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Ameliorative Roles of Vitamins C, E and DMSA on Hepatic Renal Functions in Male Albino Rats.

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Abstract:This study was carried out to estimate the toxic effect of lead acetate on hepatic and renal functions of rats , and the possible ameliorative effect of both vitamin C and E , and their combination with DMSA against lead intoxication . Thirty male albino rats were divided into five groups and administrated the following treatments for six weeks . group 1 received drinking water and served as control; group 2 received 100 ppm lead acetate; group 3 received 100 ppm lead acetate combined with 50 mg/kg b.wt of DMSA (ip) ; group 4 received 100 ppm lead acetate plus 160 mg/kg b.wt of vitamin C combined with 50 mg / kg b.wt of vitamin E; group 5 received in addition to lead acetate both vitamin C and E plus DMSA in the same previous dose . Lead acetate administrated induced a significant decrease in plasma alkaline phosphatase (ALP) , total proteins and plasma albumin , while it induced a significant increase in the plasma level of acid phosphatase (AP) , compared to the control. Significant elevation in the plasma level of urea , total lipids and triglycerides as a result of lead exposed were also observed , compared with control . DMSA , vitamin C combined with vitamin E , and DMSA combined with both vitamin C and E induced a significant elevation in the plasma level of ALP , total proteins and albumin respectively , whereas a significant decrease in the plasma level of AP , urea , total lipids and triglycerides , respectively were observed . Thus , lead acetate induced toxic effect in hepatorenal functions , whereas , administration of DMSA ,vitamin C plus E and also DMSA combined with both vitamins C and E could ameliorate the toxic effect of lead acetate on the kidney function and to somewhat on the liver function .

Keywords: Lead acetate, Vitamin C, Vitamin E, DMSA, Toxicity.

1 Introduction

Lead is a toxic heavy metals used in many industrial applications such as lead acid batteries, cosmetics and printing dyes (1) .Also, Lead is a useful metal in life and used in modern industries and agriculture(2,3). However, lead exposure alter the function of many organs inducing pathophysiological changes (4,5,6). Lead can enter the body mainly through eating, drinking or inhalation and transport to many organs such as liver, kidney and brain where it causes its toxicity (7) . Liver can be considered as the target organ for the toxic effect of lead , since liver is responsible for maintaining the body's metabolic homeostasis (8) , and the largest lead repository of soft tissues followed by kidney (9,10) .

Continuous exposure to lead even at low concentration has been established as a risk factor causing hepatic and renal intoxication (11,12) . Several studies had shown a significant increase in the liver function parameters (ALT, AST and ALP) in male rats as a result of administration of lead acetate (13,14,15,16,17,18,19,20,5,21,22,23).

However, administration of lead acetate caused a significant reduction in the plasma and serum levels of total protein and albumin, whereas it led to a significant increase in the level of acid phosphatase in the experimental animals (17,18,22,24,23,9).

Lead can be absorbed through the gastrointestinal tract and transported to the kidney where it is accumulated and caused renal injury (12) It was found that oral administration of lead acetate led to a significant elevation

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in the plasma and serum levels of creatinine , urea ,uric acid, cholesterol , total lipids and triglycerides in male rats (*25,26,27,28,29,30,23*) .

Chelating agents are commonly used to reduce or eliminate lead toxicity ; however , some of them have undesirable side effects .Dimercapto – succinic acid (DMSA) is a thiol compound and has been used to treat lead intoxication (31). Moreover clinical trials and researches on the experimental animals have established that DMSA as the major metal chelator, based on renal metal excretion (32). Generally, the aim of the chelating agents such as DMSA is to remove the toxic effect of heavy metals from the critical organs such as liver, kidney and brain (33). Treatment with DMSA caused a significant decrease in the lead levels in liver, kidney and blood in male rats (34). Administration of DMSA restored the activities of ALP and ACP in rats treated with lead (4). Also , treatment with DMSA restored the activities of kidney marker enzymes near to the control in rats which were administrated with lead (35).

Antioxidants are capable of reducing the oxidative stress of lead exposure to improving the pro-oxidant/antioxidant balance of cells. Antioxidants natural or synthetic vitamins such as vitamin C and E (36) which are naturally organic compounds with antioxidant properties (37) .Vitamin C has good potent antioxidant action against lead acetate hepatic and renal functions and structure damage . Vitamin C is a very important and powerful antioxidant and can works in aqueous environment of the body (38). Animal studies have demonstrated that an antagonistic effect of ascorbic acid (Vitamin C) on lead absorption with its excellent chelating agent towards lead (39,40) . Treatment with vitamin C to the animals exposed to lead caused a significant decrease in the serum level of ALP, AST and ALT (41,14,42,43) . Administration of vitamin C to the male rats which were exposed to lead acetate led to a significant reduction in the plasma and serum levels of cholesterol , triglycerides , phospholipids and LDL (41,14) .

Vitamin E (α- tocopherol is well known as lipid soluble antioxidant It is one of the main non - enzymatic antioxidants (44,45) .Vitamin E is considered to be the first line of the antioxidant protection (46) . It reduces membrane lipid peroxidation through scavenging free radicals such as lipid peroxyl radicals , and ameliorates oxidative stress induced damages in many tissues in experimental animals (47) . Vitamin E protects the deleterious effects of lead on the serum and tissues total cholesterol , LDL and cholesterol in rats (48).

Several studies indicated that the administration of vitamin C combined with vitamin E has a beneficial effect in altering and providing recoveries in biochemical variables in the lead exposed experimental animals . Oral administration of vitamin C combined with vitamin E resulted in a significant reduction in the plasma level of AST , ALT , ALP , cholesterol triglycerides and LDL in the lead exposed male rats (14) . Also , oral administration of vitamin C or vitamin E , or in combination caused a significant decrease in the activity of hepatic AST and ALT , while serum uric acid and creatinine levels showed non-significant change in the lead acetate - vitamin C exposed rats . On the other hand lead acetate and vitamin E exposed rats showed a significant increase in the serum uric acid and creatinine levels (49).

