and electrolytes (e.g., potassium, magnesium) [8].

Mitigating cisplatin-induced nephrotoxicity could enhance its therapeutic potential. Synthetic drugs and antioxidants are under investigation, with phytoconstituents, particularly phenolic compounds, gaining attention for their antioxidant properties. Phenolic compounds (e.g., ellagic, caffeic, ferulic, syringic acids) act as reducing agents, hydrogen donors, and singlet oxygen quenchers, scavenging free radicals [9]. Eugenol, an allyl chain-substituted guaiacol found in clove oil, cinnamon, holy basil, and nutmeg, exhibits antioxidant, anti-mutagenic, anti-genotoxic, and anti-inflammatory properties [10]. Present in essential oils of plants like *Eugenia caryophyllus* and *Ocimum sanctum*, eugenol functions as a free radical terminator, metal chelator, and singlet oxygen quencher [11]. Its potential to ameliorate cisplatin-induced nephrotoxicity, however, remains underexplored.

Given the limited understanding of eugenol's protective effects, the present study, "Cisplatin-Induced Nephrotoxicity and Its Amelioration with Eugenol in Wistar Rats," investigates eugenol's efficacy in mitigating cisplatin's nephrotoxic effects in a Wistar rat model. The study aims to elucidate clinical, hematological, biochemical, and histopathological outcomes, addressing the paucity of data on eugenol's nephroprotective potential and informing strategies to optimize cisplatin's therapeutic index in cancer treatment.

2. Material and Method

2.1 Location

The study was conducted at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar-385506, Gujarat, India, to evaluate cisplatin-induced nephrotoxicity and eugenol's ameliorative effects in Wistar rats.

2.2 Institutional Animal Ethics Committee (IAEC) Approval: The protocol, "Cisplatin-induced nephrotoxicity and its amelioration with Eugenol in Wistar rats," was approved as VETCOLL/IAEC/2022/19/PROTOCOL-06 on 09/01/2022 by the Institutional Animal Ethics Committee (IAEC) of Kamdhenu University, Sardarkrushinagar adhering to CPCSEA guidelines.

2.3 Animal Procurement

Forty Wistar rats (20 male, 20 female) were sourced from Cadila Pharmaceuticals, Dholka, Gujarat, India, and acclimatized for 8 days before the experiment.

2.4 Housing and Environmental Conditions

Rats were housed in polypropylene cages with corncob bedding in an environmentally controlled room (22 ± 3°C, 30-70% humidity, 12/12-hour light/dark cycle). Animal management followed CPCSEA guidelines to minimize stress.

2.5 Feeding and Identification

Rats had ad libitum access to standard pellet feed (VRK Nutritional Solutions, Sangli, Maharashtra; 20-21% crude protein, 4-5% ether extract, 4% crude fiber, 8% ash, 1.2% calcium, 0.6% phosphorus, 54% nitrogen-free extract, 3600 kcal/kg metabolizable energy, 12 mm pellet size) and water. Identification used tail markings (1-4 rings or none for rats 1-5) and cage labels, with five rats per cage.

2.6 Test Compounds: Cisplatin (Platinex-50, $PtCl_2(NH_3)_2$, 300.05 g/mol, yellowish-white powder, 0.253 g/100 g water solubility at 25°C; Khandelwal Laboratories, Mumbai) and eugenol (clear pale yellow liquid, 162.2 g/mol, CAS 97-53-0, slightly water-soluble, soluble in DMSO, alcohol, acetic acid, chloroform; Sigma Aldrich, China) were used.

2.7 Experimental Design

Forty Wistar rats (20 male, 20 female) were equally divided into four groups, each consisting of 5 male and 5 female rats. Group I (Control) received distilled water orally (p.o.) daily for 28 days and a single intraperitoneal (i.p.) dose of normal saline on day 21. Group II was administered eugenol (50 mg/kg body weight, p.o.) daily for 28 days. Group III received a single dose of cisplatin (6.5 mg/kg body weight, i.p.) on day 21. Group IV was treated with eugenol (50 mg/kg body weight, p.o.) daily for 28 days combined with a single dose of cisplatin (6.5 mg/kg body weight, i.p.) on day 21.

2.8 Clinical Observations

Morbidity, mortality, and behavioral changes were monitored twice daily during the 28-day experiment and once daily during acclimatization.

2.9 Body Weight

Body weights were recorded on days 0, 7, 14, 21, and 28 using an analytical balance.

2.10 Blood Collection, Necropsy, and Organ Weight

2.10.1 Blood: On day 29, blood was collected under mild isoflurane anesthesia from the retro-orbital plexus using heparinized capillary tubes into 4% K_3EDTA vials for hematology and serum clot activator vials for biochemistry. Giemsa-stained blood smears were prepared within 3 hours for platelet, erythrocyte morphology, and leukocyte counts.

2.10.2 Necropsy

Rats were fasted overnight, euthanized by cervical dislocation on day 29, and subjected to detailed necropsy. Terminal body weights were recorded before necropsy.

2.10.3 Organ Weight

Organs (liver, kidneys, lungs, heart, brain, spleen, adrenals, thymus, ovaries, epididymides, testes) were weighed for absolute values, and relative weights were calculated based on terminal body weight.

2.11 Clinical Pathology

2.11.1 Hematology: The Exigo Haematology Analyzer (Boule Medical AB, Sweden), utilizing the impedance method, analyzes a comprehensive set of hematological parameters, including Total Leukocyte Count (TLC, $10^3/\mu L$), Differential Leukocyte Count (DLC) comprising Lymphocytes (%), Monocytes (%), Neutrophils (%), Eosinophils (%), and Basophils (%), Hemoglobin (Hb, g/dL), Total Erythrocyte Count (TEC, $10^6/\mu L$), Packed Cell Volume (PCV/HCT, %), Mean Corpuscular Volume (MCV, fL), Mean Corpuscular Hemoglobin (MCH, pg), Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL), Platelets (PLT, $10^3/\mu L$), and Mean Platelet Volume (MPV, fL).

2.11.2 Biochemical Profile

The RANDOX-RX Monaco Analyzer (United Kingdom), utilizing Randox reagent kits, measures a comprehensive

panel of biochemical parameters, including Urea (mg/dL), Alanine Aminotransferase (ALT, U/L), Aspartate Aminotransferase (AST, U/L), Alkaline Phosphatase (ALP, U/L), Gamma-Glutamyltransferase (GGT, U/L), Total Protein (TP, g/dL), Albumin (g/dL), Creatinine (mg/dL), Cholesterol (mg/dL), Glucose (mg/dL), Calcium (mg/dL), Phosphorus (mg/dL), Iron (µg/dL), Magnesium (mg/dL), and Uric Acid (mg/dL).

