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In Vivo Ameliorative Impact Of Demethoxycurcumin, Epi-Orientin And Ursolic Acid On Magnesium Chloride Induced Multiple Organ Damage In Albino Wistar Rats

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ABSTRACT
The main and fastest reaction that infections use as a quick defense response is inflammation. A strong link has been established between inflammation and cancer. Animals and humans may have specific endocrine, hematological, cardiovascular, and neurological consequences from elevated serum magnesium concentrations. Magnesium compounds in mammalian cells produced aneuploidy, sister chromatid exchange, and DNA strand breaks, but they did not result in chromosomal abnormalities. Moreover demethoxycurcumin, EPI-orientin and ursolic acid produce antioxidant and hypoglycemic properties. In current study, the anti-inflammatory behavior of demethoxycurcumin, EPI-orientin, and ursolic acid has been examined after the induction of multiple organ toxicities via 100 mg/mL of $MgCl_2$, by keeping Naproxen @125mg/5mL/Kg/b.wt as standard in albino Wistar rats (150-200 g) of both genders while blood biochemistry has been done by ELISA approach. Results showed that female rats treated with demethoxycurcumin, EPI-orientin, and ursolic acid showed a considerable improvement ($p>0.005$) in the levels of AST, ALT, bilirubin, albumin, globulin, ALP, BUN, creatinine, uric acid, haemoglobin, WBC, RBC, HCT, MCV, MCH, MCHC, platelets, neutrophils, eosinophils, lymphocytes and monocytes, especially at the concentration of 250 mg/mL/ Kg. Female rats showed significant changes in ALP, BUN, and AST ($P < 0.01$), but no significant changes in ALT were seen when compared to the control group. At 100 mg/ Kg, these compounds produced significant change in cholesterol, HDL, LDL, triglycerides, and VLDL concentrations ($P < 0.05$) as compared to compared to the rats of control group at all concentrations of compound. Moreover, histopathological findings (at 100 μm) showed that $MgCl_2$ induced severe toxicity in the liver, kidney, and heart. Therefore, the combinational formulation of the three bioactive components (demethoxycurcumin, EPI-orientin and ursolic acid) was found to be the best regimen for obtaining a 95% recovery from multiple organ damage so these compounds can be used, alone and in combination, to limit multiple organ damage due to over dose of megensium (which is used in different pharmaceutical products
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Keywords: Demethoxycurcumin, EPI-orientin, Ursolic acid, Blood, Kidney, Liver

INTRODUCTION

It is thought that the system becomes fatal once magnesium chloride levels rise to a certain point, which may account for the toxicity caused by magnesium chloride. The development of the inflammation-induced malignant phenotype in cancer is closely correlated with both cancer genetics and epigenetics[1]. Magnesium has various purposes in human anatomy. It is a co-factor for over 300 enzymes that are essential to the body's essential processes. Neuromuscular transmission, muscle contraction, blood pressure regulation, glycaemic management, cardiac contraction, energy production, active transmembrane ion transport, nuclear material synthesis, and bone formation are among the crucial tasks. Animals and humans may have specific endocrine, haematological, cardiovascular, and neurological consequences from elevated serum magnesium concentrations[2]. Magnesium compounds in mammalian cells produced aneuploidy, sister chromatid exchange, and DNA strand breaks *in vitro*, but they did not result in chromosomal abnormalities. According to reports, magnesium exposure damages the kidneys and liver[3]. Research has shown that metallic magnesium powder can cause acute irritation and change capillaries, particularly in the peritoneum and lungs. This caused fluid to spill out and occasionally caused haemorrhage. Magnesium is 500 times more soluble in plasma than in saline[4]. By maintaining the stability of the transcriptional activator hypoxia inducible factor (HIF), magnesium imitates hypoxia and increases erythropoietin production[5].

The synthetic counterpart of curcumin known as dimethoxycurcumin (DiMC) has two methoxy groups in place of the two phenolic-OH groups[6]. Better metabolic stability and enhanced anti-cancer action against many cancer types are made

possible by this little structural alteration. When Kunwar et al. examined the effects of DiMC (5–50 μM) on MCF7 breast cancer cells, they discovered that the cancer cells were inhibited through a process that involved the production of reactive oxygen species (ROS), decreases in glutathione levels, induction of DNA damage, and mitochondrial dysfunction, which ultimately resulted in the induction of S-phase cell cycle arrest and apoptosis[7].