The information about the protective effect of DMSA combined with either vitamin C and/or vitamin E or both against lead intoxicity on liver and kidney functions in the experimental animals is so scare in the literatures. However , there are studies which indicated that the combination of DMSA with vitamin C had a remarkable influence in decreasing heavy metal toxicity in soft organs (36) . Also, vitamin E in combination with DMSA produced pronounced recovery in sub - chronically lead poisoning in rats (50). So, the objective of this study was to investigate the effect of lead acetate toxicity on some biochemical parameters in liver and kidney of male albino rat and to evaluate whether these effects can be reduced or ameliorated via treatments with DMSA alone , DMSA combined with both vitamin C and E , and the combination of vitamin C with vitamin E .

2 Materials and Methods

2.1 Animals

This study was carried out on 30 male albino rats *(Rattusrattus*) approximately 8-10 weeks old, their weights ranging from 160-180gm. Theanimals were obtained from the animal house ofAssuitUniversity, Egypt .The animals were housed in stainless steel cages at roomtemperature, sixrats each andacclaimizedto laboratory condition two weeksbeforethe experiment and fed commercial pellet ratfood. Foodand water were available*addlibitum*. Lightingcycle of 12 hours (light/dark) was also taken in consideration.

2.2 Chemicals

Leadacetate,vitaminC (ascorbic acid) , vitamin E (α - tocopherol)and DMSA were obtained from*Labo*chemicals , India. Allchemicals in this study were of analytical grade.

2.3 Experimental Procedure

This study was designed as an oral toxicity study .The experimental animals were randomly divided into 5 groups (six rats each):

Group 1 (G1): This group served as a control and was administrated with drinking water and food for six weeks.

Group 2 (G2) :this group were administrated with 100 ppm of lead acetate in drinking water daily for six weeks.

Group 3 (G3): This group were administrated with 100 ppm of lead acetate in drinking water daily for six weeks and 50 mg/kgb.wt .ofDMSAintraperitonallyinjectedtwo times per weekfor six weeks.

Group 4 (G4): This group was administrated with 100 ppm of lead acetate daily in drinking water and 160 mg/kgb.wtof vitamin Ccombinedwith 50 mg/kgb.wtof vitamin E two times per week orally for six weeks.

Group 5 (G5): This group were administrated with 100 ppm of lead acetate daily , and 160 mg/kgb.wtof vitamin Ccombinedwith 50 mg/kgb.wtof vitamin E plus 50mg/kgb.wtof DMSA injectedinterapritonallytwo times per week for six weeks

2.4 BiochemicalAnalysis

After six weeks of treatments ,the animals of each group werescarifiedand the blood were collected from each rat into dry cleaned tubes containing EDTA asanticoagulant ,then wascentrifugatedat 3000r.p.mfor 20 minutes to obtain plasma .The obtained plasma was stored at -20 Cuntil use for biochemical analysis.

The activities of some biochemical parameters representing liver and kidney function were determined in the blood of rats ,calorimetrically.

2.4.1 Liver Function

2.4.1.1 Alkaline Phosphatase (ALP)

Alkaline phosphatase activity was measured in plasma according to the method of(51,52).The kits provided byBicondiagnostic company.

2.4.1.2 AcidPhosphatase(AP)

Acid phosphatase activity was measured in plasma spectrophotometry at 405 nm according to the method of (53) . The kits provided byhuman company

2.4.1.3Total Proteins

The estimation of total protein in plasma was measured according to the method of (54). The kits provided by Human Company.

2.4.1.4 Albumin

The estimation of albumin in plasma was carried out according to the method of(55).Thekits provided by human company.

2.4.1.5Total Lipids

Total lipids were determined in plasma according to the method of(56). The kits provided by Bicon diagnostic company.

2.4.1.6 Triglycerides

The estimation of triglycerides in plasma was carried out according to the method of(52). The kits provided by Bicon diagnostic company.

2.4.2 KidneyFunction

2.4.2.1 Urea

Urea concentration was determined in plasmaaccording to the method of (57, 58) .The kit provided by Human Company.

3 Results

The influence of administration of lead acetate and the ameliorative effects of chelating agent (DMSA) , antioxidants (combined vitamin C with vitamin E) and the combination of the chelating agent(DMSA) with both vitamin C and E against intoxication of lead acetate on some biochemical parameters representing liver and kidney function in male albino rats were investigated.

Treatments of rats with lead acetate caused a significant ($P < 0.05$) decrease in the plasma levels of ALP , AP , total proteins and albumin , respectively compared to that of control (Fig .1 , Fig .2 , Fig .3 and Fig . 4) .

Administration of lead acetate resulted in a significant ($p<0.05$) increase in the plasma levels of urea , total lipids and triglycerides , respectively , relative to that of normal control group (Fig . 5 , Fig .6 and Fig .7).

Co- administration of DMSA with lead acetate caused a significant ($P<0.05$) increase in the plasma levels of ALP, and albumins , whereas it led to a significant ($p<0.05$) decrease in the plasma level of AP and total proteins. Vitamin C combined with vitamin E and the combination of DMSA with both vitamin C and E had a similar effect on the levels of ALP , AP and total proteins , albumin and AP , like that of DMSA relative to that of control group (Fig. 1 , Fig. 3 , Fig. 4 and Fig . 2) . Relative to the effect of lead acetate, DMSA, vitamin C combined with vitamin E and the combination of DMSA with both vitamin C and E resulted in a significant (P 0.05) decrease in the plasma

levels of ALP, total proteins and albumin, respectively, Fig,1 ,Fig.3 and Fig.4). Fig.7) On the other hand , DMSA caused a significant (P < 0.05) decrease on the plasma levels of urea , total lipids and

of the control group (Fig. 5, Fig.6 and Fig . 7). the same results were obtained on the treatments of albino rats with both vitamin C and E , and combination of DMSA with vitamin C plus vitamin E , like that of DMSA , relative to that of normal control group (Fig .5, Fig.6 and Fig .7).