2.12 Pathomorphology
After recording of all pathological gross lesions, tissues (kidneys, liver, heart, brain, spleen, adrenals, thymus, testes, epididymides, ovaries, uterus, urinary bladder, seminal vesicles, prostates, esophagus, stomach, intestines) were collected, trimmed off for any adherent tissue as appropriate and tissues were preserved in 10% neutral buffered formalin (or Modified Davidson fluid for testes/epididymides).

2.12.1 Tissue Processing and Staining
Tissues were trimmed, labelled, washed, dehydrated in isopropyl alcohol (30%, 70%, 90%, 100%), cleared in three changes of xylene and paraffin-embedded using Leica TP1020 Automatic Tissue Processor, Leica EG1160 Embedding Station, and Leica EG1150 Cold Plate. Sections (4-5 µm) were cut with Leica RM2255 Rotary Microtome, mounted on poly-L-lysine-coated slides. Sections were deparaffinized, rehydrated, stained with hematoxylin, differentiated in acid alcohol, blued in ammonia water, stained with eosin, dehydrated, cleared, and mounted with DPX [12].

2.13 Immunohistochemistry
Liver sections from male rats were analyzed for CYP3A1 and CYP2E1 expression. Four-micron paraffin-embedded sections were mounted on poly-L-lysine-coated slides, deparaffinized, rehydrated, and subjected to heat-induced epitope retrieval in EDTA buffer (pH 8.5) using a pressure cooker. Endogenous peroxidase was blocked with 3% H_2O_2 for 30 minutes. Sections were incubated with primary antibodies (CYP3A1: 1:500; CYP2E1: 1:200; Merck, polyclonal) for 1 hour at room temperature, followed by Envision rabbit/mouse reagent (DakoREALTM EnvisionTM HRP) for 30 minutes. Color was developed with 3,3'-diaminobenzidine (DAB) chromogen, counterstained with Gill's hematoxylin, dehydrated, cleared, and mounted.

2.13.1 Interpretation of Immunohistochemical Staining
Staining intensity was scored (1: weak, 2: moderate, 3: strong) in pericentral, midzonal, and periportal areas. Hepatocyte layers around the central vein were graded (1+ to 4+).

2.14 Statistical Analysis
Hematological, biochemical, body weight, and organ weight data were analyzed using two-way ANOVA with Duncan's test for pairwise comparisons. Immunohistochemistry data were evaluated using the Kruskal-Wallis test. Significance was set at $P<0.05$.

3. Result and Discussion
3.1 Symptomatology
Over 28 days, rats in a study were monitored for clinical and behavioral symptoms. Group I (control) showed no abnormalities. Group II (Eu) exhibited no significant clinical signs compared to controls. Groups III (CDDP) and IV (Eu+CDDP) displayed reduced food intake, weakness, lethargy, sluggish movement, oliguria, red nasal exudate, and diarrhea from day 2 post-CDDP induction. These findings align with Vera *et al.* (2006) [13] and Zenitani *et al.* (2021) [14], indicating CDDP's cytotoxic effects. Motor and behavioral changes, consistent with Jangra *et al.* (2016) [15], suggest central inhibitory effects. Red nasal exudate, linked to thrombocytopenia, was novel. Oliguria, indicating acute renal failure, contrasted with Kishore *et al.* (2000) [16].

3.2 Mortality
No mortality was observed in any rat group during twice-daily checks over the experimental period, consistent with findings by Uozumi *et al.* (1992) [17], Sener *et al.* (2000) [18], and Li *et al.* (2020) [19] using the same CDDP dose.

3.3 Body Weight
No significant body weight differences were observed in Groups I and II up to day 28, or in Groups III and IV compared to controls on day 21. On day 28, Groups III and IV showed significant ($P<0.05$) body weight reduction compared to Group I, consistent with Amin and Hamza (2006) [20], Famurewa *et al.* (2020) [21], and Lin *et al.* (2018) [22], likely due to reduced food intake, disrupted metabolism, gastric stasis, and stomach distention in CDDP-treated rats. (Table 1 and 2)

Table 1: Effect of CDDP and Eu on weekly body weight (gm) (Mean± S.E.,n=5) in male rats during 28 days

Day	Group I	Group II	Group III	Group IV
0 Day	168.60±8.55 (n=5)	168.60±7.58 (n=5)	166.20±4.24 (n=5)	166.00±9.44 (n=5)
7 Day	205.00±12.08 (n=5)	196.40±9.11 (n=5)	197.40±6.56 (n=5)	196.40±9.33 (n=5)
14 Day	230.20±11.84 (n=5)	224.40±11.14 (n=5)	225.00±6.44 (n=5)	214.00±8.24 (n=5)
21 Day	254.00±12.76 (n=5)	251.80±8.50 (n=5)	252.20±6.77 (n=5)	253.00±8.44 (n=5)
28 Day	$276.80±11.50^b$ (n=5)	$273.20±8.56^b$ (n=5)	$204.40±8.10^a$ (n=5)	$208.20±10.91^a$ (n=5)
	Note: Mean bearing different superscripts in row differ significantly ($P<0.05$).			

Table 2: Effect of CDDP and Eu on weekly body weight (gm) (Mean± S.E.,n=5)in female rats during 28 days

Day	Group I	Group II	Group III	Group IV
0 Day	171.60±7.69 (n=5)	170.00±2.58 (n=5)	172.60±9.70 (n=5)	172.60±3.52 (n=5)
7 Day	194.40±9.45 (n=5)	188.40±2.50 (n=5)	190.40±12.50 (n=5)	187.20±4.04 (n=5)
14 Day	208.60±9.05 (n=5)	207.00±2.44 (n=5)	203.20±10.78 (n=5)	200.40±7.78 (n=5)
21 Day	219.40±10.62 (n=5)	221.40±1.77 (n=5)	216.40±10.85 (n=5)	217.20±6.83 (n=5)
28 Day	$230.60±11.10^b$ (n=5)	$230.80±2.74^b$ (n=5)	$184.20±7.31^a$ (n=5)	$192.60±7.89^a$ (n=5)
	Note: Mean bearing different superscripts in row differ significantly ($P<0.05$).			

3.4 Clinical Pathology

Hematological and clinical chemistry data for male and female rats in Groups I, II, III, and IV are presented in Tables 3-6. Group II showed no significant hematological or biochemical differences compared to Group I (control). In Groups III and IV, male rats had significant ($P<0.05$) decreases in HCT, MCV, and platelets, while females showed decreases in RBC, Hb, HCT, platelets, and neutrophils %, and increased MCHC compared to controls. No differences were noted between Groups III and IV.

