الجمهورية الجزائرية الديمقراطية الشعبية

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

وزارة التعليم العالي و البحث العلمي

MINISTERY of HIGHER EDUCATION AND SCIENTIFIC RESEARCH

جامعة باجي مختار .عنابة.

BADJI MOKHTAR UNIVERSITY - ANNABA

FACULTY OF SCIENCES

DEPARTMENT Of BIOLOGY

SUBMITTED FOR THE OBTAIN OF DOCTORAL THESIS

IN ANIMAL BIOLOGY

ENTITLED:

The protective role of vitamin C and virgin olive oil in Wistar rats fed a Pb contaminated diet

Presented by: M^{iss} LOUDJANI Farida

Member of the Jury:

KHELILI Kamel (Pr)	**Chairman**	**University of Annaba**
ABDENNOUR Cherif (Pr)	**Supervisor**	**University of Annaba**
LALAOUI Koraichi (MC)	**Examiner**	**University of Constantine**
NECIB Youcef (MC)	**Examiner**	**University of Constantine**
MAALEM Leila (MC)	**Examiner**	**University of Annaba**

2011

Acknowledgements

Professor ***Abdennour Cherif,***

I would like to express my deepest gratitude to him for his supervision, help and guidance to prepare and complete this thesis.

Professor ***Khelili Kamel***

I am gratefully for him to be the chairman of my thesis, sincere thanks and deepest respect for him.

Doctor ***Lalaoui Koraichi,***

I would thank him for agreeing to participate in my thesis editorial board, all my gratitude and my profound respect.

Doctor ***Nacib Youcef,***

I would thank him for agreeing to participate in my thesis editorial board, all my gratitude and my profound respect.

Doctor ***Maalam Leila,***

Thanks are given for her to be an examiner in my thesis, please accept my sincere appreciation.

Finally, I would like to extend my thanks to everyone who helped me and made this work possible, who have contributed in one way or another to make this work fruitful, in particular my sister Amina.

Dedication

I dedicate this humble work:

I thanked him before, he gave me. I thank him today to give me more and more

"Allah"

I heartily offer my success to the most two persons I ever loved;

To my dear mother source of love and affection which has always shown me her blessing and her sacrifices in the most important moments.

T o my dear father source of courage and trust, who always supported and helped me, he was always by my side since my childhood that I succeed.

May Allah keep them and protect them.

To my precious pearl "TOUFIK"

To my dear brothers: Faycel and Mohamed

To my dearest sisters: Samia, Abla, Amina.

To my nieces: Mouhamed, Nadir, Adlen, Selma, Abedelrahmen, Hadil, Chames Eddin, Yaakoub.

Special inscription to my friends: Rouabhia S., Nadji S., Benhadid R., Boubsil S.

The protective role of vitamin C and virgin olive oil in Wistar rats fed a Pb contaminated diet

Abstract:

In this study, an attempt was carried out to detoxify lead poisoning by antioxidant substances; vitamin C and olive oil. Two separate experimental protocols were carried out:

The first experimental protocol:

In order to investigate the antioxidant role of vitamin C in the Wistar rat subjected to a Pb contaminated diet, females received either Pb alone or combined with vitamin C in drinking water for the first 4 weeks, where half the animals were sacrificed. In the second period, treatment method has been reversed for the remaining animals and continued for 2 other weeks (6 weeks in total).

Serum albumin, immunoglobulins (Igs), Ca, Fe, leukocytes, and relative organ weights were evaluated. During the trial of four weeks, the most important results showed a significant decrease of albumin and Neutrophils with a significant increase in Igs of Pb group. However, the group of Pb-Vitamin C resulted in significant inverse results.

Regarding the levels of Ca and Fe, no significant difference was observed between the two treatments groups, which decreased significantly compared to

control. The weight of the kidneys and spleen were significantly increased compared to control.

During the second period, a significant decrease (albumin and lymphocytes) and a significant increase (Igs, Neutrophils and monocytes) were observed in the Pb group.

By comparing the two periods, albumin, Igs, Ca and Fe were returned to normal in the C-Vitamin Pb, accompanied by a decrease (albumin), an increase (Igs, Ca and Fe) or no change (Neutrophils) in group Pb. The relative organ weights of kidneys and spleen were higher in both groups by both treatments compared to control. Results therefore indicate a significant effect of vitamin C against the toxicity of lead on the concentration of albumin and IGS.

***T*he second experimental protocol:**

Vitamin C and olive oil were supplemented separately or together to rats fed a Pb contaminated diet during a period of four weeks. The following parameters were estimated; total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, calcium, iron, immunoglobulins, leucocytes, red blood cells and haemoglobin. The histological study of liver and kidney was also carried out.

Regarding the biochemical parameters, the Pb-treated group has a decreased concentration of HDL-cholesterol and iron, accompanied by a significant increase in LDL. When vitamin C or olive oil is added, the results were reversed, especially

for LDL and HDL. However, these parameters were almost identical to the control when rats were given a combination of vitamin C and olive oil.

The most important results have revealed increased levels of white blood cells and Igs in the Pb-group. But, all groups supplemented with Vit C and olive oil showed completely opposite results. In the Pb-group there were a decreased number of red blood cell counts and the heamoglobulin concentration, which have been returned to normal values by the addition of Vit C and olive oil.

