ejpmr, 2017,4(3), 267-274.

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

AMELIORATIVE EFFECT OF *EMBLICA OFFICINALIS* IN POTASSIUM DICHROMATE INDUCED TOXICITY IN RATS

Kader Asen Fathima*[1] and Kalapuram Sivaraman Priyanka[1]

Department of Biochemistry, Bharathi women's college, Chennai-600108.

Corresponding Author: Dr. Kader Asen Fathima

Department of Biochemistry, Bharathi Women's College, Chennai-600108.

Article Received on 04/01/2017 **Article Revised on 24/01/2017** **Article Accepted on 14/02/2017**

ABSTRACT

Potassium dichromate is a common inorganic chemical reagent, most commonly used as an oxidizing agent in various laboratory and industrial applications. Potassium dichromate, a Cr (VI) compound, is the most toxic form of Cr (VI) and has been demonstrated to induce toxicity associated with oxidative stress in humans and animals.A single intra peritoneal dose of Potassium dichromate (15mg/kg body weight) dissolved in sterile saline (0.9% Nacl) increased the levels of serum creatinine, urea, activity of SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase), acid phosphatase (ACP) and alkaline phosphatase (ALP) in serum and reduced the activity of SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase),alkaline phosphatase in liver tissue and acid phosphatase in kidney. Depletion in reduced glutathione (GSH) and superoxide dismutase (SOD) was observed in liver and kidney tissue. It also caused enhancement in the levels of lipid peroxidation (LPO) in liver and kidney.Pretreatment with *E. officinalis* at doses of 250 mg/kg body weight, prior to intoxication of Potassium dichromate showed significant reduction in the levels of serum creatinine, urea, activity of SGOT, SGPT, ALP, ACP and tissue LPO. There was also increase in tissue SGOT, SGPT, ALP, ACP, reduced glutathione and SOD. The results suggest that *E. officinalis* inhibits hepatic and nephro toxicity in wistar rats.

KEYWORDS: Potassium dichromate, *Emblica officinalis*, Hepatotoxicity, Nephrotoxicity.

INTRODUCTION

Liver is an important target for toxicity produced by drugs, xenobiotics and oxidative stress.[1] Exposure to chemicals used in different industrial processes may induce liver toxicity.[2, 3] because most chemicals are metabolized in liver and toxic metabolites generated through the metabolism are the main causes of liver damage. Nephrotoxicity is caused by several xenobiotic substances which damages proximal tubule, the portion of the nephron with greater sensitivity to nephrotoxic effects. Some chemicals which cause damage to proximal tubule are cadmium, hexavalent chromium, mercury and palladium.[4]Potassium dichromate ($K_2Cr_2O_7$) is a chemical compound widely used in metallurgy, chrome plating, chemical industry, textile manufacture, wood preservation, photography and photoengraving, refractory and stainless steel industries and cooling systems.[5] Potassium dichromate is a hexavalent form of Cr and has been demonstrated to induce oxidative stress and carcinogenic in nature.[6, 7, 8] *Emblica officinalis* Garten, commonly known as amla (synonym Indian gooseberry), is one of the fruits which contain bioactive components that is thought to have antioxidative properties is widely used in India as a traditional medicine.[9, 10]The present study is aimed to evaluate the ameliorative effect of Emblica officinalis in potassium dichromate induced hepatotoxicity and nephrotoxicity in male wistar rats. Liver biomarkers ALP, SGOT, SGPT and protein and Kidney biomarkers ACP, creatinine, urea and protein in serum were evaluated. To assess oxidative damage LPO, SOD and GSH were also determined in both tissues. This study was aimed to investigate the ameliorative effect of *Emblica officinalis* on potassium dichromate toxicity.Pretreatment with *Emblica officinalis* mitigated the toxic effects of potassium dichromate indicating the therapeutic potential of *Emblica officinalis*.

MATERIALS AND METHODS

Preparation of extract

The powder of dried fruit of Emblica officinalis (EF) was obtained from Ayurvedic pharmacy, Chennai and was extracted with 50% ethanol 25 g/ 100ml in soxhlet extraction assembly. The fruit extract powder dosage was fixed as 250mg/kg from previous literature.

Animals and experimental protocol

24 Male wistar rats weighing 150-200g and the age of 12-22 weeks were obtained from BRULAC, Saveetha University, chennai-77 and were housed in a ventilated room at 25 ± 5°C under a 12 h light/dark cycle. The animals were accessed free to standard laboratory feed

and water *ad libitum*. The study was approved by the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) having Registration number and date of registration: SU/BRULAC/RD/005/2014, March 20th, 2014. CPCSEA guidelines were followed for animal handling and treatment.Rats were divided into four groups of 6 animals each.Group I (Control) received normal laboratory feed and water,Group II (toxic) received 15mg/kg/bwt of potassium dichromate dissolved in sterile saline(0.9% Nacl) as single IP injection,Group III received 250mg/kg/bwt of EF in water through oral gavage for 14 days and at the end of 14th day the rats are treated with potassium dichromate (15mg/kg/bwt) and Group IV received 250mg/kg/bwt of EF in water through oral gavage for 14 days.