Further evidence that the methoxy group may play a key role in the stability and/or activity of curcumin and its congeners comes from the observation that the order of efficacy for the production of ROS and activation of apoptosis was DiMC> Curcumin > bis-demethoxycurcumin[8]. Numerous studies show that DiMC causes cell death by a process called paraptosis, which is linked to proteosomal inhibition and causes ER stress, mitochondrial malfunction, and the generation of ROS, particularly mitochondrial superoxide.One of these bioactive compounds, called epi-orientin, is extracted from a variety of therapeutic plants, including lavender, basil, bamboo, passion flower, golden queen, flax, and dayflower[9]. Many therapeutic actions of epi-orientin include anti-inflammatory, antioxidant, antibacterial, neuroprotective, vasodilatory, and cardio-protective qualities. It has been widely used in scientific study, especially with flavonoids, as a model of hepatotoxicity linked to the release of TNF-α from activated hepatic macrophages, according to earlier investigations. Lipopolysaccharide (LPS)-induced inflammatory responses have been demonstrated to be inhibited by epi-orientin, a putative anti-inflammatory drug, improving lung injury[10]. Epi-orientin reduced the inflammatory response in osteoarthritic rats to alleviate the condition. Furthermore, epi-orientin lessens liver damage by suppressing oxidative stress. Ursolic acid has anti-inflammatory, antibacterial, antioxidant, anti-cancer, and anti-diabetic properties.Studies conducted both *in vivo* and *in vitro* show that ursolic acid (UA) has positive anti-inflammatory properties and can counteract both endogenous and external inflammatory triggers[11,12]. Reduced histamine release from mast cells, inhibition of lipoxygenase, cyclooxygenase, and phospholipase activities, decreased nitric oxide and reactive oxygen species production, blocking of signal pathway activation, downregulation of inflammatory factor expression, and inhibition of complement and elastase activities are the main anti-inflammatory mechanisms[7,2,13,18]. These pathways may provide new opportunities for the scientific community to create or enhance cutting-edge treatment strategies to address inflammatory diseases, including cancer, dermatitis, atherosclerosis, neuroinflammation, liver, kidney, and diabetic problems, as well as arthritis and bowel disorders.

MATERIALS AND METHODS

COMPOUDS USED FOR STUDY

Demethoxycurcumin (CAS No.: 36062-04-1), EPI-orientin (CAS No: 28371-16-6) and ursolic acid (CAS No: 77-52-1) were purchased from Sigma-Aldrich Corporation located in St. Louis, MO, USA.

EXPERIMENTALDESIGN

After taking ethical approval, which was given after the confirmation that experment will be done after following all Institutional Guidelines for the Care and Use of Animals for Scientific Purposes, experiment has been performed on healthy male, healthy non-pregnant and nulliparous female rats (100-150 g and 10-12 weeks old). Animals were housed in individual cages for seven days in standard conditions (22±4 °C, 30-70% humidity and 12 hours light-dark cycle), and fed with laboratory food and water. After 1 week of acclimatization, the study was conducted according to OECD 407 guidelines (Co-operation and Development, 2008). Ten groups (Table 1), with ten rats in each group, received an intraperitoneal dose of magnesium (1.0 mL/Kg b.w.) for ninety days while for one month, groups C, D, E, F, G, H, and I were fed varying dosages of demethoxycurcumin, epi-orientin and ursolic acid. Group J received treatment with the synthetic medication, Neproxen 1.0 tab/Kg @(500 mg/ Kg b.w/day) for one month for four weeks.

Table 1: Experimental Design

Groups	Treatments
A	Control
B	Only $MgCl_2$ administered
C	$MgCl_2$+ Ursolic acid (100 mg/ Kg b. w.)
D	$MgCl_2$+Demethoxycurcumin (100 mg/ Kg b. w.)
E	$MgCl_2$+ EPI-orientin(500mg/ Kg b.w.)
F	$MgCl_2$+ Urosolic acid (100 mg/ Kg b. w.)+ Demethoxycurcumin (100 mg/ Kg b. w.)
G	$MgCl_2$+ Demethoxycurcumin (100 mg/ Kg b. w.) + EPI-orientin (500 mg/ Kg b. w.)
H	$MgCl_2$+ Urosolic acid (100 mg/ Kg b. w.) + EPI-orientin (500 mg/ Kg b. w.)
I	$MgCl_2$+ Urosolic acid (100 mg/ Kg b. w.) + Demethoxycurcumin (100 mg/ Kg b. w.) + EPI-orientin (500mg/ Kg b. w.)
J	$MgCl_2$+ NAPROXEN©