Biochemically, Group III males had significant ($P<0.05$) increases in BUN, creatinine, ALT, ALP, phosphorus, cholesterol, GGT, uric acid, and iron, while females showed increases in BUN, creatinine, uric acid, ALT, ALP, and phosphorus. Both genders in Group III had decreased AST and TP. Group IV (CDDP + eugenol) showed similar alterations to Group III compared to controls. These findings, including decreased HCT, MCV, RBC, Hb, and platelets, and increased MCHC, align with Karale and Kamath (2017) [23], Marković *et al.* (2011) [24], and Geyikoglu *et al.* (2017) [25], except for neutrophils % and TLC, possibly due to a lower CDDP dose. Anemia resulted from CDDP-induced hematopoietic disruption, impaired erythropoiesis, free radicals, and defective iron metabolism, with thrombocytopenia linked to bone marrow suppression and increased MCHC due to reduced HCT.

Increased BUN, creatinine, ALT, ALP, phosphorus, cholesterol, GGT, uric acid, and iron in Group III, consistent with Al-Majeed *et al.* (2003) [26], Vasaikar *et al.* (2018) [27], and Silici *et al.* (2010) [28], indicate renal and hepatic damage, except for TP, which decreased due to reduced liver protein production. Elevated BUN and creatinine reflect renal impairment, GGT and uric acid indicate acute renal failure, and high phosphorus suggests tubular epithelium damage. Increased ALT points to hepatic damage, elevated cholesterol indicates lipid metabolism disruption and liver dysfunction, and high ALP correlates with severe diarrhea. Hemolysis caused increased iron. Group IV showed similar results, but lower phosphorus compared to Group III, possibly due to eugenol's ameliorative effect.

Table 3: Effect of CDDP and Eu on haematological parameters (Mean± S.E., n=5) in male rats after 28 days

Parameter	Unit	Group I	Group II	Group III	Group IV
RBC	10^6/ml	7.66±0.11	7.56±0.13	6.85±0.40	7.47±0.33
Hb	gm/dL	15.88±0.12	15.86±0.17	14.44±0.76	15.06±0.44
HCT	%	$39.96±0.26^b$	$38.92±0.68^b$	$33.76±2.42^a$	$36.46±1.58^a$
MCV	fL	$52.08±0.85^b$	$51.34±0.44^b$	$49.20±0.85^a$	$48.93±0.90^a$
MCH	Pg	20.69±0.21	20.95±0.53	21.13±0.23	20.26±0.49
MCHC	gm/dL	39.73±0.28	40.81±1.02	43.01±0.87	41.42±0.78
PLT	10^3/µL	$928.40±86.27^b$	$974.60±26.20^b$	$246.00±43.10^a$	$173.80±46.31^a$
MPV	fL	4.66±0.02	4.66±0.05	4.94±0.14	4.96±0.12
WBC	10^3/µL	9.52±0.42	11.04±0.42	8.30±1.49	10.20±1.59
Neutrophils	%	12.20±0.58	10.40±0.74	11.80±2.76	9.20±2.10
Lymphocytes	%	81.80±1.15	85.60±1.40	83.00±3.53	85.60±2.73
Monocytes	%	3.20±0.20	2.40±0.40	3.40±0.40	3.40±0.40
Eosinophils	%	2.80±0.66	1.60±0.40	1.80±0.48	1.80±0.37
Basophils	%	0±0	0±0	0±0	0±0
Note: Mean bearing different superscripts in row differ significantly ($P<0.05$).					

Table 4: Effect of CDDP and Eu on haematological parameters (Mean± S.E., n=5) in female rats after 28 days

Parameter	Unit	Group I	Group II	Group III	Group IV
RBC	10^6/µL	$7.40±0.07^b$	$7.36±0.14^b$	$7.05±0.10^a$	$6.72±0.22^a$
Hb	gm/dL	$15.64±0.18^b$	$15.90±0.17^b$	$15.60±0.29^b$	$14.66±0.43^a$
HCT	%	$36.76±0.83^b$	$36.78±0.59^b$	$34.16±0.67^a$	$32.44±0.84^a$
MCV	fL	49.60±0.77	50.06±1.37	48.38±0.72	48.19±0.77
MCH	Pg	21.11±0.27	21.62±0.50	22.09±0.25	21.76±0.15
MCHC	gm/dL	$42.59±0.64^a$	$43.23±0.45^a$	$45.71±1.09^c$	$45.17±0.41^{bc}$
PLT	10^3/µL	$898.40±47.80^b$	$977.20±63.76^b$	$404.20±92.39^a$	$366.00±79.57^a$
MPV	fL	4.70±0.12	4.80±0.15	4.78±0.22	4.90±0.10
WBC	10^3/µL	10.18±0.35	11.28±1.16	10.36±0.92	9.12±0.50
Neutrophils	%	$11.60±1.17^b$	$9.80±0.73^b$	$7.60±0.74^a$	$8.00±0.63^a$
Lymphocytes	%	83.40±1.86	86.20±1.24	88.20±1.15	88.00±1.00
Monocytes	%	2.60±0.24	2.40±0.24	2.80±0.37	2.40±0.24
Eosinophils	%	2.40±0.60	1.60±0.40	1.40±0.24	1.60±0.24
Basophils	%	0±0	0±0	0±0	0±0
Note: Mean bearing different superscripts in row differ significantly ($P<0.05$).					

Table 5: Effect of CDDP and Eu on biochemical parameters (Mean±S.E.,n=5)in male rats after 28 days

Parameter	Unit	Group I	Group II	Group III	Group IV
Alanine Aminotrans-ferase (ALT)	U/L	$60.46±2.68^a$	$58.66±3.41^a$	$127.18±5.12^b$	$123.24±10.99^b$
Aspartate Aminotrans-ferase (AST)	U/L	$267.30±12.57^b$	$270.54±13.90^b$	$145.74±17.31^a$	$124.64±5.75^a$
Alkaline Phosphatase (ALP)	U/L	$487.20±27.96^b$	$557.20±39.10^b$	$781.14±21.73^a$	$671.20±22.82^{ab}$
Total Protein	gm/dL	$7.00±0.10^b$	$7.18±0.07^b$	$6.08±0.42^a$	$6.50±0.15^{ab}$
Albumin	gm/dL	3.46±0.04	3.50±-0.06	3.32±0.04	3.46±0.06

Urea	mg/dL	16.91±2.71^{a}	13.80±2.48^{a}	67.94±8.75^{b}	75.52±5.36^{b}
Creatinine	mg/dL	0.64±0.02^{a}	0.67±.02^{a}	1.69±0.17^{b}	2.36±0.53^{b}
Uric Acid (UA)	mg/dL	1.68±0.13^{b}	1.68±0.24^{b}	3.68±0.37^{a}	3.46±0.44^{a}
Calcium	mg/dL	10.02±0.38	10.16±0.15	10.62±0.47	11.16±0.15
Phosphorous	mg/dL	10.06±0.46^{a}	9.92±0.29^{a}	14.38±0.36^{c}	11.46±0.38^{b}
Magnesium	mg/dL	3.62±0.11	3.65±0.16	3.35±0.08	3.64±0.10
Cholesterol	mg/dL	61.80±4.14^{a}	60.60±3.68^{a}	77.18±3.89^{b}	78.24±2.77^{b}
Gamma Glutamyl Transferase (GGT)	U/L	1.60±0.40^{a}	1.40±0.24^{a}	4.00±0.83^{b}	1.60±0.24^{a}
Iron	mg/dL	111.34±5.42^{a}	103.50±2.72^{a}	200.22±12.57^{b}	230.84±9.26^{c}
Note: Mean bearing different superscripts in row differ significantly (P<0.05).					