Blood collection

Blood was collected from retro-orbital plexus of the animal by capillary pipetting after anesthetizing the animal.Serum was separated by centrifuging at 3200 rpm for 10 mins and it was used for the study.

Tissue homogenate preparation

All the animals were sacrificed by cervical dislocation, Liver and kidney tissues were dissected, washed in physiological saline, (0.9% Nacl) homogenised for 5 min in ice-cold 0.1M Tris-HCl buffer solution (pH 7.2; 1:5 w/v) and centrifuged at 8000 rpm for 30 min. Supernatant were used for estimation of selected enzyme activities.

Biochemical parameters

Serum urea was determined by the method of Geyer and Dabich [11] and creatinine was estimated by the method of Broad and Sirota.[12] The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed following the method of Reitman and Frankel method,[13] Alkaline phosphatase (ALP) and acid phosphatase (ACP) were estimated by the method of King[14.15] in serum and tissue. The total reduced glutathione was determined by the method of Moron et al,[16]Lipid peroxide level by Yogi et al[17] and protein by Lowry et al [18].The activity of superoxide dismutase was determined by Misra and Fridovich method.[19]

Statistical analysis

Results are expressed as Mean ± SEM (from 6 experiments from each group). The statistical significance of differences between the experimental groups was calculated using "t"test. Analyses were performed using the statistical software Graph Pad InStat. Results were considered significant when $P < 0.05$. Group II compared with Group I, Group III compared with Group II and Group IV compared with Group I.*p<0.01,**p<0.001,***p<0.0001 and NS= Not significant

RESULTS

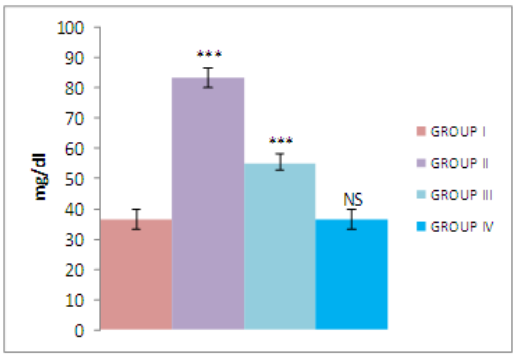

Fig-1: Effect of $K_2Cr_2O_7$ induced changes in serum urea level and its ameliorative effect by Emblica officinalis

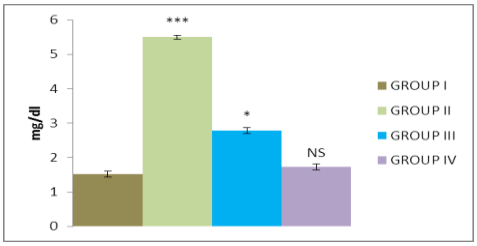

Fig-2: Effect of $K_2Cr_2O_7$ induced changes in serum creatinine level and its ameliorative effect by Emblica officinalis

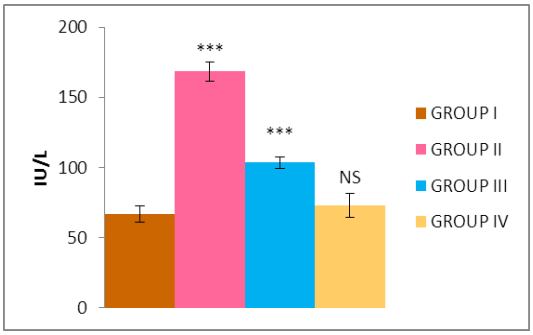

Fig-3: Effect of $K_2Cr_2O_7$ induced changes in serum ALP activity and its ameliorative effect by Emblica officinalis.

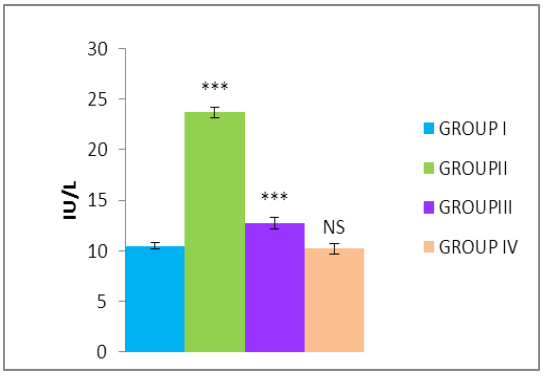

Fig-4: Effect of $K_2Cr_2O_7$ induced changes in serum ACP activity and its ameliorative effect by Emblica officinalis.