Note: $MgCl_2$@ (1 mL/Kg B.w. per week for three months), E1+E2+E3@ (2.5 mg/ Kg b.w./ for one month), NEPROXEN© @ (500 mg/ Kg b.w/day) for one month

SERUM AND BLOOD SEPARATION

Five millilitres of blood were drawn from the rat heart during the dissection, and the serum was separated by centrifugation for ten minutes at 1500 rpm through Benchmark Scientific C2570 centrifuge machine. Serum was separated and then kept at -60°C for biochemical examination.

DETERMINATION OF BODY AND ORGAN WEIGHT

Body weights were measured every week during 28 days on laboratory weighing balance in animal house. On 29th day, rats were fasted overnight and blood was obtained through cardiac puncture after using ketamine (1cc injection /mL/Kg body weight). Heart, liver and kidney were removed by open chest method, cleaned with 10% normal saline and weighed individually on same weighing balance.

Biochemical Assays

alanine transaminase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, globulin, albumin, creatinine, blood urea nitrogen (BUN), Uric acid, Lipid Profile (cholesterol, Triglycerides, high density lipopolysaccharides (HDL), low density lipopolysaccharides (LDL) , very low density lipopolysaccharides (VLDL), haemoglobin, white blood cells (WBC), red blood cells (RBC Hematocrit (HCT), mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelets, neutrophils, eosinophils, lymphocytes and monocytes were measured using com mercially available ELISA kits from Sigma-Aldrich Corporation (St. Louis, MO, USA) in hematology lab of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore.

Histopathological evaluation

Organs, which were fixed in 10% formalin solution at room temperature were embedded in paraffin wax and prepared at 4 mm then stained with hematoxylin and eosin[25]. Slides were observed under 100 µm resolution of compound microscope, equiped with digital camera in the Zoology lab of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore.

Statistical analysis

All findings were presented as mean±SEM. Statistical significant difference between control and treated groups was assessed via two way analysis of variance (ANOVA) using bonferroni post-tests in Graph pad prism version 5.

RESULTS

EFFECT ON BODY AND ORGAN WEIGHT

Over the course of 28 days, the body weight of male rats in the control group increased gradually from 1st day to 28th day (123.67±1.28 g and 176±1.18 g respectively). Rats treated with demethoxycurcumin @100 mg/ Kg and EPI-orientin @ 200 mg/ Kg showed a considerable ($P < 0.001$) increase in body weight compared to the control group (118.17±1.35 g to 157.17±0.95 g in males and 131.67±0.67 g to 162.50±0.76 g in female rats respectively). The difference in body weights of male rats treated with EPI-orientin @ 100 mg/ Kg (157.17±0.95) and the control group (176±1.18 g) was not statistically significant. The weight of the female rats increased from 135.33±1.26 g to 183±0.58 g.in control groupM while female rats treated with demethoxycurcumin @100mg/Kg, EPI-orientin @200mg/Kg, and ursolic acid @ 100mg/ Kg, the weight increased significantly ($P >0.001$) from 112.50±0.76 g to 148.33±0.88 g, 127.50±0.76 g to 132.50±0.67 g, and 133.67±0.49 g to 140.50±0.89 g, respectively . In comparison to the normal group, male rats treated with EPI-orientin @200mg. Kg^{-1} showed a significant ($P < 0.001$) change in the weight of all organs. On the other hand, male rats treated with Ursolic acid @100 mg/ Kg of compound showed a considerable ($P < 0.001$) increase in heart and liver weight (0.54±0.01 g and 2.92±0.01 g). However, at 200 mg/Kg of EPI-orientin and 100mg/ Kg of demethoxycurcumin, there was no discernible change in the kidney's weight of male rats. Demethoxycurcumin @100mg/Kg resulted in a significant change in both liver and heart weight ($P = 0.01$) and ($P = 0.05$), respectively, while after administration of EPI-orientin @200mg/Kg, female rats showed a change in liver and kidney weight relative to the normal control group. Rats administered with EPI-orientin and urosolic acid showed a notable shift in both the liver and heart weights (0.53 ±0.01g and 0.67 ±0.01g respectively) (Table 2).