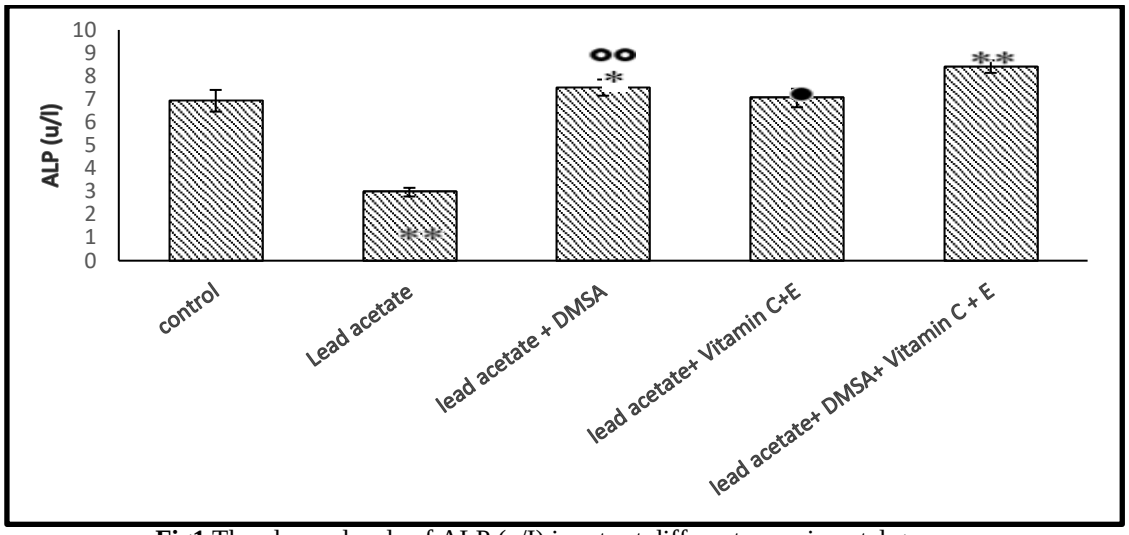

Fig1. The plasma levels of ALP (u/I) in rats at different experimental groups.

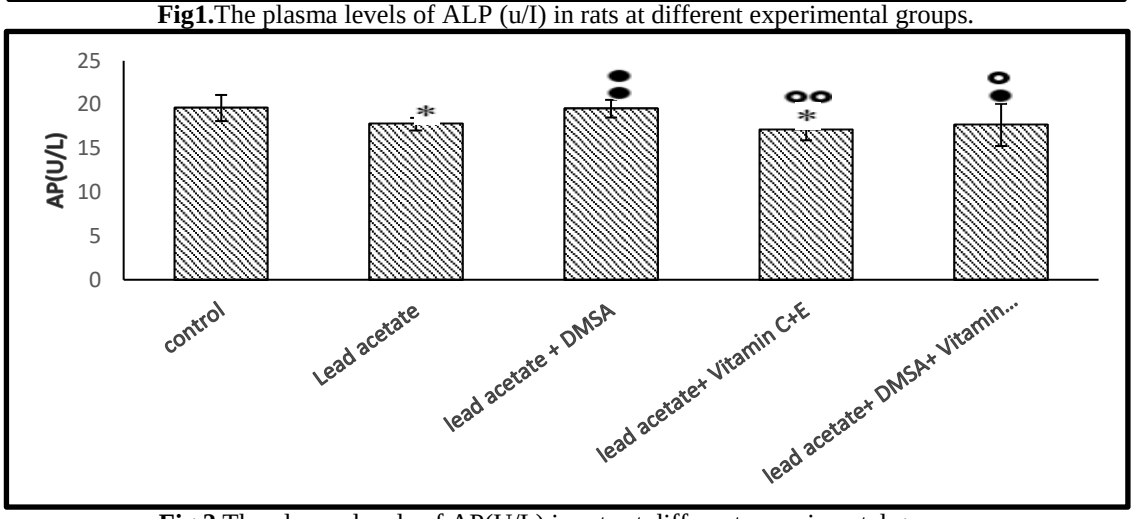

Fig 2. The plasma levels of AP(U/L) in rats at different experimental groups.

The number of rats in each series were 6.

- ● p> 0.05(Non-significant).
- ∗P <0.05(Significant difference with respect to control group).
- ∗∗P <0.009(Highly Significant difference with respect to control group).
- ❍P< 0.05 (Significant difference with respect to lead acetate treated group).
- ❍❍P< 0.009 (Highly Significant difference with respect to lead acetate treated group).

But, these treatments caused a significant (P 0.05)increase in the plasma level of AP. Relative to the effect of lead acetate , vitamin C combined with vitamin E, and the combination of DMSA with vitamin C plus vitamin E caused a significant (P 0.05) increase in the plasma levels of urea, total lipids and triglycerides, respectively ,(Fig. 5, Fig. 6 and triglycerides , respectively , compared with that

