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Effects of melatonin, vitamin Cand E alone or in combination on lead-induced injury in liver and kidney organs of rats

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Abstract
***Context** Lead is known to disrupt the biological systems by altering the molecular interactions, cell signaling, and cellular functions.*
***Objective**: The present study was designed to investigate the potential protective effect of melatonin, vitamin C, vitamin E and their combinations against the lead induced hepatic and renal injuries in male rats.*
***Materials and Methods:** Fifty four adult male albino rats were used in this study. The experimental rats were randomlydistributed into nine groupsand they were treated for 10 weeks as the following: Group 1: Control rats, Group 2: Sodium acetate(0.1mg/L drinking water), Group 3: Lead acetate (Pb)(0.1mg/L drinking water), Group 4: Pb + Melatonin(60 mg of melatonin/kg diet), Group 5: Pb+ Vitamin E. (1000 I.U of vitamin E /kg diet),Group 6: Pb +Vitamin C (1mg of vitamin C/L drinking water), Group 7(Combination): Pb +Melatonin + Vitamin E,Group 8 (Combination): Pb + Melatonin +Vitamin C, and Group 9(Combination): Pb +Vitamin E+ Vitamin C.*
***Results**: Significant elevations in malondialdehyde (MDA) levels wererecorded after10 weeksinPbexposedratsin comparison with control group. Melatonin, vitamin C and vitamin E caused significant reduction in serum MDA levels. Interestingly, the significant reduction in MDA level caused by melatonin-vitamin E combination was more than that caused by melatonin and vitamin E alone.**Conclusion**, long term lead-treated rats exhibited remarkable elevation in oxidative stress, which consequently causes liver and kidney injuries. These alterations were nearly restoredby the anti-oxidative effects of vitamin E alone or in combination with melatonin in comparison with melatonin, vitaminC and their combinations.*

***Keywords**—Lead (Pb), Oxidative stress,liver, kidney, Melatonin, Vitamin Eand C*

I. INTRODUCTION

Lead is known to disruptbiological systemsbyalteringthe molecular interactions, cellular signaling, and cellular functions. Several studiessuggestedthat lead is responsibleforexcessivereactive oxygen species (ROS) production in experimentalanimals (Gonick*et al*.

1997). Significant increasesin aspartateaminotransaminase (AST), alaninaminotransaminase (ALT) and creatininelevels were reported in lead acetateexposedrats (Ozsoy*et al*., 2011).Liu et al. (2005) reported that Pbis involved in increasing lipopolysccaharides (LPS)-induced liver damage, along with increased nitric oxide (NO)-initiated oxidative stress and tumor necrosisfactoralpha(TNF-α)production in rats.

Abdel Moneim*et al*. (2011) concluded that lead induced injury of the renal tissue is mediated by an increase in lead concentrations in the kidney and eventually an increase in lipid peroxidation, nitric oxide and reactive oxygen species production.

On the other hand, melatonin considered as a potent antioxidant agent because it has a high potential in scavenging hydroxyl, superoxide, peroxynitite anion, singlet oxygen and nitric oxide free radicals (Paulis and Simko, 2007). The anti-oxidative action of melatonin is mediated byactive stimulation or synthesis of enzymes that metabolize toxic reactant such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase (Leon*etal*., 2004). Unlike other antioxidantcompoundsmelatoninis notgoingthroughrodoxcycling. Once melatonin is oxidized it cannot be reduced to its former state because melatonin forms several stable end products upon reacting with free radicals (Pechanova*et al*., 2006).

Melatonin can easily cross cell membrane and blood brain barrier (Hardeland, 2005). Melatonin has a protective effect against lipid peroxidation in rats (Rodriguez *etal*., 2004).In their study Quiroz *et al*.(2008) concluded that melatonin ameliorates oxidative stress, inflammation, proteinuria,and progression of renal damage in rats with renal mass reduction.