The Figure 1 and 2 indicates the levels of urea and creatinine in serum. The urea level (P<0.0001) and creatinine level (P<0.0001) were increased in Group II when compared to control. The levels of urea (P<0.001) and creatinine (P<0.01) was lowered in group III when compared to group II. The Figures 3 and 4 represents the activities of alkaline phosphatase (ALP) and Acid phosphatase (ACP) in serum. A highly significant (P < 0.0001) increase in levels of ALP and ACP were observed in group II due to potassium dichromate treatment. In group III ($K_2Cr_2O_7$ + EF treated) ALP and ACP activity were lowered to normal levels significantly (P<0.0001) compared to group II.

Table-1: Effect of Potassium dichromate induced changes in protein level in serum, liver and kidney homogenate and its ameliorative effect by Emblica officinalis

S.No	GROUP	TREATMENT	PROTEIN(g/dl)	PROTEIN(mg/g of liver tissue)	PROTEIN(mg/g kidney tissue)
1.	I	Control	3.15±0.2	3.76±0.3	3.44±0.1
2.	II	$K_2Cr_2O_7$	0.93±0.2***	0.74±0.1***	0.93±0.1***
3.	III	$K_2Cr_2O_7$ + EF	2.48±0.4***	1.88±0.1***	1.78±0.1***
4.	IV	EF	3.49±0.3NS	3.61±0.2NS	3.60±0.2NS

Table-2: Effect of Potassium dichromate induced changes in Aspartate aminotransferase activity in serum and liver homogenate and its ameliorative effect by Emblica officinalis

S.No	GROUP	TREATMENT	AST(IU/L)	AST(µm/min/mg protein)
1.	I	Control	48.0±0.5	62.67±1.7
2.	II	$K_2Cr_2O_7$	120.0±4.0***	18.3±0.6***
3.	III	$K_2Cr_2O_7$ + EF	69.67±4.6***	42.5±2.9***
4.	IV	EF	48.67±0.9NS	63.1±1.5NS

Table-3: Effect of Potassium dichromate induced changes in Alanine aminotransferase activity in serum and liver homogenate and its ameliorative effect by Emblica officinalis

S.No	GROUP	TREATMENT	ALT(IU/L)	ALT(µm/min/mg protein)
1.	I	Control	64.6±1.7	68.3±1.8
2.	II	$K_2Cr_2O_7$	109.1±2.2**	18.5±0.8**
3.	III	$K_2Cr_2O_7$ + EF	78.8±1.5***	44.33±1.9***
4.	IV	EF	65.8±2.1NS	66.3±2.4NS

The result in Table 1 depicts the protein level in serum, liver and kidney homogenate. The protein level (P< 0.0001) in group II was reduced significantly when compared to control group. The level of protein returned to normal in (P<0.0001) group III when compared to group II.

The results in Table 2 and 3 represent the activities of Aspartate amino transferase (AST) and Alanine amino transferase (ALT) in serum. A highly significant increase in levels of AST (P < 0.0001) and ALT(P < 0.001) were observed in group II due to potassium dichromate treatment whereas in group III ($K_2Cr_2O_7$ + EF treated) showed a significant (P<0.0001) change to normal levels in AST and ALT activity when compared to group II. The Aspartate amino transferase (AST) (P<0.0001) and Alanine amino transferase (ALT) (P<0.001) activity in liver homogenate was reduced significantly in group II when compared to group I. There was a significant change in the enzyme activity (P<0.0001) in group III when compared with group II as represented in the table 2 and 3.

Table-4: Effect of Potassium dichromate induced changes in reduced glutathione level in liver and kidney homogenate and its ameliorative effect by Emblica officinalis

S.No	GROUP	TREATMENT	GSH(nmoles/min/mg protein)	GSH(nmoles/min/mg protein)
1.	I	Control	14.1±0.4	14.6±0.3
2.	II	$K_2Cr_2O_7$	8.3±0.1***	8.4±0.1***
3.	III	$K_2Cr_2O_7$ + EF	11.0±0.3***	10.8±0.4***
4.	IV	EF	14.6±0.2NS	14.6±0.1NS

Table-5: Effect of Potassium dichromate induced changes in Lipid peroxide level in liver and kidney homogenate and its ameliorative effect by Emblica officinalis