Table 6: Effect of CDDP and Eu on biochemical parameters (Mean±S.E., n=5) in female rats after 28 days

Parameter	Unit	Group I	Group II	Group III	Group IV
Alanine Aminotrans-ferase (ALT)	U/L	59.12±3.28^{b}	84.00±20.71^{b}	128.02±13.91^{a}	114.82±9.06^{a}
Aspartate Aminotrans-ferase (AST)	U/L	277.64±10.49^{b}	286.98±19.36^{b}	171.78±13.48^{a}	184.38±8.71^{a}
Alkaline Phosphatase (ALP)	U/L	245.72±16.96^{b}	220.40±10.45^{b}	516.60±31.46^{a}	477.54±26.71^{a}
Total Protein	g/dL	7.46±0.13^{bc}	7.86±0.09^{c}	7.22±0.23^{ab}	6.82±0.15^{a}
Albumin	g/dL	3.60±0.06	3.76±0.02	3.70±0.05	3.58±0.10
Urea	mg/dL	28.66±1.97^{a}	29.73±3.71^{a}	95.11±4.65^{c}	77.43±6.15^{b}
Creatinine	mg/dL	0.77±0.01^{a}	0.78±0.04^{a}	2.72±0.51^{b}	1.26±0.08^{a}
Uric Acid (UA)	mg/dL	2.86±0.36^{b}	2.10±0.14^{b}	3.2±0.24^{a}	3.86±0.28^{c}
Calcium	mg/dL	10.44±0.15	9.98±0.27	10.26±0.11	10.40±0.12
Phosphorous	mg/dL	11.32±0.50^{b}	11.38±0.20^{b}	13.64±0.62^{a}	12.64±0.29^{ab}
Magnesium	mg/dL	3.52±0.04	3.36±0.14	3.62±0.14	3.34±0.17
Cholesterol	mg/dL	67.10±5.93	67.67±4.93	57.90±2.36	67.62±4.34
Gamma Glutamyl Transferase (GGT)	U/L	1.20±0.20	1.40±0.24	1.80±0.37	2.40±0.50
Iron	mg/dL	233.16±18.29^{a}	250.60±5.68^{b}	274.02±3.75^{bc}	275.12±14.98^{bc}
Note: Mean bearing different superscripts in row differ significantly (P<0.05).					

3.5 Organ Weight: Absolute and relative organ weights for male and female rats in Groups I, II, III, and IV are presented in Tables 7-10. Group II showed no significant differences in organ weights compared to Group I (control). In Group III, males had significantly (P<0.05) reduced liver, spleen, and testes weights and increased adrenal weights, while females had reduced ovary weights and increased kidney weights. Relative weights in Group III males showed significant (P<0.05) increases in kidneys, heart, brain, adrenals, testes, and epididymides, and in females, liver and brain, with thymus weights significantly reduced in both genders. Group IV showed similar significant alterations compared to Group III. These findings align with Pratibha *et al.* (2006)[29], Yousef *et al.* (2009) [30], Amin *et al.* (2012) [31], Owoeye *et al.* (2018) [32], and Purena *et al.* (2018) [33].

Increased kidney weight may result from glomerular cell proliferation and protein casts. CDDP accumulation in the liver, inhibiting DNA biosynthesis, likely reduced liver weight. Immunosuppression caused reduced spleen and thymus weights, while effects on rapidly dividing cells led to reduced testes and ovary weights.

Table 7: Effect of CDDP and Eu on absolute organ weight (Mean± S.E., n=5) inmale rats after 28 days

Organ	Group I	Group II	Group III	Group IV
Liver	7.51±0.52^{bc}	7.82±0.24^{c}	6.31±0.21^{a}	6.48±0.30^{ab}
Lungs	1.56±0.10	1.57±0.09	1.41±0.19	1.27±0.11
Kidneys	1.71±0.08	1.40±0.35	1.90±0.07	1.76±0.02
Spleen	0.56±0.01^{c}	0.59±0.01^{c}	0.41±0.02^{b}	0.35±0.02^{a}
Heart	0.97±0.02	0.93±0.05	0.88±0.03	0.82±0.01
Brain	2.02±0.08	1.85±0.02	1.81±0.03	1.87±0.05
Adrenals	0.03±0.002^{a}	0.03±0.003^{a}	0.06±0.005^{c}	0.04±0.003^{b}
Thymus	0.57±0.03^{c}	0.57±0.03^{c}	0.30±0.02^{b}	0.20±0.01^{a}
Testes	2.87±0.07^{b}	2.82±0.06^{b}	2.55±0.04^{a}	2.85±0.05^{b}
Epididymis	0.94±0.05	0.95±0.04	0.89±0.008	0.96±0.04
Note: Mean bearing different superscripts in row differ significantly (P<0.05).				

Table 8: Effect of CDDP and Eu on relative organ weight (Mean± S.E., n=5) inmale rats after 28 da

Organ	Group I	Group II	Group III	Group IV
Liver	2.71±0.14	2.86±0.05	3.10±0.15	3.13±0.18
Lungs	0.20±0.008	0.21±0.01	0.20±0.01	0.17±0.01
Kidneys	0.35±0.006^{a}	0.34±0.01^{a}	0.43±0.03^{b}	0.40±0.02^{ab}
Spleen	0.56±0.02	0.57±0.03	0.68±0.07	0.61±0.05
Heart	0.61±0.008^{a}	0.63±0.01^{a}	0.93±0.06^{b}	0.85±0.04^{b}
Brain	0.73±.04^{a}	0.68±0.02^{a}	0.89±0.04^{b}	0.90±0.04^{b}
Adrenals	0.01±0.0008^{a}	0.01±0.001^{a}	0.03±0.003^{c}	0.02±0.002^{b}
Thymus	0.20±0.01^{c}	0.21±0.01^{c}	0.14±0.01^{b}	0.09±0.001^{a}
Testes	1.04±0.02^{a}	1.03±0.04^{a}	1.25±0.04^{b}	1.38±0.06^{b}
Epididymis	0.33±0.01^{a}	0.34±0.01^{a}	0.44±0.01^{b}	0.46±0.03^{b}
Note: Mean bearing different superscripts in row differ significantly (P<0.05).				