Table 2.Effect of Demethoxycurcumin, EPI-orientin and Urosolic acid on body and organ weight of rats

Weight (g) of animals and organs during the course of study/ Groups		Control group		Phytoactive compounds treated groups					
		Vehicle		Demethoxycurcumin @100mg/Kg		EPI-orientin @200mg/Kg		Urosolic acid@100mg/Kg	
		M	F	M	F	M	F	M	F
Animals	On 1st day	123.67±1.28	135.33±1.26	118.17±1.35***	112.50 ±0.76***	131.67 ±0.67***	127.5± 0.76***	123.50 ±1.12	133.67 ±0.49
	On 7th day	131.33±1.15	143.50±1.26	127±1.53**	121.83 ±0.95***	141.33 ±1.15***	142.83± 0.60	130.83 ±0.6	127.83 ±0.60***
	On 14th day	148.33±1.05	157.67±0.76	140.83±0.83***	130.83 ±0.83***	150 ±0.58	132.83 ±0.60***	137.50 ±0.76***	123.50 ±0.76***
	On 21th day	157±0.97	164.33±0.88	147.17±1.47***	137.33± 0.80***	158.33 ±0.67	128.17± 0.48***	146.33 ±0.92***	131.83 ±0.60***
	On 28th day	176±1.18	183±0.58	157.17±0.95***	148.33± 0.88***	162.50 ±0.76***	132.5± 0.67***	157.33 ±0.62***	140.50 ±0.89***
Organs	Liver	2.88±0.01	2.92±0.01	3.32±0.01***	3.15 ±0.01***	3.04 ±0.01***	3.08 ±0.01***	2.92 ±0.01**	2.97 ±0.01***
	Kidney	0.52±0.01	0.59±0.01	0.67±0.01***	0.64 ±0.01***	0.53 ±0.01	0.59 ±0.01	0.54 ±0.01	0.57 ±0.01
	Heart	0.54±0.01	0.60±0.01	0.73±0.01***	0.62 ±0.01	0.68 ±0.01***	0.57 ±0.01***	0.51 ±0.01*	0.53 ±0.01***

Values are expressed as mean±S.E.M ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ as compared to control group.

EFFECT ON BIOCHEMICAL PARAMETERS

Male rats treated with EPI-orientin @200mg/Kg shwoed significant changes in the levels of AST (P >0.01), ALT, ALP, and BUN (P >0.001) from those of the control group. 200mg/Kg of EPI-orientin and 100mg/ Kg demethoxycurcumin treated groups showed considerably (P ˃0.001) more changes in AST, ALT, ALP, and BUN in male and female rats than in the control group, but these changes were still within normal ranges as mentioned in Table 3. (At 200 mg/ Kg of EPI-orientin, female rats showed significant changes in ALP, BUN, and AST (P < 0.01), but no significant changes in ALT were seen when compared to the control group. However, no statistically significant differences in biochemical profile has been observed between normal control rats and treatment groups of either sex in terms of bilirubin, albumin, globulin, serum creatinine, and uric acid concentrations (Table 3).

Table 3. Effect of demethoxycurcumin, epi-orientin and urosolic acid on liver and kidney function tests of rats

Biochemical parameters	Control group		Treatment groups					
			Demethoxycurcumin @100mg/Kg		EPI-orientin @200mg/Kg		Urosolic acid@100mg/Kg	
	M	F	M	F	M	F	M	F
AST (µ/L)	68.33± 2.73	78.33± 2.95	62±2.54**	87±3.76**	88.50±2.46***	113.17±2.97***	121.17±5.01***	154.33±7.91***
ALT (µ/L)	24.67± 2.33	29.67± 2.17	39±2.02***	26.50±1.60	46.50±1.73***	47.83±1.96***	58.83±2.30***	49.67±145***
Bilirubin (mg/dL)	0.24±0.01	0.29±0.02	0.36±0.02	0.37±0.01	0.21±0.02	0.37±0.02	0.43±0.02	0.43±0.01
Albumin (g/dL)	3.96±0.07	4.12±0.11	4.11±0.11	4.27±0.08	3.58±0.12	4.52±0.13	4.48±0.14	4.61±0.07
Globulin (g/dL)	2.68±0.09	2.92±0.13	3.70±0.10	3.27±0.07	2.78±0.08	3.28±0.08	3.26±0.08	2.97±0.06
ALP (µ/L)	69±1.37	75±1.63	84.83±2.23***	94.50±1.26***	107.67±1.84***	115.67±1.65***	82.67±2.42***	95±1.53***
BUN (mg/dL)	10.85± 1.01	13.33± 1.20	17.29±0.82***	18.21±0.69***	24.17±1.08***	21.83±1.08***	21.33±1.15***	24.83±0.95***
Creatinine (mg/dL)	0.29±0.02	0.37±0.01	0.33±0.02	0.42±0.03	0.71±0.03	0.54±0.06	0.46±0.02	0.68±0.05
Uric acid (mg/dL)	0.63±0.05	0.77±0.08	0.86±0.03	0.81±0.06	0.98±0.05	0.79±0.03	1.32±0.08	0.86±0.02