Successful recoveries were reported after using melatonin in various nephrotoxic models (Parlakpinar*et al*., 2003) and against ischemia/reperfusion injuries in rats (Rodriguez-Reynoso*et al*., 2004).Deterioration of renal function (plasma creatinine and protein urea) and structure (glomerulosclerosis) in rats,as consequence of chronic renal failure, were ameliorated with melatonin treatment (Quiroz *et al*., 2007). Yousef*et al*.,(2011) reported that melatonin has a protective effectagainstcolistin-induced nephrotoxicity, whichis caused by oxidative stress.

It is suggested that melatonin has hepatoprotective effects against toluene toxicity primarilyviaantioxidativeproperties(Tas*et al*.,2011).

While in regard to antioxidative power, melatonin has a better antioxidative potential in comparisonwith other antioxidants such as vitamin Eagainst free radicals induced by radiation in rat testis (Yalcinkaya*et al*., 2009).

Vitamin E attenuated the progression of renal fibrosis in obstructed kidneys. The renoprotective effect of vitamin E could be mediated by inhibition of TGF-beta/Smad2/3 signaling pathway (Tasanarong*et al*., 2011). Yurdakul*et al*., (2010) reported that pretreatment with α-tocopherolantioxidantreducing lipid peroxidation of renal cellular membranes in normothermic renal ischemia-reperfusionmodelrats.

Oxidative stress related damages areoccurring earlier than necrosis in the kidney and heart. In case of steatosis, prior to vitamin E consumption and increased lipid peroxidation, no necrosis is observed in the liver (Repetto*etal.,* 2010). Wang*et al*., (2007) foundthatVitaminCcan lessen the damage in liver cells from lead-induced oxidative stress. The present study was designed to investigate the potential protective effect of melatonin, Vitamin C, vitamin E and their combination against the hepatic and renal toxicity in lead exposed rats.

II. MATERIALS AND METHODS

Animals and housing

Fifty four adult female albino rats (*Rattusnorvegicus*) were used in this study. All rats were weighing about (240 - 280 gm) and (7-9 weeks old) at the time when the experiment started. Animals were housed in plastic cages bedded with wooden chips. They were housed under standard laboratory conditions, 12:12 light/dark photoperiod at 22 ± 2 ºC. The animals were given standard rat pellets and tap water *ad libitum*.

Experimental Design

This experiment was planned to study the effects of melatonin, vitamin E ,vitamin C and their combinations on SBP, heart rate, serum NO,sodium, potassium , calcium and body weight in rats treated with lead acetate. The experimental rats were divided into nine groups, each with six individuals and the treatments were continued for 10 weeks as following:

Group 1: Control. The rats were given standard rat chow and tap water *ad libitum.*

Group 2: Sodium acetate. The rats were given standard rat chow and sodium acetateat dose (0.1mg/L drinking water).

Group 3: Lead acetate (Pb). The rats were given standard rat chow and lead acetateat dose (0.1mg/L drinking water).

Group 4: Pb + Melatonin. The rats were supplied with standard rat chow with melatonin (60 mg/kg diet) and Pb

Group 5: Pb +Vitamin E. The rats were supplied with standard rat chow with vitamin E (1000 I.U/kg diet) and Pb

Group 6: Pb +Vitamin C. The rats were given standard rat chow withPband vitamin C at dose (1mg/L drinking water).

Group 7: Pb + Melatonin + Vitamin E. The rats were supplied with standard rat chow with melatonin, vitamin E andPb.

Group 8: Pb + Melatonin +Vitamin C. The rats were supplied with standard rat chow with melatonin, vitamin CandPb.

Group 9: Pb +Vitamin E+ Vitamin C. The rats were supplied with standard rat chow with vitamin E,vitaminandPb.

III. COLLECTION OF BLOOD SAMPLES

At the end of the experiment, the rats were anesthetized with ketamine hydrochloride (50 mg/kg). Blood samples were taken by cardiac puncture into chilled tubes and centrifuged at 3000 rpm for 20 minutes; then sera were stored at -85C^0 until assay.