Table 9: Effect of CDDP and Eu on absolute organ weight (Mean± S.E., n=5) infemale rats after 28 days

Organ	Group I	Group II	Group III	Group IV
Liver	5.68±0.21	5.75±0.09	5.42±0.11	5.55±0.23
Lungs	1.41±0.06	1.41±0.10	1.32±0.02	1.26±0.02
Kidneys	1.27±0.04^{a}	1.41±0.02^{ab}	1.54±0.07^{bc}	1.58±0.04^{c}
Spleen	0.49±0.04	0.54±0.04	0.38±0.02	0.44±0.03
Heart	0.87±0.04	0.81±0.03	0.79±0.02	0.75±0.01
Brain	1.92±0.02	1.82±0.02	1.86±0.03	1.82±0.03
Adrenals	0.05±0.002	0.05±0.004	0.05±0.005	0.05±0.008
Thymus	0.57±0.05^{b}	0.58±0.04^{b}	0.25±0.04^{a}	0.20±0.03^{a}
Ovaries	0.13±0.006^{c}	0.11±0.004^{bc}	0.10±0.006^{ab}	0.09±0.007^{a}
Note: Mean bearing different superscripts in row differ significantly (P<0.05).				

Table 10: Effect of CDDP and Eu on relative organ weight (Mean± S.E.,n=5) in female rats after 28 days

Organ	Group I	Group II	Group III	Group IV
Liver	2.47±0.11^{a}	2.49±0.04^{a}	2.96±0.13^{b}	2.88±0.06^{b}
Lungs	0.21±0.02	0.23±0.01	0.21±0.01	0.22±0.01
Kidneys	0.38±0.02	0.35±0.01	0.43±0.02	0.39±0.01
Spleen	0.61±0.02	0.61±0.04	0.72±0.03	0.66±0.03
Heart	0.55±0.01	0.61±0.01	0.84±0.05	0.82±0.04
Brain	0.84±0.03^{a}	0.79±0.02^{a}	1.01±0.02^{b}	0.95±0.04^{b}
Adrenals	0.02±0.001	0.02±0.001	0.02±0.004	0.02±0.004
Thymus	0.25±0.02^{b}	0.25±0.01^{b}	0.13±0.02^{a}	0.10±0.01^{a}
Ovaries	0.05±0.004	0.05±0.002	0.05±0.001	0.05±0.003
Note: Mean bearing different superscripts in row differ significantly (P<0.05				

3.6 Pathomorphology

3.6.1 Gross Pathology

No gross lesions were observed in Group II compared to Group I (control). Groups III and IV showed whitish foci on lungs, atrophied thymus and spleen (Fig. 1-2), and paler kidneys in some male and female rats compared to controls.

Fig 1-2: Gross photograph of atrophied thymus and spleen of Group III as compared to thymus and spleen of control group.

3.6.2 Histopathology

Group II kidneys, testes, spleen, thymus, and adrenals showed normal architecture compared to controls. Groups III and IV exhibited significant histopathological changes which are summarized in Table 11 and 12.

Table 11: Effect of CDDP and Eu on histopathological alterations in male rats after 28 days

Organ	Observation	Severity	GroupI	Group II	Group III	Group IV
Liver	Infiltration of inflammatory cells	Minimal	2	0	0	0
	Hepatocellular necrosis (pericentral)	-	0	0	0	0
Lungs	Perivascular cuffing	Minimal	4	3	3	2
		Mild	1	2	0	0
	Alveolar histiocytosis	Minimal	3	5	0	0
	Proliferation of type-II pneumocytes	Minimal	1	1	0	0
Kidneys	Tubular regeneration	Mild	0	0	1	0
		Moderate	0	0	4	5
	Tubular cast	Mild	0	0	1	0
		Moderate	0	0	4	5
	Presence of megalocytes	Mild	0	0	1	0
		Moderate	0	0	4	5
	Tubular vacuolation	Mild	0	0	1	0
		Moderate	0	0	4	5
	Tubular dilation	Minimal	0	1	0	0
		Mild	0	0	0	1

	Proliferation of mesangial cells in glomeruli	Mild	0	0	1	0
	Epithelial metaplasia of Bowman's capsule	-	0	0	0	0
Spleen	Depletion of follicles in white pulp	Minimal	0	0	1	4
		Mild	0	0	4	1
	Reduction in cellularity in red pulp	Minimal	0	0	1	4
		Mild	0	0	4	1
Thymus	Cortical lymphocytes depletion	Moderate	0	0	5	5
	Starry sky appearance in cortex	Moderate	0	0	5	5

Table 12: Effect of CDDP and Eu on histopathological alterations in female ratsafter 28 days

Organ	Observation	Severity	GroupI	Group II	Group III	Group IV
Liver	Infiltration of inflammatory cells	Minimal	1	0	0	0
	Hepatocellular necrosis (pericentral)	Minimal	0	2	0	0
Lungs	Perivascular cuffing	Minimal	4	4	4	2
		Mild	1	1	0	0
	Alveolar histiocytosis	Minimal	4	4	2	1
	Proliferation of type-II pneumocytes	-	0	0	0	0
Kidneys	Tubular regeneration	Mild	0	0	5	5
	Tubular cast	Mild	0	0	5	5
	Presence of megalocytes	Mild	0	0	5	5
	Tubular vacuolation	Mild	0	0	5	5
	Tubular dilation	Mild	0	0	2	0
	Proliferation of mesangial cells in glomeruli	-	0	0	0	0
	Epithelial metaplasia of Bowman's capsule	Minimal	0	0	0	2
Spleen	Depletion of follicles in white pulp	Minimal	0	0	1	0
		Mild	0	0	2	1
		Moderate	0	0	2	4
	Reduction in cellularity in red pulp	Minimal	0	0	1	0
		Mild	0	0	2	1
		Moderate	0	0	2	4
Thymus	Cortical lymphocytes depletion	Moderate	0	0	5	5
	Starry sky appearance in cortex	Moderate	0	0	5	5
Heart	Infiltration of inflammatory cells	Minimal	0	1	0	0
Adrenals	Vacuolation in cells of *Zona fasciculata*	-	0	0	0	0

Kidneys: Groups III and IV showed renal lesions including tubular regeneration, dilatation, cytoplasmic vacuolation, tubular casts, and megalocytes in proximal convoluted tubules (Fig. 3-6), consistent with Chopra *et al.* (1982) [34], Ravindra *et al.* (2010) [35], Al-Salam *et al.* (2020) [36], Atessahin *et al.* (2006) [37], Tikoo *et al.* (2007) [38], and Prabhu *et al.* (2013) [39]. Al-Salam *et al.* (2020) [36] noted additional interstitial fibrosis, possibly due to prolonged CDDP exposure. Epithelial metaplasia of Bowman's capsule was novel. Lesions, linked to CDDP accumulation in the S3 segment, reduced glomerular filtration, and free radicals, were less severe in females, possibly due to a CDDP resistance gene. Group IV showed no ameliorative effect from eugenol (Eu).Biochemical findings support histopathological changes.