ALT: Alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphate BUN: blood urea nitrogen, M: male, F: female. Values are expressed as mean±S.E.M ***p < 0.001, **p < 0.01 as compared to control group.

EFFECT ON HEMATOLOGICAL PARAMETERS

As compared to normal control rats, male rats treated with demethoxycurcumin, EPI-orientin, and Urosolic acid showed a significant (P < 0.001) drop in RBC levels (13.57 g/dL) as compared to the female rats (14.57 g/dL). Rats treated with EPI-orientin showed no statistically significant differences in Haemoglobin, WBC, HCT, MCV, MCHC, lymphocytes, monocytes, neutrophils, eosinophils, MCH, and platelets. Male rats administered with 100 mg/ Kg demethoxycurcumin showed no significant changes in any of the hematolgical parameters, with the exception of RBC (18.78 g/dL), which also decreased significantly in female rats (P < 0.001, 16.60 g/dL) in comparison to the control group. Platelets increased dramatically (P <0.001) in rats administered with 200 mg/Kg EPI-orientin but stayed within the normal range. Compared to the control group, the 100mg/ Kg demethoxycurcumin treated group had a considerably lower RBC level (P < 0.001), while there was a considerable (P ˆ 0.001) rise in monocytes, platelets, and neutrophils. A significant (P < 0.001) decrease in red blood cells was seen in female rats treated with all three compared to the normal control group. At 100mg/ Kg of ursolic acid, eosinophils and monocytes showed substantial changes (P ˆ 0.001) (506.83 g/dL in female rats and (P ˆ 0.01) 476.50 g/dL in male rats, respectively), although these changes were not clinically significant (Table 4).

Table 4. Effect of Demethoxycurcumin, Epi-orientin and Urosolic acidon hematological parameters of rats

Hematological profile	Control group		Treatment groups					
	Vehicle		Demethoxycurcumin 100 mg/ Kg		EPI-orientin (200 mg/ Kg)		Ursolic acid (100 mg/ Kg	
	M	F	M	F	M	F	M	F
Haemoglobin (g/dL)	13.18±0.33	12.16±0.29	13.70±0.09	14.57±0.12	18.78±0.46	16.60± 0.10	15.68±0.15	14.93±0.23
WBC ($\times10^3$/L)	10.15±0.59	9.53± 0.58	11.72± 0.45	9.42± 0.18	15.02± 0.14	14.78± 0.08	11.61± 0.15	12.19± 0.33
RBC ($\times10^{12}$/L)	8.29± 0.22	9.59± 0.14	6.75± 0.08***	8.30± 0.01***	6.31± 0.08***	7.71± 0.07***	5.76± 0.07***	7.25± 0.08***