Determination of serum malondialdehyde (MDA)

The level of serum MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief to 150µl serum sample added the followings: 1ml trichloroacetic acid (TCA) 17.5 %, 1ml of 0.66 % TBA, mixed well by vortex,incubate it in boiling water for 15 minutes, & then allowed to cool. Then add 1ml of 70 % TCA, and let the mixture to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, & take out the supernatant for scanning spectrophotometrically. The concentration of MDA calculated as follow:

MDA (µmol/L) = Absorbance at 532 nm x D/ L x Eo

Where L: light bath (1cm)

Eo: Extinction coefficient 1.56 x 105 M-1.Cm-1

D: Dilution factor = 1 ml Vol. used in ref. / 0.15 =6.7

Histological procedure:

Liver and kidney were removed and fixed in Bouins fluid, dehydrated, cleared, embedded in paraffin and cut into 4-5µm thick section,then stained by hematoxylin and eosin (Murice-Lambert et al., 1989).

Statistical analysis

All data were expressed as means ± standard error (SE) and statistical analysis was carried out using available statistical soft ware (SPSS version 15). Data analysis was made using one-way analysis of variance (ANOVA). The comparisons among groups were done using Duncan post hoc analysis. P values <0.05 were considered as significant

IV. RESULTS

As seen in Figure (1), significant elevation in MDA (µmol/L) levels were resulted after10 weeksofPb administration (2.485 ±0.118) compared to control group (1.835± 0.032).Melatonin, vitamin C and E caused significant reduction in serum MDA levels. Interestingly, both vitamin E alone and combination of melatonin with vitamin E statistically reduced serum MDAmore than the other treatments in comparison to Pb group.

As shown in Fig (2a), control group showed normal histological structure of rat liver. This structure is disturbed when lead acetate was administered. The most characteristic features of this hepatotoxicity were dilatation of sinusoid lumen, fatty changes in hepatocytes and inflammation in region adjacent to blood vessels (Fig 2a & b). Fatty changes and degeneration of hepatocytes were seen in melatonin treated rat liver but dilatation of blood sinusoid was attenuated (Fig 2d).As shown in Fig (3a), little dilatation is still seen in the liver of vitamin C plus E as well as few dead hepatocytes, while in melatonin plus vitamin C treated rats, the lumen of the sinusoids was returned to normal, but large number of pyknotic nuclei were still exist (Fig. 3b). Approximately normal histological structure of liver was observed in the remaining groups(Fig. 3c-f) especially the melatonin plus vitamin E group in which no inflammation, blood sinusoids lumen dilatation and hepatocytes degeneration were seen(Fig. 3f). Signs of restoring normal structure were seen in some sections in which mitotic figures were detected (Fig. 3e).

Signs of kidney tubule lumen dilatation, inflammation and kidney cells degeneration were detected in the kidney of rats exposed to lead acetate in comparison to the natural histological structure of control group (Fig. 4a & b). These abnormal features didn't attenuated after administrating the studied antioxidants or their combinations except the melatonin plus vitamin E which showed approximately normal histological structure compared to other groups (Fig. 4c-f).

V. DISCUSSION

After 10 weeks of Pb administration, serum MDA levels were significantly increased compared to the control group.Several studies have suggested the primary involvement of the increased production of ROS in lead-exposed animals (Gonicket al., 1997).The possible mechanism of this elevation may be related to an increase in NO levels by lead administration.Liu et al.,2005 concluded that Pb increased LPS-induced liver damagemight be associated with increased NO-initiated oxidative stress andTNF-alpha in rats .As mentioned from the present results, melatonin caused a significant reduction in serum MDA levels. This result may be due to antioxidant properties of melatonin which it can scavenge free radicals. The same result was obtained by Paulis and Simko, (2007), they concluded that the potent antioxidant ability of melatonin can be explained by the potential to scavenge hydroxyl, superoxide, peroxynitite anion, singlet oxygen but also nitric oxide free radical. Furthermore, the antioxidative action of melatonin may be mediated by the active stimulation or synthesis of enzymes that metabolize toxic reactant such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase (Leon et al., 2004).