Testes/Epididymides/Prostate: Minimal spermatid retention, apoptosis of pachytene spermatocytes, and atypical mitotic figures in Groups III and IV (Fig. 7 and 9), with sperm depletion and prostatic fluid atrophy, align with Turk *et al.* (2008) [42] and Favareto *et al.* (2011) [43]. Lesions resulted from CDDP's action on dividing germ cells and reactive oxygen species.

Spleen: Groups III and IV exhibited lymphoid depletion (Fig. 9 and 10), reduced megakaryocytes, and extramedullary hematopoiesis, aligning with Milicevic *et al.* (1994) [40], due to CDDP's antiproliferative and immunosuppressive effects, correlating with reduced spleen weight.

Thymus: Marked lymphocyte depletion and starry sky appearance in Groups III and IV (Fig. 11 and 12), consistent with Kouchi *et al.* (1996) [41], indicated CDDP's immunosuppressive effects and reduced thymus weight.
Groups III and IV showed similar lesion severity, indicating no ameliorative effect of Eu, likely due to low dose and short study duration.

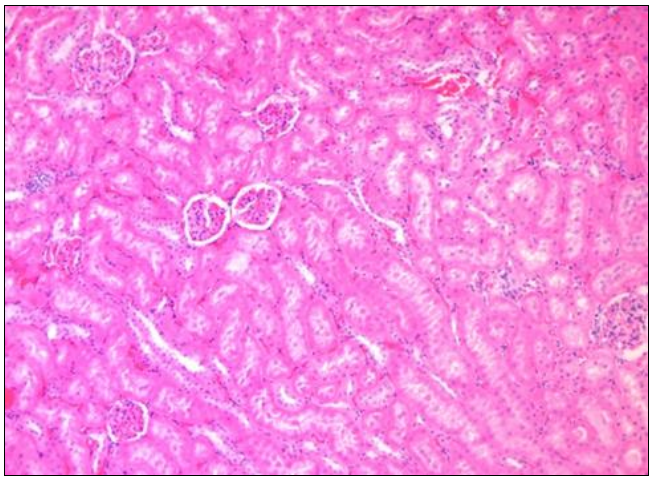

Fig 3: Group I: Photomicrograph of kidney showing normal architecture. H&E, X100 control group.

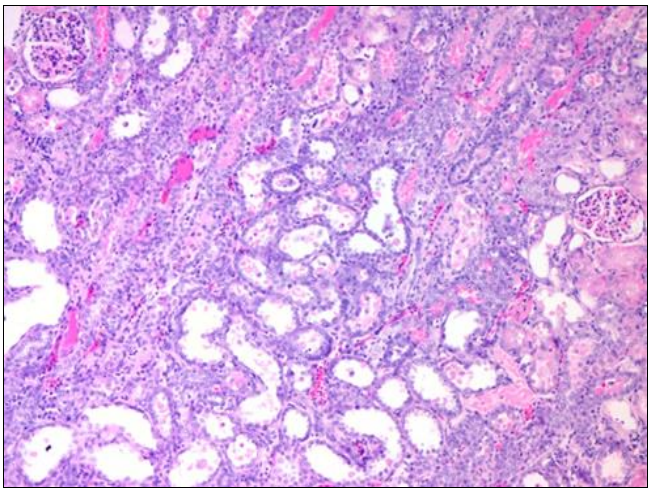

Fig 4: Group IV: Photomicrograph of kidney showing tubular regeneration tubular dilation and tubular casts in outer strip of outer medulla. H&E, X200

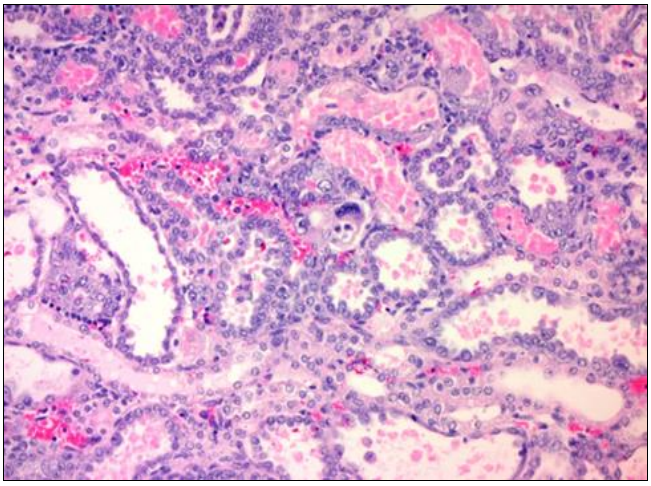

Fig 5: Group IV: Photomicrograph of kidney showing dilated regenerative tubule with atypical mitotic figure. H&E, X400

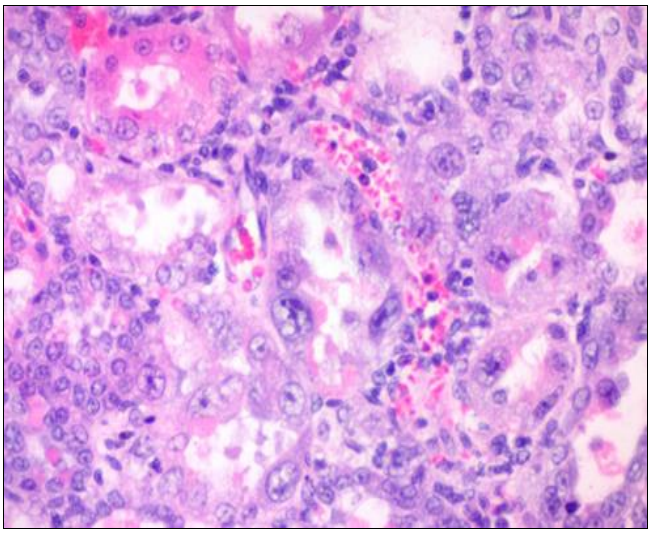

Fig 6: Group III: Photomicrograph of kidney showing multifocal presence of megalocytes within tubules. H&E, X400