S.No	GROUP	TREATMENT	LIPID PEROXIDE(µmoles/g)	LIPID PEROXIDE(µmoles/g)
1.	I	Control	3.23±0.09	3.30±0.1
2.	II	$K_2Cr_2O_7$	6.61±0.3***	6.97±0.2***
3.	III	$K_2Cr_2O_7$ + EF	5.31±0.1**	4.56±0.2***
4.	IV	EF	3.15±0.06NS	3.35±0.09NS

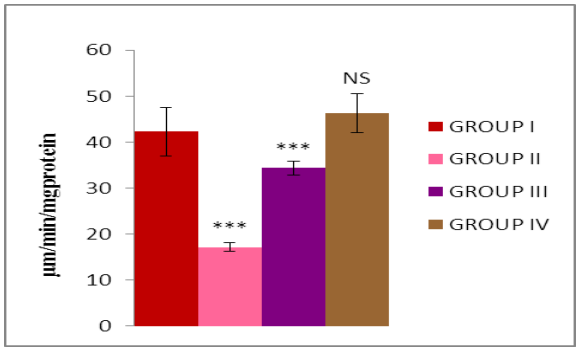

Fig-5: Effect of $K_2Cr_2O_7$ induced changes of ALP activity in liver homogenate and its ameliorative effect by Emblica officinalis.

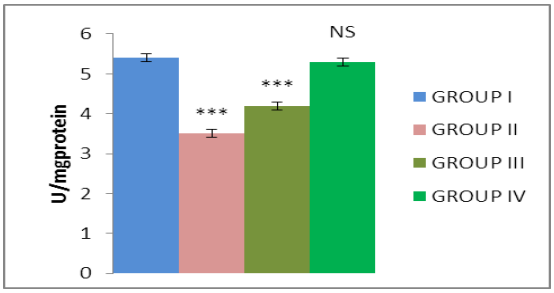

Fig-6: Effect of $K_2Cr_2O_7$ induced changes of SOD activity in liver homogenate and its ameliorative effect by by Emblica officinalis.

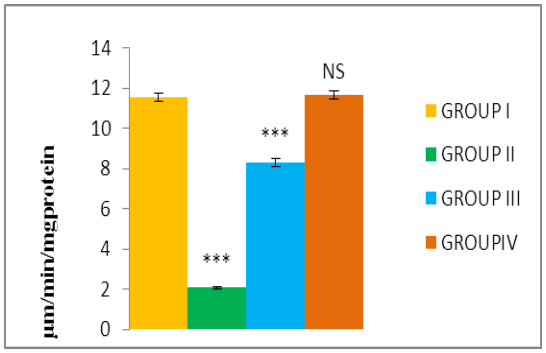

Fig-7: Effect of $K_2Cr_2O_7$ induced changes of ACP activity in kidney homogenate and it's ameliorative effect by Emblica officinalis

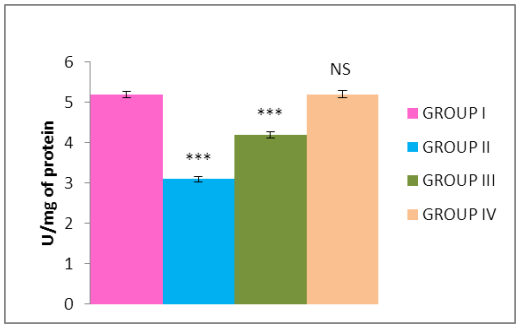

Fig-8: Effect of $K_2Cr_2O_7$ induced changes of SOD activity in kidney homogenate and its ameliorative effect by Emblica officinalis

The level of reduced glutathione in group II ($K_2Cr_2O_7$ treated) was reduced significantly (P<0.0001) when compared to non-treated group I in liver and kidney homogenate. The reduced glutathione level returned to normal significantly (P<0.0001) in group III when compared to the group II as represented in table 4.The table 5 represents the lipid peroxide level in liver and kidney homogenate in the group II ($K_2Cr_2O_7$ treated)significantly elevated (P<0.0001) when compared to group I. In group III LPO level were significantly (P<0.001) lowered in liver and kidney homogenate (P<0.0001) compared to the group II.

The alkaline phosphatase (ALP) activity (P<0.0001) and super oxide dismutase (SOD) activity in liver homogenate (P<0.0001) were reduced significantly when compared to control. The activity of Alkaline phosphatase (P<0.0001) and super oxide dismutase (P<0.0001) in group III were enhanced to normal when compared to group II as represented in the figure 5 and 6. The Figures 7 and 8 represents the activities of Acid phosphatase (ACP) and Super oxide dismutase (SOD) in kidney homogenate. A highly significant (P < 0.0001) decrease in levels of ACP and SOD were observed in group II due to potassium dichromate treatment whereas in group III ($K_2Cr_2O_7$ + EF treated) observed a significant (P<0.0001) change in ACP and SOD activity to normal when compared to group II.