Vitamin C and E also reduced serum MDA levels significantly.Jewo*et al.,* (2012) obtained that vitaminC normalizeMDAlevelsand attenuate changes in the levels of catalase and superoxide dismutase.Also Ferreira et al., (2012) resulted thatadministration of vitamins C significantly prevented the increase of lipid peroxidation.UlasandCay(2011) obtained the same result and concluded that alpha-tocopherolsupplementations

to diabetic rats may strengthen the antioxidant defense system by reducing lipid peroxidation.Interestingly, melatonin in combination with vitamin E statistically reduced serum MDA compared to Pb group more than melatonin alone and vitamin in combination with vitamin E.

Histologically, the results have supported the above biochemical finding whether for the effect of lead acetate or the protective effect of the antioxidants. The degenerative effect of lead acetate on liver and kidney, according to the oxidation-antioxidation action is due to the production of ROS which induces cell death (Ramaekers*et al*., 1997). Many modes of cell death wereexpected(i.e. apoptosis and necrosis) but this must be further checked using special staining. From the histological slides of liver and kidney, it seemed that vitamin E showed the best results with respect to the protective role against lead acetate effect. This is related to the strong antioxidant power of this vitamin(Piechota and Goraca 2009), but also may refer to the chelating property of this vitamin for washing out metals from the blood circulation (Jomova and Valko2011).The good attenuating results obtained in the combination of melatonin with vitamin E may be due to the good protective role of vitamin E rather to the effect of melatonin.Piechota and Goraca 2009 work has supported the higher antioxidant power of vitamin C and E in comparison to melatonin. Although vitamin C and E and melatonin are considered as good antioxidants, but the present investigation showed conflicting results concerning their protective role against lead toxicity and this may be related to the effect of this metal on vitamins concentration in certain rat organs. Such effect has been detected in cadmium treated rats in which itinduced a decreased vitamin C concentration in the liver and kidneys, while itincreased the concentration of Vitamin E in the rat liver, kidneys and plasma (Ognjanović*et al*., 2003). We are not certain about the effect of lead on the antioxidant system especially vitamin C and E and this is left for future studies.

REFERENCES

[1] Abdel Moneim AE, Dkhil MA, Al-Quraishy S.(2011).The protective effect of flaxseed oil on lead acetate-induced renal toxicity in rats . J Hazard Mater, 30, 194:250-5.

[2] El-Sokkary GH, Abdel-Rahman GH, Kamel ES. (2005) Melatonin protects against lead-induced hepatic and renal toxicity in male rats.Toxicol, 15, 213(1-2):25-33.

[3] Ferreira AG, Stefanello FM, Cunha AA, da Cunha MJ, Pereira TC, Bonan CD, Bogo MR, Netto CA, Wyse AT.(2011) Role of antioxidants on Na(+),K (+)-ATPase activity and gene expression in cerebral cortex of hyperprolinemicrats.Metab Brain Dis,26(2):141-7.

[4] Gonick, H.C, Ding Y, Bondy S.C, Ni Z, VaziriN.D.(1997). Lead-inducedhypertension: interplay of nitric oxide and reactive oxygen species. Hypertension 30, 1487–92.

[5] Hardeland, R.. (2005). Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine, 27: 119-130.

[6] Jewo PI, Duru FI, Fadeyibi IO, Saalu LC, Noronha CC. (2012) .The protective role of ascorbic acid in burn-induced testicular damage in rats.Burns,38(1):113-9

[7] Jomova K, Valko M. (2011).Advances in metal-induced oxidative stress and human disease. Toxicol, 10, 283(2-3):65-87.

[8] Leon, J, Acuna-Castroviejo D, Sainz R.M, Mayo JC, Tan DX. and Reiter RJ.(2004). Melatonin and mitochondrial function. Life Sci, 75: 765-790.

[9] Murice-Lambert, E., A. Banford and R. Folger. (1989). Histological Preparation of Implanted Biomaterials for Light Microscopic Evaluation of the Implant-Tissue Interaction. Biotech Histoche, 64(1):19-24.

[10] Ognjanovic BI, Pavlovic SZ. , MaleticSD..Ikic RV., Stain AS., Radojicic RM.,SaicicZS.,Petrovic VM. (2003). Protective Influence of Vitamin E on Antioxidant DefenseSystem in the Blood of Rats Treated with Cadmium. Physiol. Res, 52: 563-570.