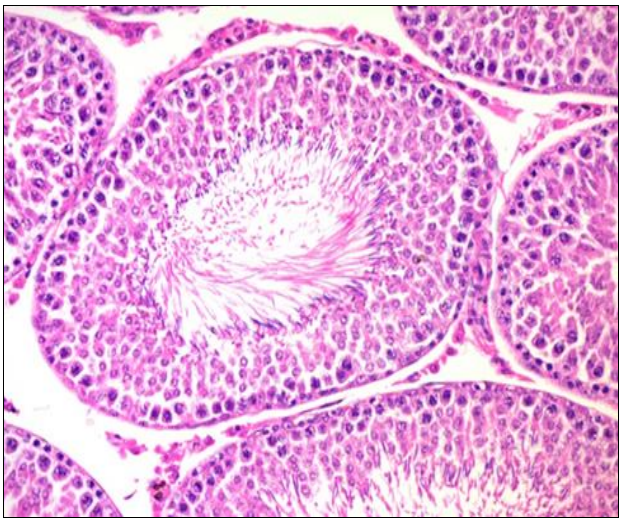

Fig 7: Group I: Photomicrograph of testes showing normal stage eight of seminiferous tubule. H&E, X200

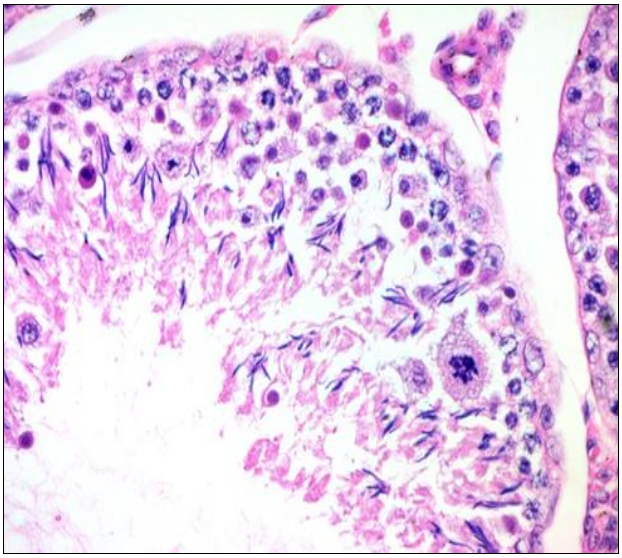

Fig 8: Group IV: Photomicrograph of testes showing stage ight of seminiferous tubule with retention of elongated spermatid, apoptosis of pachytene spermatocytes and atypical mitotic figures. H&E, X400.

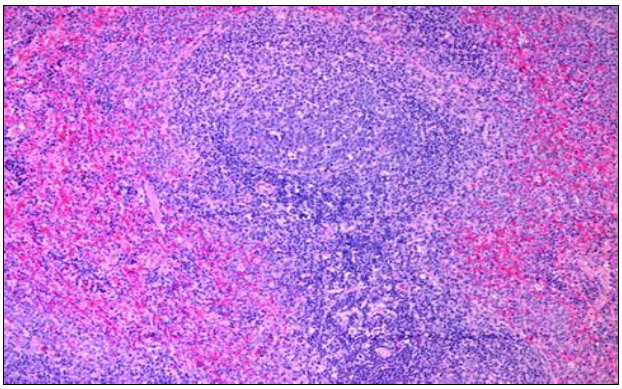

Fig 9: Group I: Photomicrograph of spleen showing normal architecture. H&E, X100

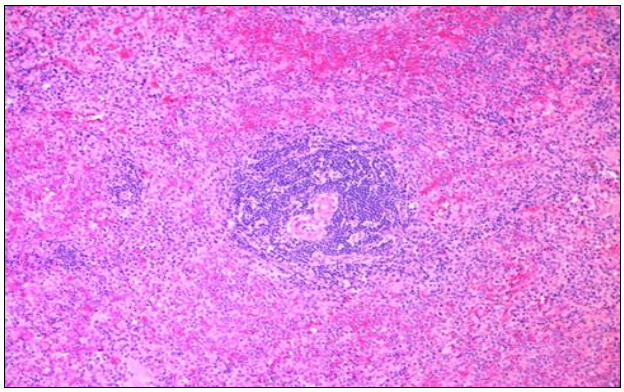

Fig 10: Group III: Photomicrograph of spleen showing lymphoid depletion in follicular area of white pulp. H&E, X50

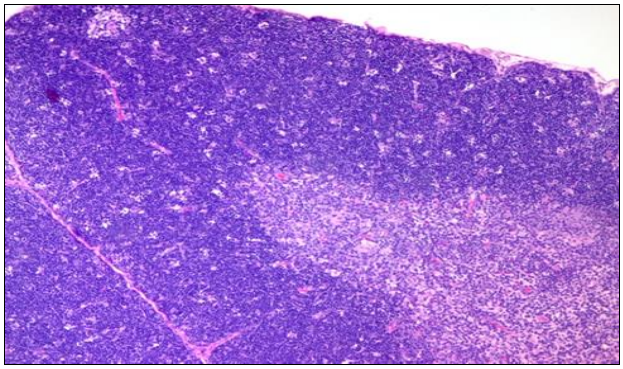

Fig 11: Group I: Photomicrograph of thymus showing normal architecture. H&E, X100

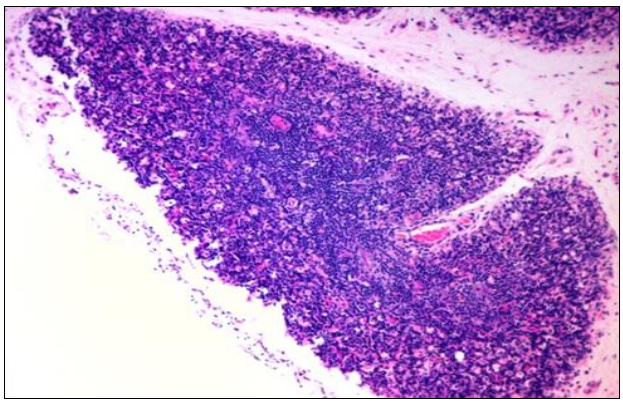

Fig 12: Group IV: Photomicrograph of thymus showing diffused lymphocytes depletion and starry sky appearance in cortical area of thymic lobule. H&E, X100

3.7 Immunohistochemistry: Liver immunohistochemistry for CYP3A1 showed no significant changes across all male groups compared to controls, though Groups III and IV had higher staining intensity around the central vein (Table 13). For CYP2E1, Group IV exhibited a significant ($P<0.05$) increase in chromogen binding intensity in the midzonal area and more affected hepatocyte layers around the central vein compared to controls (Table 14) (Fig. 13 and 14), likely due to higher specificity and sensitivity of the CYP2E1 antibody and increased enzyme expression from combined eugenol and cisplatin treatment. These findings align with Buehler *et al.* (1992) [44] but contrast with Hwang *et al.* (2020)[45], possibly due to higher dose and shorter study duration. Group III showed strong pericentral, moderate midzonal, and weak periportal CYP2E1 expression. Due to less severe histopathological lesions in females, immunohistochemistry was conducted only in male liver tissues.