DISCUSSION

Potassium dichromate ($K_2Cr_2O_7$) is a chemical compound widely used in metallurgy, chrome plating, chemical industry, textile manufacture, wood preservation, photography and photoengraving, refractory and stainless steel industries and cooling systems.[5] Emblica officinalis Gaertn (commonly known in India as Amla, Syn. Phyllanthus emblica L.; Family: Euphorbiaceae) is regarded as "one of the best rejuvenating herbs" in the Ayurveda: an Indian traditional medicinal science. Emblica officinalis extract contains several antioxidants such as emblicanin A and B, gallic acid, ellagic acid, ascorbic acid that possesses strong antioxidative activity.[20, 21] The fruit extract has many pharmacological activities for the treatment of a number of diseases and is a constituent of many hepatoprotective formulations.[22]This study was undertaken to elucidate the protective effect of Emblica officinalis in potassium dichromate induced Hepatotoxicity.

The levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and acid phosphatase are largely used to assess organ damage induced by potassium dichromate.Studies reported both hepatic markers (alkaline phosphatase, alanine and aspartate aminotransferases) and kidney function markers (urea and creatinine) were found to be significantly increased in the serum of rats treated with potassium dichromate (10 mg/kg b.w, i.p.) as compared to the control, suggesting hepatic and renal stresses by potassium dichromate. Renal damage induced by $K_2Cr_2O_7$ has been previously reported to be associated with oxidative stress.[7,23,6.24,25] Elevation of AST,ALT

and ALP is due to the leakage of this enzymes from organ into blood stream due to organ damage.These enzymes are normally located in the cytoplasm and released into the circulation after cellular injury.[26] ALP is a membrane bound enzyme,[27] while ALT and AST are cytosolic enzymes.[28] These enzymes are highly concentrated in the liver and kidney and are only found in serum in significant quantities when the cell membrane becomes leaky and even completely ruptured.[29,30] A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of damage to the liver cells.[31]Elevation of aspartate transaminase and alanine transaminase have been reported in serum of Potassium dichromate treated rats at 24 and 48 hr.[32]

Serum urea and creatinine (serum markers of kidney functions) have been considered the most important manifestation of severe tubular necrosis of kidney.[33,34] Hany A.,et al.[35] reported subcutaneous injection of a single dose of potassium dichromate caused a significant elevation ($P < 0.05$) in serum urea and creatinine. Administration of dried fruit extract of *E. officinalis* (EO) 600 mg/kg, significantly normalized ($P < 0.01$) serum creatinine and urea levels as compared to cisplatin (8 mg/kg; i.p.) - control group rats.[36]

Liver cells synthesize albumin, fibrinogen, prothrombin, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, transferrin, alpha foeto proteins and acute phase reactant proteins. The blood levels of these plasma proteins are decreased in extensive liver damage.[37]

GSH is a tripeptide (L-γ-glutamyl-L-cysteinylglycine) responsible for protection against ROS and other reactive species and detoxification of endogenous and exogenous toxins of an electrophilic nature. [38] Depletion of GSH decreases the antioxidant capacity and leads to oxidative stress. [39,40] Rats treated with $K_2Cr_2O_7$ presented low GSH levels in comparison with control, probably due to the oxidative stress induced for the $K_2Cr_2O_7$ exposure.[41] and reduction in GSH levels might be due to its consumption in the scavenging of free radicals generated by $K_2Cr_2O_7$.

Emblica officinalis extract was found to increase the level of reduced glutathione thus showing an antimutagenic activity in mice exposed in vivo to cyclophosphamide.[42] Studies on aqueous extract of Emblica officinalis revealed increase in the level of hepatic ascorbic acid and glutathione in rats.[43]

Excessive ROS production that exceeds critical levels can overwhelm all antioxidant defense strategies, causing oxidative stress.[44] As a result of ROS formation, the antioxidant defense mechanism of the cells including SOD and GSH prevent the cell death caused by these toxic radicals so their levels in the tissue homogenate were decreased specially at higher doses.[45] The significant reduction in renal SOD activity ($P <0.05$) and renal GSH content ($P< 0.05$) following potassium dichromate administration was reported by Hany A et al. [35] Potassium dichromate treated group showed increase $P< 0.05$ in hepatic tissues in the levels of MDA and a decrease $P< 0.05$ in the levels of GSH at 48h. [32] The SOD activity in the liver showed significant increase in group (Cd + 200 mg Amla) ($P < 0.0001$), in comparison to group (Cd alone),[46, 36] reported increase in the activities of GSH ($P < 0.01$) and SOD ($P < 0.05$) in kidney tisues of EO (600 mg/kg) pretreated group compared to the cisplatin (8 mg/kg; i.p.) -control rats.