[11] Ozsoy SY, Ozsoy B, Ozyildiz Z, Aytekin I.(2011) Protective effect of L-carnitine on experimental lead toxicity in rats: a clinical, histopathological and immunohistochemical study. Biotech Histochem , 86(6),436-43.

[12] Parlakpinar H, Ozer M, Sahna E, Vardi N, Cigremis Y, Acet A. (2003).Amikacin-induced acute renal injury in rats: protective role of melatonin. J Pineal Res, 35:85–90

[13] PaulisL. andSimko F. (2007). Blood pressure modulation and cardiovascular protection by melatonin: Potential mechanism behind. Physiol Res, 56: 671-687.

[14] Pechanova, O, Zicha J, Paulis L, Zenebe W, Dobesova Z, Kojsova S, Jendekova L, Sladkova M, Dovinova I, Simko F and Kunes J. (2006). Effect of N-acetylcysteine and melatonin in adult SHR with established hypertension. Submitted to Eur J Pharmacol.PediatrNephrol, 22: 2011–2022.

[15] Piechota A, Goraca A.(2009).The comparison of alpha-lipoic acid, melatonin, vitamin C and trolox effectiveness in decreasing DNA stand brakes and increasing plasma antioxidant power. Pol MerkurLekarski, 27(157),19-21.

[16] Quiroz Y,Atilio F, Freddy Ro, Nosratola DV, and Bernardo R.(2008). Melatonin ameliorates oxidative stress, inflammation, proteinuria, and progression of renal damage in rats with renal mass reduction, Am J Physiol Renal Physiol, 294,F336–F344.

[17] Ramaekers, V., B. Bosman, G. Jansen and R. Wanders.(1997). Increased plasma malondialdehyde associated with cerebellar structural defects. Arch Dis Child, 77: 231-234.

[18] Repetto MG, Ossani G, Monserrat AJ, Boveris A.(2010).Oxidative damage: the biochemical mechanism of cellular injury and necrosis in choline deficiency.ExpMolPathol, 88(1),143-9.

[19] Rodriguez, C., Mayo J.C., Sainz R.M., Antoli I., Herrera F., Martin V. and Reiter R.J. (2004). Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal. Res, 36: 91-9.
[20] Rodriguez-Reynoso S, Leal C, Portilla-de Buen E, Castillo J, Ramos- Solano F. (2004). Melatonin ameliorates renal ischemia/reperfusion injury. J Surg Res, 116:242–247
[21] Tas U, Ogeturk M, Meydan S, Kus I, Kuloglu T, Ilhan N, Kose E, Sarsilmaz M.(2011) Hepatotoxic activity of toluene inhalation and protective role of melatonin.ToxicolInd Health, 27(5),465-73.
[22] Tasanarong A, Kongkham S, Duangchana S, Thitiarchakul S, Eiam-Ong S.(2011) Vitamin E ameliorates renal fibrosis by inhibition of TGF-beta/Smad2/3 signaling pathway in UUO mice.J Med Assoc Thai.7:S1-9.
[23] Ulas M, Cay M (2011) Estradiol and vitamin E modulates oxidative stress-induced kidney toxicity in diabetic ovariectomizedrat.Biol Trace Elem Res,144(1-3):821-31
[24] Wang C, Liang J, Zhang C, Bi Y, Shi X, Shi Q (2007) Effect of ascorbic Acid and thiamine supplementation at different concentrations on lead toxicity in liver. Ann OccupHyg, 51(6), 563-9.
[25] YalcinkayaFR ,GokceA. , Guven EO. ,Davarci M. ,Cikim G , Yekelerand H., Balbay MD.(2009). Protective Effect of Vitamin E and Melatonin Against Radiation Induced Damage in Testes of Rats.J Animal VeterAdv, 8(11): 2335-40
[26] Yousef ,M. J, Gong C, Prue AH, Roger LN, and Jian L (2011). Melatonin Attenuates Colistin-Induced Nephrotoxicity in Rats, Antimicrob. Agents Chemoth, 4044–49.
[27] Yurdakul T, Kulaksizoglu H, Pişkin MM, Avunduk MC, Ertemli E, Gokçe G, Barişkaner H, Byükbaş S, Kocabas V.(2010).Combination antioxidant effect of α-tocoferol and erdosteine in ischemia-reperfusion injury in rat model.IntUrol Nephrol,42(3), 647-55.