HCT (%)	52.50± 3.96	54± 1.88	50.17± 1.47	49.50± 1.41	53.50± 0.76	52.50± 0.76	61.67± 1.05	62.50± 0.76
MCV (fL)	53± 3.31	55.83± 3.26	47.50± 0.76	47.50± 0.76	57± 0.97	62.83± 0.95	61.50± 0.76	67.50± 0.76***
MCH (Pg)	16.28± 0.90	16.56± 0.51	15.17± 0.29	15.62± 0.14	15.58± 0.14	17± 0.28	18.64± 0.15	17.78± 0.07**
MCHC (%)	38.33± 2.17	50.17± 3.84	42.33± 2.29	52.22± 3.01	49.50± 1.93	54.67± 0.88	65.50± 1.52***	63.50± 0.76
Platelets ($\times 10^9$/L)	460.50± 16.28	549.83± 24.30	475.83± 1.58	489.67± 1.59	534.50± 1.61***	539.67± 2.91	476.50± 17.15**	506.83± 1.78
Neutrophils (%)	26.50± 4.29	30.67± 0.80	25.50± 1.61	27.50± 0.76	16.17± 1.52	22.50± 0.76	8.69± 0.34*	17.33± 0.80
Eosinophils (%)	2.15 ±0.51	2.97 ±0.29	3.15 ±0.29*	3.58 ±0.14	2.65 ±0.14	2.55 ±0.14	1.37 ±0.22	1.66 ±0.15***
Lymphocytes (%)	66.17 ±1.52	67 ±0.99	71.50± 1.18	75.33± 1.02	57.67± 0.84	59± 0.97	66.83± 1.08	52.50± 0.76
Monocytes (%)	2.67± 0.44	2.55± 0.47	2.57± 0.14	2.83± 0.33	3.57± 0.16	2.53± 0.15	4.36± 0.15***	1.63± 0.15**

HCT: hematocrit, RBC: red blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell, M: male, F: female. Values are expressed as mean±S.E.M ***p < 0.001, **p < 0.01, *p < 0.05as compared to control group.

EFFECT ON LIPID PROFILE

When male rats (treated with 200 mg/ Kg of EPI-orientin) were compared to normal control rats, there was a substantial change in cholesterol, LDL (P > 0.05), and triglycerides (P >0.001) levels, but these changes were still within the reference range. The amount of cholesterol, (after treatment with 200 mg/ Kg of EPI-orientin) significantly changed (P < 0.05) in male rats. At 100mg/ Kg of demethoxycurcumin, there was a significant (P>0.001) change in LDL and cholesterol levels of male rats. Significant differences were seen in cholesterol, HDL, LDL, triglycerides, and VLDL (P < 0.05) as compared to the control group in female rats treated with 100 mg/Kg of demethoxycurcumin, 200mg/Kg of EPI-orientin and 100mg/Kg of ursolic acid, however these differences were not clinically significant (Table: 05).

Table 5. Effect of Demethoxycurcumin, Epi-orientin and Ursolic acidon lipid profile of rats

Lipid profile (mg/dL)	Control group		Treatment groups					
	Vehicle		Demethoxycurcumin@100 mg/ Kg		EPI-orientin @200 mg/ Kg		Ursolic acid@100 mg/ Kg	
	M	F	M	F	M	F	M	F
Cholesterol	75.17 ±1.08	90 ±0.58	99.33 ±1.54*	110.50 ±0.89***	99.50 ±1.26*	112.17 ±2.81***	117.17 ±0.95***	111 ±1.71***
Triglycerides	90±0.58	95.83 ±1.64	136±1.07***	113.17 ±3.10***	97.50 ±0.76	125.67 ±1.65***	83.59 ±0.71	108.33 ±0.88***
HDL	36.33 ±1.63	38.33 ±0.88	35.83 ±1.30	33.50 ±0.76***	38.33 ±0.67	30.50 ±0.76***	25.83 ±0.95	22.50 ±0.76***
LDL	113.83 ±1.17	123.50 ±0.76	90.50 ±1.48*	85.17 ±1.35***	94.83 ±0.95	76.50 ±0.76***	78.33 ±0.67***	83.50 ±0.76***
VLDL	16.17 ±0.60	24.67 ±1.05	27.50 ±0.76	19.50 ±0.76*	28.17 ±1.35	17.67 ±0.88*	21.50 ±0.76	32.50 ±0.76

HDL: High density lipoprotein, LDL: low density lipoprotein VLDL: very low density lipoprotein, M: male, F: female.Values are expressed as mean±S.E.M ***p < 0.001, *p < 0.05as compared to control group.

HISTOPATHOLOGICAL ANALYSIS

Liver of normal control group displayed the portal triad, which included the interlobular bile duct and branches of the hepatic artery portal veins. Hepatocytes in demethoxycurcumin treated rats were normal, while endothelial cells bordered the hepatic sinusoids. In rats treated with EPI-orientin, there was no evidence of hepatic congestion, regeneration, or necrosis (including eosinophilic cytoplasm and keratolysis). Normal glomerulus, proximal and distal convoluted tubules, Bowman's capsule, and macula densa were observed in kidney sections of normal control rats. When compared to controls, ursolic acid treated rats did not exhibit much renal congestion, apoptosis, or vacuolar degeneration. Demethoxycurcumin treated rats displayed slight renal congestion but EPI-orientin treated rats show no significant vacuolar degeneration. Cardiac tissues in heart of rats treated with demethoxycurcumin, ursolic acid and in control group rats, displayed normal muscular fibres with intercalating discs (Figure 1 and Figure 2).