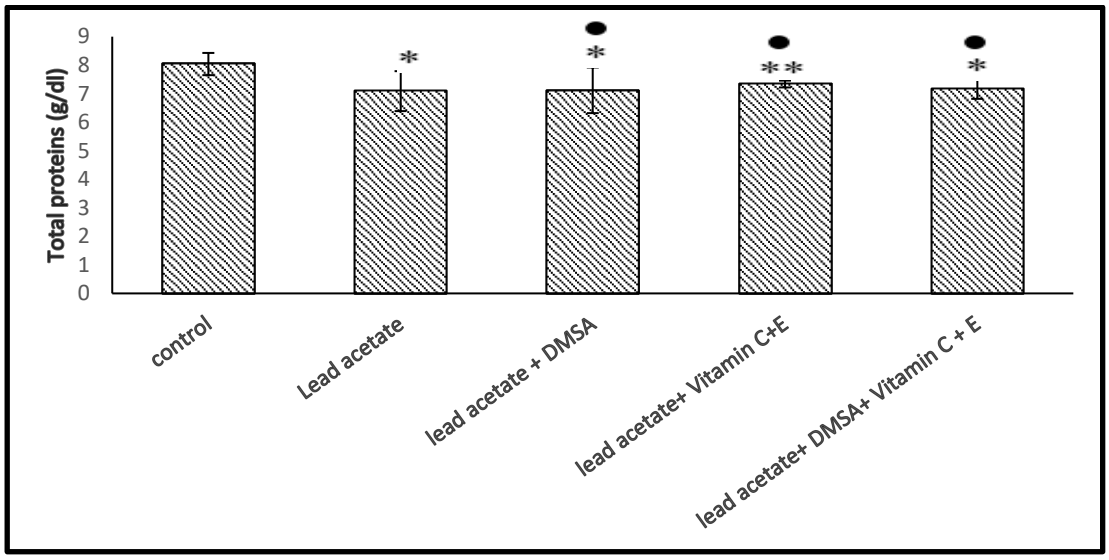

Fig 3.the plasma levels Total proteins (g/dl) in rats at different experimental groups

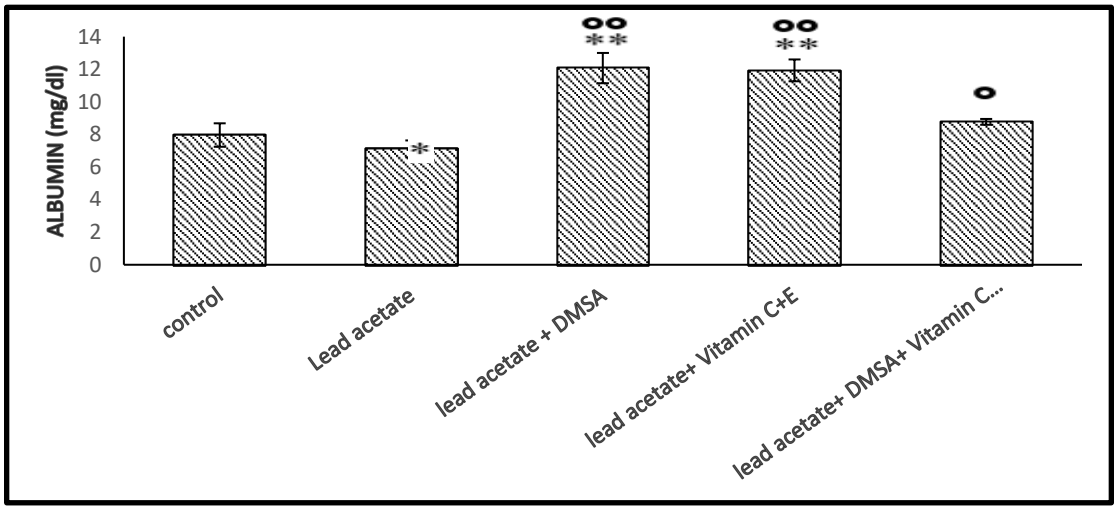

Fig4.the plasma levels of ALBUMIN (mg/dl) in rats at different experimental groups.
The numbers of rats in each series were 6.

● p> 0.05(Non-significant).
∗P <0.05(Significant difference with respect to control group).
∗∗P <0.009(Highly Significant difference with respect to control group).
⭘ P< 0.05 (Significant difference with respect to lead acetate treated group).
⭘⭘P< 0.009 (Highly Significant difference with respect to lead acetate treated group).

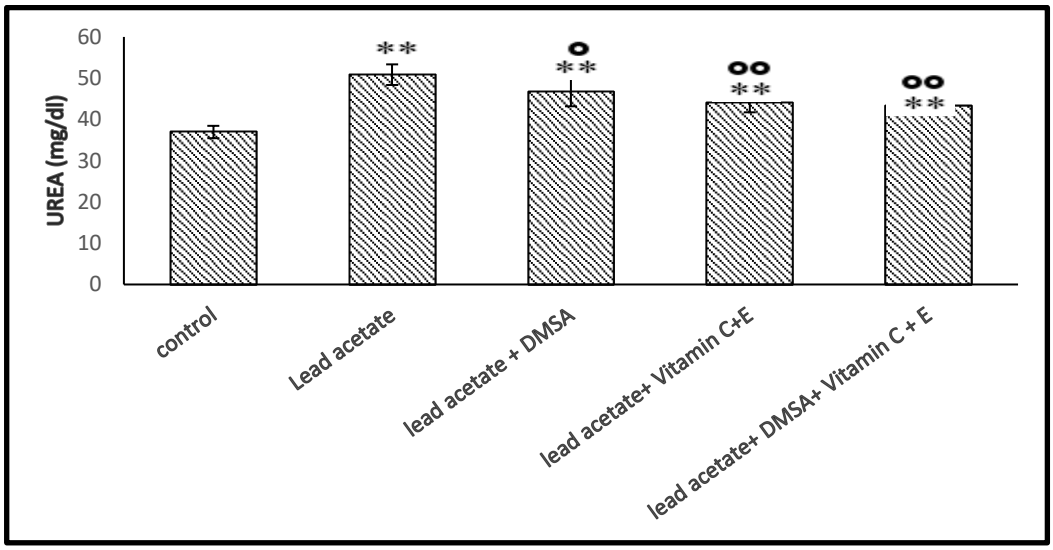

Fig 5. The plasma levels of UREA (mg/dl) in rats at different experimental groups.