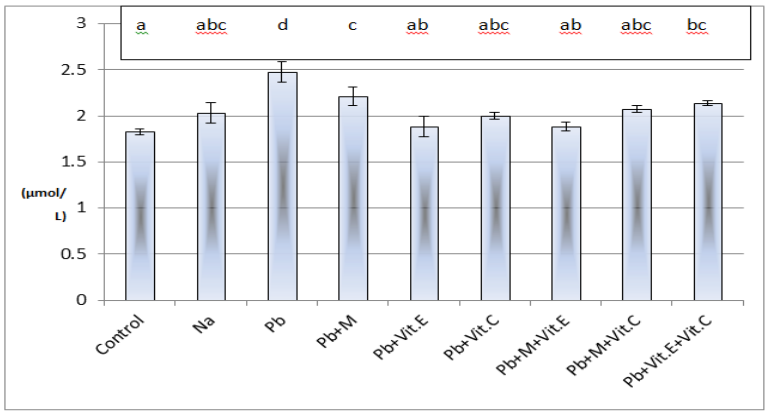

Figure 1: Effect of melatonin, vitamin C, vitamin E and their combination on serum MDA levels in

Pbtreated rats. The data presented as mean ± SE measured after 10 weeks of the treatments in all groups (Control rats.Sodium acetate, Lead acetate (Pb), (Pb + Melatonin.,Pb+ Vitamin E.,Pb +Vitamin C,Pb +Melatonin + Vitamin E,Pb + Melatonin +Vitamin C, andPb +Vitamin E+ Vitamin C. The same letters mean no significant differences while the different letters mean significant differences at $p<0.05$

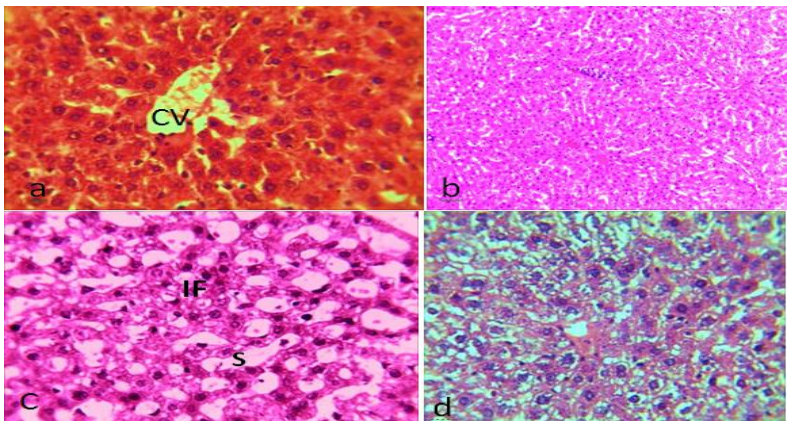

Fig 2: Liver sections in: a) control showing healthy hepatocytes, sinusoids and central vein (CV), b) Lead acetate treated group showing pyknotic hepatocytes, fatty changes and blood sinusoid dilatation (S), c) Lead acetate treated group showing inflammatory foci (IF), d) Melatonin-treated group showing degenerated and fat accumulated hepatocytes, , all magnification= 400X.