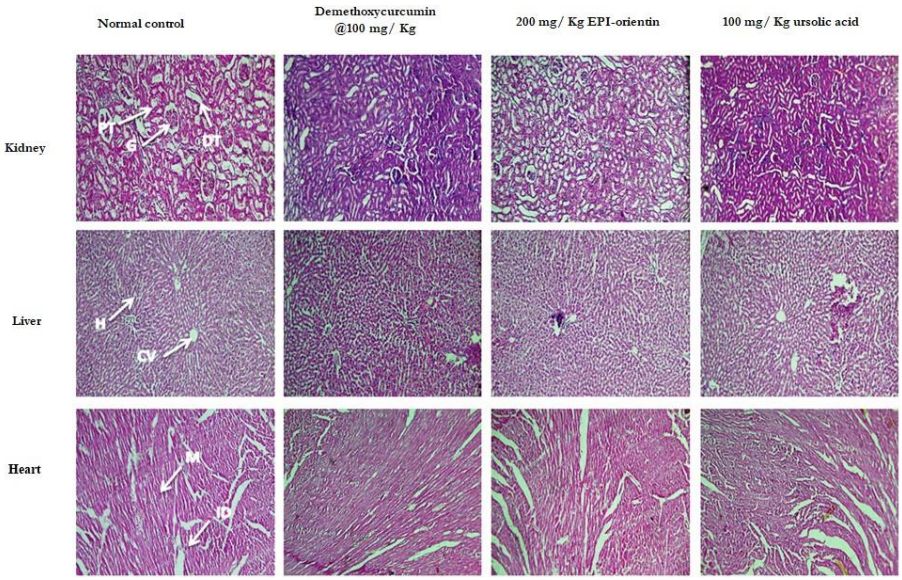

Figure 1: Histopathological examination of kidney, liver and heart tissues of male rats in normal control group rats and demethoxycurcumin, EPI-orientin and ursolic acid treated groups (@100, 200 and 100 mg/ Kg respectively) treated groups. G: glomerulus, PT: proximal tubules, DT: distal tubules, CV: central vein, H: hepatocytes, M: myocardium, ID: intercalated disc (40 XH & E stain)