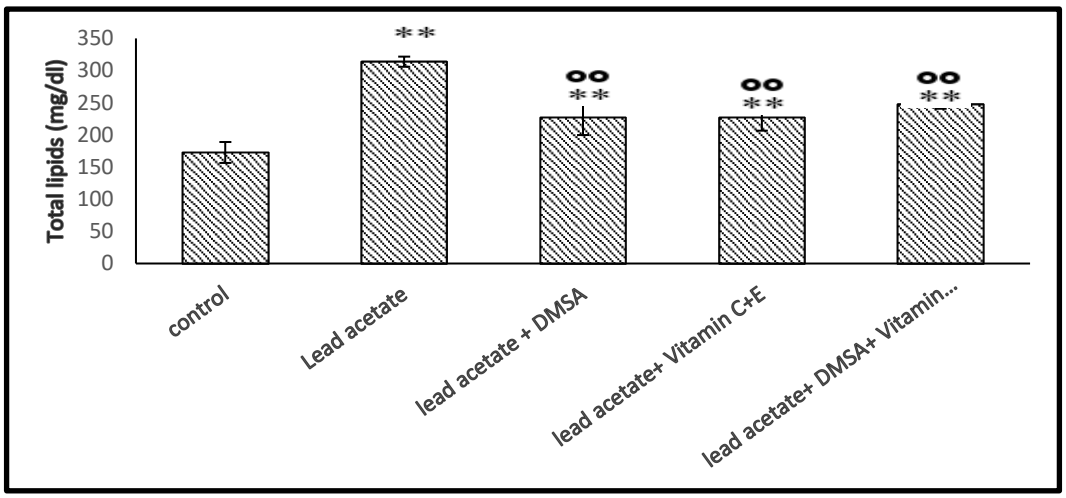

Fig 6. The plasma level of Total lipids (mg/dl) in rats at different experimental groups.

The number of rats in each series were 6.

● P> 0.05(Non-significant).

∗P <0.05(Significant difference with respect to control group).

∗∗P <0.009(Highly Significant difference with respect to control group).

⭘ P< 0.05 (Significant difference with respect to lead acetate treated group).

⭘⭘ P< 0.009 (Highly Significant difference with respect to lead acetate treated group).

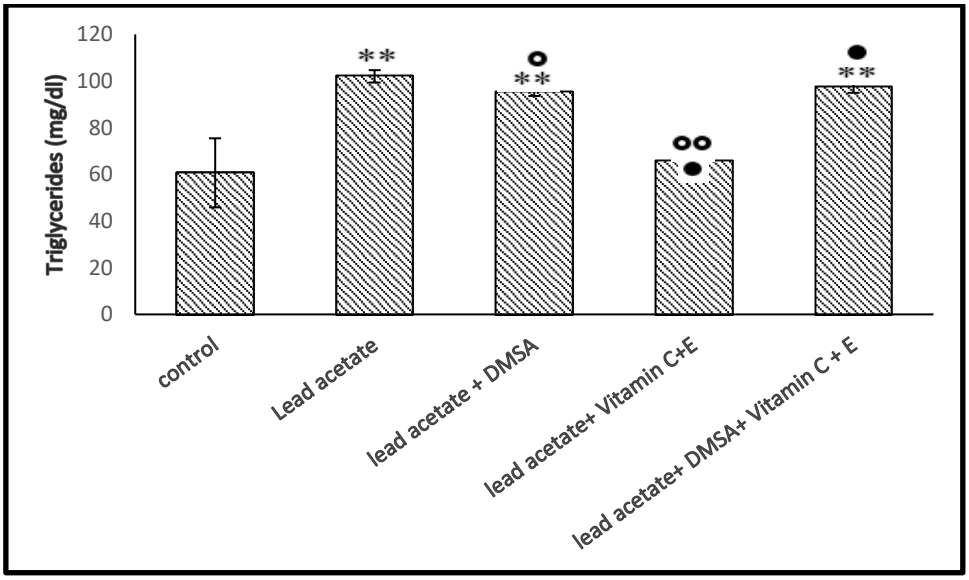

Fig 7. The plasma level of Triglycerides (mg/dl) in rats at different experimental groups.
The number of rats in each series were 6.

● P> 0.05(Non-significant).
∗P <0.05(Significant difference with respect to control group).
∗∗P <0.009(Highly Significant difference with respect to control group).
O P< 0.05 (Significant difference with respect to lead acetate treated group).
OO P< 0.009 (Highly Significant difference with respect to lead acetate treated group).

4 Discussions

The current study was aimed to evaluate the ameliorative effects of DMSA as a chelating agent, vitamin C combined with vitamin E , as antioxidants and the combination of DMSA with vitamin C plus vitamin E against lead toxicity on some biochemical parameters representing liver and kidney functions in male albino rats.

It is well known that lead is one of the most toxic heavy metals. The main body organs affected by lead exposure include liver , kidney and erythrocytes (4). Liver has been considered as a target organ for the toxic effect of lead , since it is responsible for maintaining the body's metabolic homeostasis (8) followed by kidney which is the largest lead repository (9,10) .As a further justification for this current work , there is currently scarce information on the efficacy of the combination of DMSA with both vitamin C and E in the treatment of lead poisoning in the experimental animals .

Hepatotoxicity is one of the most reasons which impair the metabolic functions of liver .The diagnosis of hepatoxicity is still a difficult area because of the lack of reliable marker for use in general clinical practice (59) . It is well known that liver plays a crucial role in lead mechanism .Liver is one of the major organs in the stoppage of biotransformation and detoxification of lead (35) .

In the present work , oral administration of lead acetate caused a decrease in the plasma level of ALP, this result is in disagreement with those reported in many studies (20,18,2015 60,16,43,61). However , our result is in accordance with that reported by (24) who stated that lead acetate caused a significant decrease in the level of ALP in the liver cultures of goat .

It has been stated that lead acetate caused a significant decrease in the plasma level of acid phosphatase (AP) in the rats (43,24) . These results are in agreement with the results obtained in the current study . It has been reported that administration of lead acetate resulted in a significant decrease in the plasma level of total proteins and albumin in rats (62, 63, 64, 17, 16, 25). The results obtained in the current work are in full agreement with the previous stated studies that lead has a very toxic effect on the liver function through its binding to plasmatic proteins where it causes alterations in a high number of enzymes representing liver functions . It can also perturb protein synthesis in hepatocytes (65) by inducing lipid peroxidation indirectly through damage to the protective antioxidant barrier (8).