Table 13: Effect of CDDP and Eu on expression of CYP3A1 enzyme inmale rats after 28 days [Median (minimum value-maximum value), n=5]

Parameter	Groups				P value
	I	II	III	IV	
LHA	1 (1-3)	2 (1-4)	3 (1-4)	2 (1-3)	0.178
PC	2 (2-3)	3 (2-3)	3 (3-3)	3 (3-3)	0.068
MD	1 (1-2)	1 (1-2)	2 (1-3)	2 (1-3)	0.605
PP	1 (1-2)	1 (1-2)	1 (1-3)	1 (1-1)	0.410
Grade:	Week-1, Moderate-2 and strong-3 for staining intensity in PC, MD and PP area. 1+, 2+, 3+ and 4+ for nlayers of hepatocytes affected around central vein.				

Table 14: Effect of CDDP and Eu on expression of CYP2E1 enzyme in male rats after 28 days [Median (minimum value-maximum value), n=5]

Parameter	Groups				P value
	I	II	III	IV	
LHA	3 (2-3)	2 (1-3)	3 (2-4)	4 (3-4)	0.049
PC	2 (2-3)	2 (1-3)	3 (2-3)	3 (2-3)	0.327
MD	1 (1-2)	1 (1-2)	2 (1-3)	3 (1-3)	0.045
PP	1 (1-2)	1 (1-1)	1 (1-2)	1 (1-2)	0.426
Grade:	Week-1, Moderate-2 and strong-3 for staining intensity in PC, MD and PP area. 1+, 2+, 3+ and 4+ for numbers of layers of hepatocytes affected around central vein.				

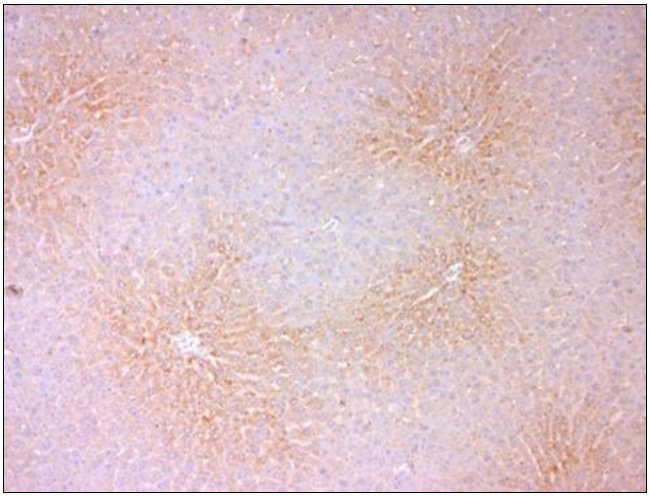

Fig 13: Group I: Photomicrograph of liver showing moderate pericentral, week midzonal and periportal expression of CYP2E1. Immunohistochemistry, DAB chromogen, Gill hematoxylin counterstain, X100

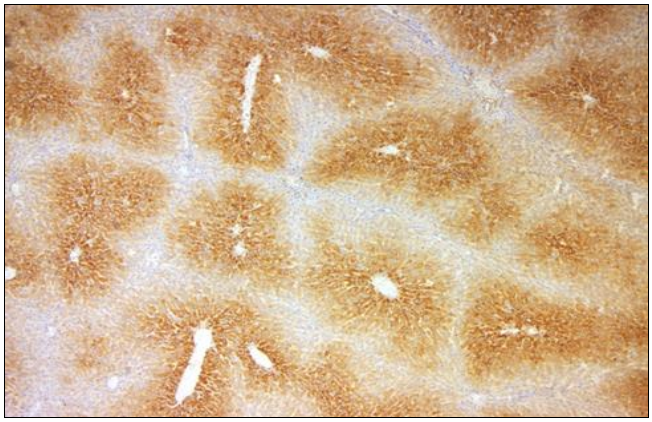

Fig 14: Group IV: Photomicrograph of liver showing strong pericentral and midzonal and moderate periportal expression of CYP2E1. Immunohistochemistry, DAB chromogen, Gill hematoxylin counterstain, X100

4. Summary and Conclusions

Cancer is a leading cause of death in India, with cisplatin (CDDP) being a key chemotherapy drug that forms DNA adducts to induce cancer cell apoptosis but is limited by nephrotoxicity and drug resistance. Eugenol (Eu), derived from clove oil and other sources, has antioxidant and anti-inflammatory properties, but its effect on CDDP-induced nephrotoxicity is unclear. A study with 40 Wistar rats (20 male, 20 female) divided into four groups (I: control, II: Eu, III: CDDP, IV: Eu+CDDP) investigated this over 28 days. Groups III and IV showed reduced food intake, lethargy, red nasal exudate, oliguria, and diarrhea, with no mortality. Body weight in Groups III and IV was significantly ($P<0.05$) reduced by day 28, with decreased liver, spleen, testes (males), and ovary (females) weights, increased adrenal (males) and kidney (females) weights, and reduced thymus weight in both sexes. Hematologically, Groups III and IV had significant ($P<0.05$) decreases in HCT, MCV, platelets (males), and RBC, Hb, HCT, platelets, neutrophils % (females), with increased MCHC (females). Biochemically, Group III showed significant ($P<0.05$) increases in BUN, creatinine, uric acid, ALT, ALP, phosphorus (both sexes), and cholesterol, GGT, iron (males), with decreased TP. Histopathologically, Groups III and IV exhibited renal tubular regeneration, casts, vacuolation, dilatation, and megalocytes; testicular spermatid retention, apoptosis, and atypical mitotic figures; epididymal sperm depletion; prostatic atrophy; splenic lymphoid depletion; thymic lymphocyte depletion; and adrenal vacuolation, with less severity in females. Group IV showed significantly ($P<0.05$) increased CYP2E1 expression in the liver midzonal area. Eu showed no ameliorative effect against CDDP-induced toxicity, possibly due to low dose and short study duration.

5. Acknowledgement

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