There was a significant increase in the concentration of MDA after 48 h of potassium dichromate administration. These results are in accordance with those obtained by[47] who detected oxidative lipid metabolites in K562and J774 cells exposed to Cr (VI). The increase observed in lipid peroxidation may be due to the formation of hydroxyl radical through a Fenton/Haber-Weiss reaction, catalysed by chromium.This radical is capable of abstracting a hydrogen atom from a methylene group of polyunsaturated fatty acids enhancing lipid peroxidation.The significant elevation $P < 0.05$ of renal MDA content following potassium dichromate administration was reported by Hany A.,et al**.**[35] It is known that tannoid compounds found in Emblica officinalis fruit had inhibitory effect of lipid peroxidation by its free radical scavenging nature.[48] The TBARS levels in the liver and kidney were reduced significantly ($P< 0.0001$) in Cd + 200 mg Amla) treated group rats compared to the group exposed to Cd alone.[46] And reduction in the level of MDA ($P < 0.05$) was reported in kidney tisues of EO (600 mg/kg) pretreated group compared to the cisplatin (8 mg/kg; i.p.) -control rats.).[36]

Emblica officinalis contains effective broadspectrum antioxidants and free radical scavengers, helping to reduce disease and slow the aging process. The use of amla as an antioxidant has been examined by a number of authors.[49,50,51,52,53,54,55.56,57] The presence of phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, ascorbic acids and many other compounds in *Emblica officinalis* with diverse pharmacological activities have been reported in previous literature.[58]

In the present study, hepatic and renal markers were preferred because liver and kidney are major target organs of toxicity. Liver is vulnerable to toxicity by different chemicals as it is the primary organ of biotransformation of xenobiotic compounds. The present study clearly shows that pre-treatment of experimental rat models with *Emblica officinalis* prior to potassium dichromate injection caused decrease in serum levels of hepatic and renal markers, thereby providing an insight towards its effect as hepatoprotective and nephroprotective agent.The ameliorative effect suggest *Emblica officinalis* as a potential therapeutic in Indian medicine.