It has been stated that the kidney is the target organ after long term occupational or environmental exposure to lead . Excessive exposure to lead may cause acute or chronic nephrotoxic effects (39,66) . Lead intoxication caused an elevation in the kidney lead level which may led to alteration in the kidney function (27) . Several studies

indicated that administration of lead acetate caused a significant increase in the plasma and serum level of urea in rats(63,67,64,17,68,20,25) . Also , it has been reported that supplementation of lead acetate caused a significant increase in the plasma and serum level of total lipid sand triglycerides in rats (64,48,65). However , it has been reported that supplementation of lead acetate caused a significant decrease in the level of triglycerides (17,69,25).The result of the current study were in agreement with the findings that lead acetate caused an increase in the plasma level of urea and total lipids . On the other hand , the present results were disagreed with the results of the studies stated that lead acetate caused a decrease in the plasma level of triglycerides . 67,showed that prolonged lead exposure induces generation of free radicals and lipid peroxidation in kidney which subsequently led to loss of membrane integrity and inactivation of tubular constituents . Also, it has been stated that lead induces lipid peroxidation via ROS such as H_2O_2 and OH, and the involvement of NO (20). Enhanced level of ROS can be attributed lead - induced distribution in the antioxidant system; thereby lead alters the antioxidant defense system of cells leading to the changes in plasma level of urea, total lipids and triglycerides . So , it can be concluded that lead through inducing oxidative stress is the cause of the changes in the plasma level of kidney enzymes in the present study .

In the current work, the co- administration of DMSA, vitamin C plus vitamin E and DMSA combined with vitamin C plus vitamin E caused a significant increase in the plasma level of ALP, total protein and albumins, whereas they caused a significant decrease in the plasma level of AP, and restored the levels of these enzymes near to those of control . It has been stated that the most suitable chelator used for lead elimination from the soft organs is DMSA, because of its high effectiveness and low toxicity (70) It has been reported that the supplementation of lead acetate followed by DMSA affected the level of ALP in the liver of rats (34) . Administration of DMSA after supplementation of lead acetate caused a non - significant increase in the plasma level of AP in albino rats (43). In the current study, it was found that DMSA caused a significant increase in the plasma level of total proteins and albumin . So, we can conclude that DMSA acts as a chelating agent and prevent the accumulation of lead in the hepatocytes , and hence the protein synthesis did not affect .

As indicated above the co - administration of vitamin C plus vitamin E caused a non - significant increase in the plasma levels of ALP , a significant increase in albumin , relative to the control . These results are in accordance with those reported in many studies (71, 72, and 43). It was found that the beneficial role of co - administration of vitamin C and vitamin E is shown in the main target organ , liver . However, studies indicated that vitamin C and vitamin E have some protective effects against lead intoxication , have a significant chelating capacity for lead and declined the biochemical alternations induced by lead intoxication via its action as antioxidants (72,71,48). So , it can be concluded that the combination of vitamin C and E (as antioxidant and chelating agents) has an ameliorative effects on lead - induced changes in the plasma levels of the enzymes representing the liver functions .

It has been found that administration of antioxidants (e .g. vitamins C and E during chelating treatments, DMSA) play more beneficial role in increasing lead mobilization and provide recovery effect in altered biochemical variables than that the administration of vitamin C and E (70,3) . This is the case in the current study , since co - administration of DMSA with both vitamin C and E resulted in an increase in the plasma levels of ALP , total proteins and albumin , whereas a decrease in the plasma level of AP was observed . It means that the treatment with DMSA plus vitamin C and E had recovery effect on altered biochemical variables induced by lead . So , in the light of these results obtained in the current study , it can be speculated that combined DMSA (as a thiol chelator) with both vitamin C and E can be used in reducing or preventing the oxidative stress inducing by lead acetate in liver tissue of albino rats .

In the current work, lead acetate caused an increase in the plasma level of urea which is an indicator of kidney function. However, the administration of DMSA caused a significant decrease in the plasma levels of urea , total lipids and triglycerides . These results are in accordance with those studies which stated that DMSA as a chelating agent increased urinary and fecal elimination and decreased the concentration of lead in the kidney of rats, and reduced the toxic effect of lead in albino rats (12,26) . Hence, DMSA has its action a chelating agent in reducing the toxic effect of lead on the kidney function.

It has been reported that antioxidants(e .g . vitamin C and vitamin E) are capable of reducing the oxidative stress of lead exposure to improve the pro- oxidant / antioxidant balance. Also, antioxidants have a protective effect against toxicity by chelating metal ion and preventing the reaction with ROS and maintaining in a redox state leading to its incompetency to reduce molecular oxygen (36,9) . This is the case in the current study, since the co -administration of both vitamin C and E resulted in a significant decrease in the plasma level of urea , total lipids and triglycerides , relative to that of lead acetate influence .

It has been stated that combination of DMSA with the antioxidants like vitamin C and E may showed a marked improvement of biochemical findings in the target organ , kidney (71,7) .Administration of DMSA as a chelating agent , and vitamin C plus vitamin E as antioxidants may resulted in the reversal of oxidative stress in the kidney (7) and protect the kidney against lipid peroxidation resulted from the toxic effect of lead (1) . Our results are in agreement with those stated above, since the co -

administration of DMSA with both vitamin C and E caused a significant decrease in the plasma levels of urea,Hence, it can be speculated that DMSA combined with both vitamin C and E reduced and ameliorate the toxicity of lead in the kidney .

So, it can be concluded that lead acetate as a heavy metal had a toxic effect on liver enzymes (ALP , AP , total proteins and albumin ,total lipids and triglycerides) , and kidney biochemical parameter (Urea), through inducing oxidative stress and lipid peroxidation . However, DMSA as a chelating agent alone, vitamin C plus vitamin E as antioxidants , and the combination of DMSA with both vitamin C and E have ameliorating effect against lead toxicity by improvement of liver and kidney function .