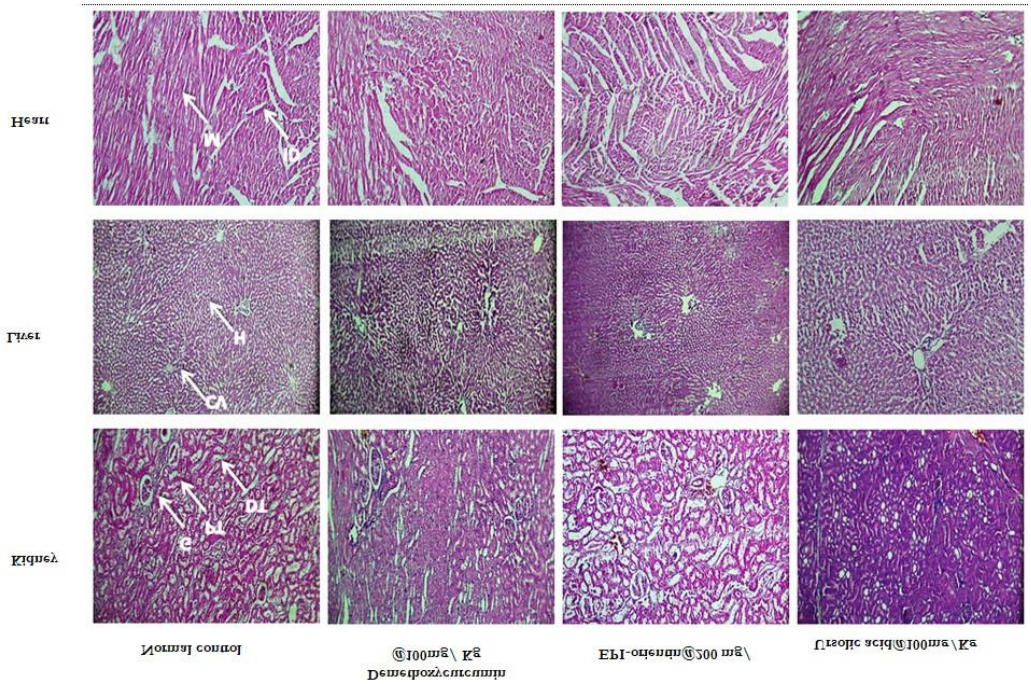

Figure 2: Histopathological examination of kidney, liver and heart tissues of female rats in normal control group rats and demethoxycurcumin, EPI-orientin and ursolic acid treated groups (@100, 200 and 100 mg/ Kg respectively) treated groups. G: glomerulus, PT: proximal tubules, DT: distal tubules, CV: central vein, H: hepatocytes, M: myocardium, ID: intercalated disc (40 XH & E stain)

DISCUSSION

The primary active component that was isolated from *Curcumae longae radiatx* is demethoxycurcumin. EPI-orientin and ursolic acid is isolated from *P. frutescens*. All the three bioactive compounds have been shown in recent studies to have anti-inflammatory and antioxidative properties. In the present study, we assessed the effects of demethoxycurcumin, EPI-orientin and urosolic acid on Liver and kidney profile, Lipid profile and hematological parameters. A drop in albumin levels could be an indication of infection[19]. The differences in albumin, globulin, and bilirubin between the groups of male and female rats were not statistically significant.. According to these results, changes in urea, creatinine, and uric acid can all be used to assess kidney function[20]. There were no significant differences in BUN, serum creatinine, or uric acid levels among all groups of male and female rats. The results of the current study support previous research in indicating that demethoxycurcumin did not cause nephrotoxicity. Additionally, ursolic acid acid and EPI-orientin exhibited the hepatoprotective and nephroprotective effects. Triglycerides, cholesterol, and LDL changed in both males and females after

bioactive compounds administration, however these changes were not clinically significant. The foresaid bioactive compounds' anti-inflammatory and antihyperlipidemic properties corroborate our findings. Both EPI-orientin and urosolic acid have the ability to lower cholesterol through a variety of metabolic routes, such as boosting the conversion of cholesterol into bile acid [21] and facilitating the outflow of cholesterol. Since haematopoietic profile is a sensitive biomarker of toxicity in both people and animals, it is significant for identifying pathological and physiological status[22]. While MCH shows the concentration of haemoglobin in average red cells, MCV shows the volume of average red cells in the sample. Red blood cells' mean haemoglobin content is known as MHC. The immune system is composed of WBC and its subtypes, which include neutrophils, eosinophils, monocytes, and lymphocytes[23]. Platelets are involved in blood coagulation and are the primary markers of thrombo-embolic illnesses. With the exception of RBC, which significantly decreased in demethoxycurcumin treated groups of male rats compared to normal control groups, treatment with EPI-orientin significantly alter the hematological and lipid profile of both male and female rats. A reduction in red blood cell counts may result in anaemia. Examining the histopathological changes in the organs is thought to be a fundamental analysis in the therapeutic evaluation of the bioactive compounds[24]. The kidneys from the control and demethoxycurcumin, EPI-orientin and Ursolic acid treatment groups of both male and female rats showed typical appearance of the tubular degeneration, vacuolar alterations, and proximal and distal convoluted tubules without necrosis. Similar to the control group, liver slices from the EPI-orientin and demethoxycurcumin treated groups showed normal architecture of hepatocytes, portal triad, sinusoids, and central vein. Heart tissues from the bioactive compounds treated groups of both and female displayed heart muscle fibres with normal shape and central degeneration and fragmentation.

CONCLUSION

The current study concluded that the demethoxycurcumin, EPI-orientin and ursolic acid offered more potent organo-protective activity and with minimum side effects in magnesium-induced toxicicated males and females rats as compared to the commercially used drugs. Therefore, these bioactive compounds in suitable dose regimen would be more useful as anti-inflammatory agents than the commercial renal and hepatic drugs and can be used as a better theranostic approach towards treating hepatic, renal and cardiac inflammation in the future.

Ethical Approval: The Ethical Committee approved current study for Scientific Research (as a part of Ph.D. Dissertation) at the University of Lahore (Approval No: U.S.M./Animal Ethics approval/2009/45/140).

Supplementary Materials: Not Applicable
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