REFERENCES

1. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity.Toxicol Sci, 2002; 65: 166 -76.
2. Franco G, Fonte R, Candura F.Hepatotoxicity of organic solvents.Br J Ind Med, 1986; 43: 139 -2.
3. Franco G.New Perspectives in biomonitoring liver function by means of serum bile acids: Experimental and hypothetical biochemical basis.Br J Ind Med, 1991; 48: 557-561.
4. Cristofori P, Zanetti E, Fregona D, Piaia A &Trevisan A Renal proximal tubule segment – Specific nephrotoxicity: an overview on biomarkers and histopathology.Toxicol pathol, 2007; 35(2): 270-5.
5. Barceloux D G, Chromium. Journal of toxicology-clinical toxicology , 1999; 2(37): 173 – 194.
6. Stohs SJ and Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med, 1995; 18(2): 321-36.
7. Bagchi D, Balmoori J, Bagchi M, Ye X, Williams C B and Stohs SJ.Comparative effects of TCDD, endrin, naphthalene and chromium VI on oxidative stress and tissue damage in the liver and brain tissues of mice, Toxicology, 2002; 175: 73 - 82.
8. Bagchi D, Stohs S J, Downs BW, Bagchi M and Preuss H, Cytotoxicity and oxidative mechanisms of different forms of chromium.Toxicol, 2002; 180: 5-22.
9. Ghosal S, Tripathi VK, Chauhan S. Active constituents of Emblica *officinalis*. Part I, the Chemistry and antioxidative effects of two hydrolysable tannins, emblicanin A and B Indian J Chem, 1996; 35(B): 941–948.
10. Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK. Antioxidant actavity of active tannoid principles of *Emblica officinali*s (amla) Ind J Exp Biol, 1999;37:676–680.
11. Guyer JW, Dabich G. Rapid method for the determination of arginase activity in tissue homogenates, Annals of Biochemistry, 1971; 39: 412.
12. Broad.J, Sirota JH. Renal clearance of endogenous creatinine in man, Journal of clinical Investigation, 1948; 27: 645.
13. Reitman, S; Frankel, S.Glutamic – pyruvate transaminase assay by colorimetric method. Am. J. Clin. Path, 1957; 28: 56.
14. King, J. The transferase alanine and aspartate transaminase. In: Van, D. (Ed.). Practical clinical enzymology, London; Nostrand Company Ltd: 1965a, 363-395.
15. King, J. The dehydrogenases or oxidoreductases lactate dehydrogenase. In: Van, D. (Ed.), Practical clinical enzymology, London; Nostrand Company Ltd: 1965b; 83-93.
16. Moron MS, Despierre JW & Minnervik B Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica Biophysica Acta Biomembranes, 1979; 582: 67 -78.
17. Yogi K., et al. Lipid peroxide and human diseases. Chemistry and Physics of Lipids, 1999; 45: 337–351.
18. Lowry OH, Rosenbrough NJ, Farr AI & Randall RJ. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry, 1951; 193: 265 -275.
19. Misra HP, and Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem, 1962; 247: 3170- 3174.
20. Pozharitskaya ON, Ivanova SA, Shikov AN, Makarov VG. Separation and evaluation of Free radical-scavenging activity of phenol components of *Emblica officinalis* extract by using an HPTLC-DPPH method. J Sep Sci, 2007; 30: 1250 -1254.
21. Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C. Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation *Emblicaofficinalis Gaertn*. J Ethnopharmacol, 2006; 104: 113–118.
22. De S, Ravishankar B, Bhavsar GC.Plants with hepatoprotective activity - a review. Indian Drugs, 1993; 30: 355 - 363.
23. Liu KJ and Shi X. In vivo reduction of chromium (VI) and its related free radical generation.Mol Cell Biochem, 2001; 222(1-2): 41-7.
24. Huang YL et al. Lipid peroxidation in workers exposed to hexavalent chromium. J Toxicol Environ Health A, 1999; 56(4): 235-47.
25. Sengupta T et al. Impact of chromium on lipoperoxidative processes and subsequent operation of the glutathione cycle in rat renal system. Indian J BiochemBiophys, 1992; 29(3): 287-90.
26. Nam-Hun Lee, Chang-Seob Seo, Ho-young Lee, Da-Young Jung, Jun-Kyung Lee, Jin-Ah Lee, Kye Yong Song, Hyeun-kyoo Shin, Mee-Young Lee, Young Bae Seo, Hokyoung Kim, and Hyekyung Ha.Hepatoprotective and antioxidative activities of Cornusofficinalis against Acetaminophenol induced hepatotoxicity in mice. Evidence-Based Complementary and Alternative Medicine, 2012: 1-8.
27. Wright P. J. and D. J. Plummer.The use of urinary enzyme measurements to detect renal changes caused by nephrotoxic and treatment. compounds. Biochem.Pharmacol, 1974; 12: 65- 68.
28. Braunstein A E.In: The Enzymes, 3rd edition (P.D.Boyer, Ed), New York; Academic Press: 1973; 9: 379 - 481.
29. Cotran R., Kumar V and Robins S. Robin's pathological basis of disease.4th edn. Harcour; W.B Saunders Co: 1989; 212-217.
30. Ngaha E.O. Renal effects of potassium dichromate in the rat: composition of urinary excretion with corresponding tissue pattern. Gen Pharmacol, 1981; 12: 291-358.