References

[1] Pan, I. T.; Wang, P. W ; AL- Suwayeh, S. A.; Chien, C. C. and Fang J .Y., Toxic . Letters. , **197**, 19-28, 2010.

[2] Hsu, P. C. and Guo, Y. L. ,Toxicol. ,**180**,33-44, 2002

[3] Woolf, A.D.; Goldman, R. and Bellinger, D. C., Pediat. Clinic. of North America . , **54(2)**, 271- 294, 2007

[4]Pande, M ; Mehta , A ; Pant , B . P . and Flora, S . J . S . , Environ . Toxicol . Pharmacol . ,**4(4)**, 173-184, 2001.

[5] Reckziegel, P.; Dias , V. T. ; Benvegnu , D.M. ; Boufleur , N.; Barcelos, R.C.S.; Segat , H.J.; Pase , C.S.; Santos, C.M . M . ; Flores , E. M . M . and Burger , M.E ., Toxicol. Report. ,**3**,351 – 365, 2016

[6] AL-Caraz- Contreras , Y.; Mendoza- Lozano, R.P.; Martinez-ALCaraz , E.R.; Martinez - Alfaro , M .; Gallegos - Corona , M.A.; Ramires - Morales , M.A.and Vazquez - Guevara, M.A. , Human Exper. Toxicol., **35 (4)**, 398- 403, 2016

[7] AL-Saleh, l. ; Naseir, M.; Mashhoun, A.; Moncari, L.; Shinwari, N: Mohamed, G. and Rabah, A. J. Environ. Pathol. Toxicol., 28,283-302, 2009

[8] Patra, R . C ; Swarap , D . and Dwivedi , S . K. ,Toxicol. , **162**, 81-88, 2001.

[9] Kumar, V. M.; Henley, A. K.; Nelson, C. J.; Indumati, O.; Roa, Y. P.; Rajanna, S. and Rajanna, B., Enivron. SCi. Pollul. Res.,**24**, 1544-1552, 2017.

[10] Patrick , L, Altern . Med. Rev . ,**11(2)**, 114 – 127, 2006

[11] El-Tantawy, W. ,. J. Tradit. Complement. Med.,**02**, 001, 2015.

[12] Salama, S.A.; Arab , H.H.; Maghrabi, I.A. ; Hassan, M .H . and AL-Saeed, M.S., Biol . Trace Elem. Res. , **173**, 96-107, 2016.

[13] Flora, S. J. S. ; Panda, M.; Kannan G .H . and Mehta, A., Molec . Biol , 50 OL *54* OL, 2004.

[14] Bashandy, S. A. E.,Egypt . J. Hospt .Med .,**23**, 371-384,2006.

[15] Jarrar, B. J. and Tiab, N. T., Experim. Toxicol. Pathol.,**64** , 291- 295, 2012.

[16] Mehana, E. E.; Meki,A.M.A. and Fazili, K.M. , Experim. Toxicol. Pathol . ,**64**, 291-295, 2012.

[17] Dewanjee, S; Sahu, R : Karmakar, S. and Gangopadhyay ,Environ.Toxicol. Pharmacol, **40**, 568-574, 2013

[18] Kalender, S. ; Appaydin, F. G. ; Bas, H. and Kalender, Y., Environ.Toxicol. Pharmacol, **40**, 568-574,2015.

[19] Tomaszewska, E; Winiarska. Meiczon, A. and Dobrowolski, P. ,Experm . Toxicol . Pathol, **67**, 331-339, 2015.

[20] Abdel- Moneim, A. E. Plos one, 1- 18, 2016.

[21] Gani, M.U.; Siddiqui , S.I.; Rashid , H .; Islam , K.; Moonmoon , S.;Ahmed , S . and Mostafa , M ., Asian J . Med. Biol. Res ., **2(2)**, 183 -189, 2016.

[22] Jalali, S. M. ; Najafzadeh, H. and Bahmei, S., Toxicol. and Indust . Health.,**33(6)**, 512 – 518, 2017.

[23] Offor, S. j.; Mbagwu, H.O.V. and Orisakwe, O., Frontiers in Pharmacol., **8(07)**, 1-10,2017.

[24] Shan, C. F. and Jain, N. K. ,UK J. pharmacol. Biosc.,**4(2)**, 31-37,2016

[25] EL-Nekeety, A. A ; EL-Kady, A. A.; Soliman, M. S. and Hassan, N. ,Food Chem. Toxicol., **47**, 2209-2215, 2009.

[26] Wang, L; Wang, Z. and Liu, J. ,Experim . Toxicol., **29(7)**, 581 –591,2011.

[27] El-Kishin, I. A; EL- Fakharany, Y. M. M. and Abdel Hamid, O.I.,Toxicol. Rep,**2**, 824-832, 2015.

[28] Ahmad, M. A .; AL-Kohtani, M.A. and Abdel - Moneim, A.M, Drug Design. Develop. Ther,**10**, 3425 – 3434, 2016.

[29] Cokce-Apaydin, F.; Bas, H.; Kalender, S. and Kalender, Y. , Environ. Toxicol. Pharmacol, **41**, 219 – 224, 2016.

[30] Fihri, A. F.; Waili, N. S. ; EL- Haskoury, R.; Bakour, M ; Amarti, A.; Ansari, M. J. and Lyoussi, B. , . Cell physiol. Biochem., **39**, 115- 122, 2016.

[31] Bradbery, S. and Vale, A. ,Clin.Toxicol, 42, 841-852, 2009.

[32] Miller, A. L. , Altern. Med.Rev., **3(3)**, 199-207, 1998.

[33] Andersen, O. and Aaseth, J. (2016): A review of pitfalls and progress in ,J. Trace Elem. Med. Biol., **38**, 74-80, 2016.