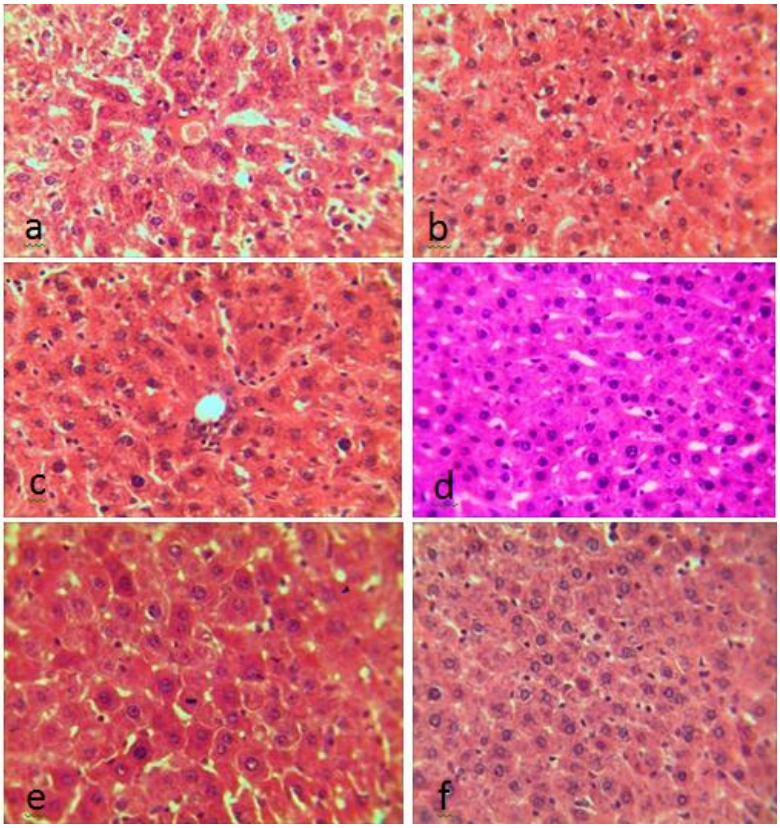

Fig 3: Liver sections in: a) Vit C+E treated group showing swelled of hepatocytes, b) Melatonine +Vit C treated group, c) sodium acetate treated group, d)Vit C treatedgroup, e) Vit E treated group, note the mitotic figure, f)Melatonin +Vit E treated group, All mag.=400X.

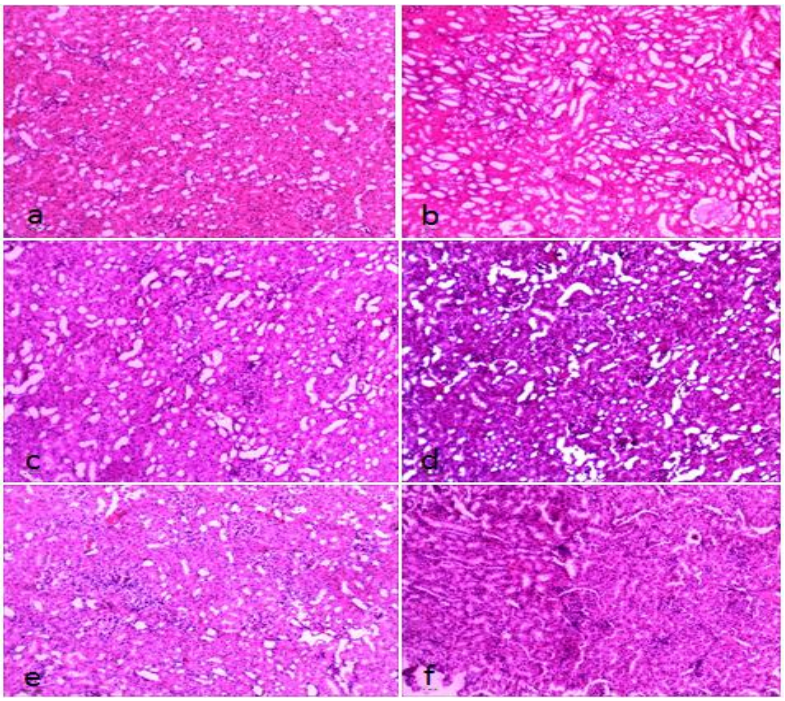

Fig 4: Sections in rat kidney: a) control, b) lead acetate treated group,c) VitC+Etreated group, d) Melatonin + Vit C treated group,e) Vit C treated group showing little dilatation but inflammation stillexist, f) Melatonin + Vit E treated group showing approximately normalkidney structure.