31. Moss D.W and Rosalki SBv. Enzyme Tests in Diagnosis.2nd Edition. London; Edward Arnold: 1996, Pp: 201-213.
32. WR, Tapia E, Cecilia Zazueta C, Zyanya Lucía Zatarain -Barron ZL, Hernandez-Pando R, Vega-García CC, andJose Pedraza-Chaverrí J**.** Curcumin Pretreatment Prevents Potassium Dichromate-Induced Hepatotoxicity, Oxidative Stress, Decreased Respiratory Complex I Activity, and Membrane Permeability Transition Pore Opening**.**Evidence-Based Complementary and Alternative Medicine Volume 2013 (2013): 19 pages.
33. Ali B H, Ben Ismail T H and Bashir A A, Sex difference in the suspectibility of rats to the gentamicin nephrotoxicity: Influence of gonadectomy and hormonal replacement therapy, Indian J pharmacology, 2001; 33: 369-373.
34. Afzal M, Khan NA, Ghufran A, Iqbal A, Inamuddin M. Diuretic and nephroprotective effect of Jawarish Zarooni Sada, a polyherbal formulation. J. Ethnopharmacol, 2004; 91: 219-23
35. Hany A. Mehany, Amira M. Abo-youssef, Lamiaa A. Ahmed, El- Shaimaa A. Arafa, Hekma A. Abd El-Latif. Protective effect of vitamin E and atorvastatin against potassium dichromate-inducednephrotoxicity in rats. Beni-Suef University Journal of Basic and Applied Sciences, 2013; 2(2): 96-102.
36. Malik S, Suchal K, Bhatia J, KhanSI, Vasisth S, Tomar A, Goyal S,Kumar R, Arya DS, and Ojha SK Therapeutic Potential and Molecular Mechanisms of *Emblica officinalis* Gaertn in Countering Nephrotoxicity in Rats Induced by the Chemotherapeutic Agent Cisplatin. Front Pharmacol, 2016; 7: 350.
37. Harsh Mohan. Inflammation and Healing, Text book of Pathology, New Delhi; Ed Jaypee Publication: 2002; 114-121.
38. Lushchak VI, Glutathione homeostasis and functions: potential targets for medical interventions. Journal of Amino Acids, 2012; 2012: 26 pages.
39. Gutierrez LLP, Mazzotti NG, Araujo ASR, Klipel RB, Fernandes T RG, Llesuy S F and Bello-Klein A.Peripheral markers of oxidative stress in chronic mercuric chloride intoxication, Brazilian Journal of Medical and BiologicalResearch,2006; 39(6): 767–772.
40. Whillier S, Kuchel P, and Raftos J, "Oxidative stress in type II diabetes mellitus and the role of the endogenous antioxidant glutathione," in Role of the Adipocyte in Development of Type 2 Diabetes, InTech Europe, Croatia, C. Croniger, Ed: 2011: 129–152.
41. Soudani N, Ben Amara I, Sefi M, Boudawara T, Zeghal N.Effects of selenium on chromium (VI)-induced hepatotoxicity in adult rats,Exp. Toxicol. Pathol, 2011; 63: 541–548.
42. Sharma N, Trikha P, Athar M, Raisuddin S. Inhibitory effectof Emblicaofficinalis on the in vivo clastogenicity of benzo (α) pyrene and cyclophosphamide in mice. Human Exp Biol, 2000; 19: 377–84
43. Anilakumar KR, Nagaraj NS, Santhanam K. Protective effects of amla on oxidative stress and toxicity in rats challenged with dimethylhydrazine. Nutrition Res, 2004; 24: 313–9.
44. Sikka SC .Relative impact of oxidative stress on male reproductive function, Curr Med Chem, 2001; 8: 851–862.
45. Amin KA and Abd Elsttar AH. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats, 2010; 48(10): 2994-9.
46. Virk P, Elobeid M, Hamad S, Korany Z, Al-Amin M, Daghestani M, Omer S, AlOlayan E, Siddiqui MI and Mirghani NM. Ameliorative effects of *Embilica officinalis* and *Rosmarinus officinalis* on cadmium-induced oxidative stress in Wistar rats.Journal of Medicinal Plant Research, 2013; 7(14): 805-818.
47. Bagchi D, Bagchi M and Stohl SJ. Chromium (VI)-induced oxidative stress, apoptotic cell Death and modulation of p53 tumor suppressor gene.Mol. Cell Biochem, 2001; 222, 149-158.
48. Verma R and Chakraborty D. Emblicaofficinalis aqueous extract ameliorates ochratoxin- induced lipid peroxidation in the testis of mice. Acta Pol Pharm, 2008; 65: 187–194.
49. Kumaran A and Karunakaran RJ. Nitric oxide radical scavengingactive components from Phyllanthus emblica L. Plant Foods Hum Nutr, 2006; 61(1): 1-5.
50. Rao TP, Sakaguchi N, Juneja LR, Wada E, Yokozawa T. Amla (Emblica officinalis Gaertn.) extracts reduce oxidative stress in streptozotocin-induced diabetic rats. J Med Food, 2005; 8(3): 362-368.
51. Naik GH, Priyadarsini KI, Mohan H. Evaluating the antioxidant activity of different plant extracts and herbal formulations. Res ChemIntermed, 2005; 31(1-3): 145-151.
52. Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contentsand antioxidant activity of some food and medicinal plants. Int J Food SciNutr, 2005; 56(4): 287-291.
53. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Effect of bioactive tannoid Principles of Emblicaofficinalis on ischemia reperfusion-induced oxidative stress in rat heart. Phytomedicine, 2002; 9(2): 171-174.
54. Anila L, Vijayalakshmi NR. Flavonoids from Emblica officinalis and Mangifera indica – effectiveness for dyslipidemia.JEthnopharmacol, 2002; 79(1): 81-87.
55. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnopharmacol, 2000; 71(1-2): 23-43.
56. Bandyopadhyay SK, Pakrashi SC, Pakrashi A. The role ofantioxidant activity of Phyllanthusemblica

fruits on prevention fromindomethacin induced gastric ulcer. J Ethnopharmacol, 2000; 70(2): 171-176.
57. Chaudhuri, Ratan K. Guttierez, Gilles and Serrar, Mustafa: LowMolecular-Weight Tannins of Phyllanthusemblica: A New Class of Anti-Aging Ingredients. Proceedings Active Ingredients Conference, Paris.2003
58. Meena A K, Singh A, Rao MM, Evaluation of physicochemical and preliminary phytochemical studies on the fruit of Emblica officinalis gaertn. Asian J of pharm, 2010: 3(3): 242-243.