[34] Malvezzi, C. K; Vassilef , I. ; Moreita , E. G. ; Vassilef , V. S. and Cordellini, S . , plumbism.Brazilian J . Med . Biol . Res.,**34**, 1341-1346.

[35] Sivaprasad, R ; Nagaraj , M . and Varalakshmi , p., J . Nutr . Biochem.,**15**,18-23, 2003.

[36] Flora, S. J. S. ,Oxidative Med. Cell. Long,**2(4)**, 191-206, 2009.

[37] Lachapelle, M. Y. and Drouin, G . , Genetica., **139(2)**, 199-207, 2010

[38] Young, I . S. and woodside , I . S. , J. Clin . Pathol ,54, 176-186, 2001

[39] Goyer, R. A. , Toxicol. Lett., **46**, 153-162, 1989

[40] Mahalfey, K. R and Michaclson, J. A. , The clinical implication of current Research (Needleman HL ,ed.) Raven press New York.**,159**-200,1980 .

[41] Upasani, C. D. and Balaraman, R. , J. Pharmacol., **33**, 185-191, 2001.

[42] Ajayi, G. O. ; Adenjyi, T. T. and babayemi. D. O., J. Med. Sci,**143**, 64-67, 2009.

[43] Hamadouche, N.; Slimani, M. and Aoues, A. , Nat. Sci. Biol. , **4(3)**, 7-13, 2012.

[44] Uzun, F. G.; Kalender, S.; Durak, D.; Demir, F. and Kalender Y. ,Food Chem. Toxicol., **47**, 1903- 1908, 2009.

[45] EL-Shawei, R. A and Saleh, R.M. , Biomed pharmacother., **84**, 314-322, 2016.

[46] Spodniewska, A.; Baiski, D. and Gizejevska, A. , Environ. Toxicol . pharmacol.,**40**,587 – 591, 2015,

[47] El-Demerdash, F. M.; Yousef. M. I.; Kedwany .F. S. and Baghdadi, H. H. ,Food chem. Toxicol., **42**,1563-1571, 2004.

[48] Sajitha, G. R.; Augusti, K. T. and Jose, R . , Ind. J.Biochem., **31(3)**, 260 – 269, 2016

[49] Abdel-Kader, M. M ; Afify, A. A. and Hegazy, M . M ., Aust. J. Bas. Appl. Sci., **5(5)**, 1178-1183, 2011.

[50] Flora, S. J. S.; Pande, M. and Mehta, A.Chemico-Biol. Interactions .,**145**, 267-280,2003.

[51] Principato, G. B. ; Asia, M. C; Telesa, V, ;Rosi,G .and Giavannini, E. ,Comp. Biochem Physiol. , **B80**, 801-804, 1985.

[52] Bablok, W.; passing, H.; Bendea, R. and Scheider, B. , J. Clin. Chem. Clin. Biochem., **26(11)**, 738-790, 1988.

[53] Moss, D. W. In: Bergmeyer, HU.(Ed).; Methods of enzymatic analysis . Verlag-Chemie.,**4**, 92-106, 1984.

[54] Henry, R. J. ,Clinical chemistry-Harber and Row publisher, NewYork ; 181, 1964.

[55] Doumas, B. T. and Biggs, H. G. (1976) : Standard method of clinical chemistry. Acad. press. New York, 175.

[56] Friedman, H. S. , serum. Clinica Chemical Acta ,**19 (2)**, 291-295, 1968.

[57] Chaney, A. L.; Marbach, C. P. and fawectt, J. K. , J. Clin. Chem. ,**8**, 130-133, 1962

[58] Patton, C. J. and Crouch, S. R., Anal. Chem. ,**49**, 464-469, 1972

[59] Andrade, R. J.; Robles, M. C. and Lucena, M. I, World J. Gastroenterol . , **13**, 329-340, 2007.

[60] Omotoso, B. R..; Abiodum, A. A. ; Ijomone ,O.M. and Adewole, S. O. , J. Biochem .Med.. (2008). Biophysical. , **3**, 27-35, 2015.

[61] Moussa, S. A. and Bashandy, S. A .,Romanian J. Biophys. , **18 (2)**,123-123, 2008.

[62] Ibrahim, N. M.; Eweis, E. A.; EL-Beltagi, H. S. and Abdel. Mobdy, K. E., Asian Pac .J.Trop. Biomed. , **2**, 41-46, 2012.

[63] Soliman, M. M. ; Baiomy, A. A. and Yassin, M. H. , Biol. Trace Elem.Res. , 167 , 91-102.

[64] Abdou, H. M. and Hassan, M. A. , Biomed. Res. Int.1-11, 2014.

[65] Shalan, M. G.: Mostafa, M. S.; Hassouna, M. M. ; Hassab EL-Nabi, S. E. and EL-Refaie, A. ,Toxicol. , **206(1),** 1-15, 2005 ,

[66] Rastagi, S. K. , Indian J. Occup. Environ .Med. ,**12**, 103-106, 2008

[67] Sharma, S. ; Raghuvanshi, B. P. and Shukla, S. , J. , Environ. Path. Toxicol. Oncol., **33(1)**, 19-32, 2014.

[68] Lakshmi, B. V. S.; Sudhakar, M and Apama, M. ,Toxicol. Pharmacol . , **35(3)**, 361-368, 2013.

[69] Heidarian, E. and Rafieian–kopaei, M. , Pharmacol. Biol. , **51(9)**, 1-13, 2013

[70] Blaurock–Busch, E. and Busch, Y. M. , Brit .J. of Med. and Med . research. ,**4(9)**, 1821 – 1835, 2014

[71] Tandon, S. K.; Singh, S.; Perasad, S; Srivastava, S. and Siddiqui, M. K. , J. Environ. Res. , **90 (1)**, 61-66, 2002.

[72] EL-Neweshy, M. S. and EL-Sayed, Y. S ., J. Exp. Toxicol. Pathol., **63**, 221-227, 2011.