Concerning the histological study, necrosis and swelling to hepatocytes were observed in the Pb-group. Pb has caused also renal degeneration and necrosis, mainly at the proximal tubules. However, the presence of Vit C has reduced necrosis of both organs, but olive oil was more efficient than Vit C.

In conclusion, Pb contaminated food containing a sufficient amount of vitamin C could reduce the toxicity of metals to some extent if the supplementation was given at the beginning or at the end of intoxication.
In addition, after the removal of vitamin C during the second treatment, the toxicity of Pb was evident in some cases.

This study also suggests that virgin olive oil is also useful in protecting rats from intoxication of lead by strengthening the immune system.

The findings prove the nutritional benefits of virgin olive oil and vitamin C to preserve the health of animals exposed to chronic Pb intoxication.

Key Words: Lead, rats, olive oil, vitamin C, immunotoxicity

In this study, an attempt was carried out to detoxify lead poisoning by antioxidant substances; vitamin C and olive oil. Two separate experimental protocols were carried out:

The first experimental protocol:

In order to investigate the antioxidant role of vitamin C in the Wistar rat subjected to a Pb contaminated diet, females received either Pb alone or combined with vitamin C in drinking water for the first 4 weeks, where half the animals were sacrificed. In the second period, treatment method has been reversed for the remaining animals and continued for 2 other weeks (6 weeks in total).

Serum albumin, immunoglobulins (Igs), Ca, Fe, leukocytes, and relative organ weights were evaluated. During the trial of four weeks, the most important results showed a significant decrease of albumin and Neutrophils with a significant increase in Igs of Pb group. However, the group of Pb-Vitamin C resulted in significant inverse results.

Regarding the levels of Ca and Fe, no significant difference was observed between the two treatments groups, which decreased significantly compared to control. The weight of the kidneys and spleen were significantly increased compared to control.

During the second period, a significant decrease (albumin and lymphocytes) and a significant increase (Igs, Neutrophils and monocytes) were observed in the Pb group.

By comparing the two periods, albumin, Igs, Ca and Fe were returned to normal in the C-Vitamin Pb, accompanied by a decrease (albumin), an increase (Igs, Ca and Fe) or no change (Neutrophils) in group Pb. The relative organ weights of kidneys and spleen were higher in both groups by both treatments compared to control. Results therefore indicate a significant effect of vitamin C against the toxicity of lead on the concentration of albumin and IGS.

***T*he second experimental protocol:**

Vitamin C and olive oil were supplemented separately or together to rats fed a Pb contaminated diet during a period of four weeks. The following parameters were estimated; total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, calcium, iron, immunoglobulins, leucocytes, red blood cells and haemoglobin. The histological study of liver and kidney was also carried out.

Regarding the biochemical parameters, the Pb-treated group has a decreased concentration of HDL-cholesterol and iron, accompanied by a significant increase in LDL. When vitamin C or olive oil is added, the results were reversed, especially for LDL and HDL. However, these parameters were

almost identical to the control when rats were given a combination of vitamin C and olive oil.

The most important results have revealed increased levels of white blood cells and Igs in the Pb-group. But, all groups supplemented with Vit C and olive oil showed completely opposite results. In the Pb-group there were a decreased number of red blood cell counts and the heamoglobulin concentration, which have been returned to normal values by the addition of Vit C and olive oil.

Concerning the histological study, necrosis and swelling to hepatocytes were observed in the Pb-group. Pb has caused also renal degeneration and necrosis, mainly at the proximal tubules. However, the presence of Vit C has reduced necrosis of both organs, but olive oil was more efficient than Vit C.

In conclusion, Pb contaminated food containing a sufficient amount of vitamin C could reduce the toxicity of metals to some extent if the supplementation was given at the beginning or at the end of intoxication.
In addition, after the removal of vitamin C during the second treatment, the toxicity of Pb was evident in some cases.

This study also suggests that virgin olive oil is also useful in protecting rats from intoxication of lead by strengthening the immune system.

The findings prove the nutritional benefits of virgin olive oil and vitamin C to preserve the health of animals exposed to chronic Pb intoxication.

Résumé

Nous avons tenté d'étudier le rôle détoxifiant du plomb inorganique en utilisant des antioxydants tels que la vitamine C et l'huile d'olive, pour cela nous avons suivi les deus protocoles expérimentaux suivants :

***P*remier protocole expérimental :**

Afin d'étudier l'effet antioxydant de la vitamine C chez le rat Wistar soumis à un régime alimentaire pollué de plomb, en premier temps période dans laquelle, les femelles ont reçu pendant 4 semaines soit le Pb seule ou combinée à la vitamine C dans l'eau potable, après cela la moitié des animaux a été égorgée . Dans la deuxième période, la méthode de traitement a été inversée pour les rats restants et poursuivie pendant 2 autres semaines (6 semaines en total). L'albumine sérique, des immunoglobulines (Igs), le Ca^{++}, le Fe^{++}, les leucocytes, et le poids relatif des organes ont été évalués. Au cours du procès de 4 semaines, les résultats les plus importants ont montré une diminution significative de l'albumine et des neutrophiles avec une augmentation significative des Igs du groupe traité par le Pb. Toutefois, le groupe de Pb-Vit C a provoqué d'importants résultats inverses.

En ce qui concerne les niveaux de Ca et de Fe, aucune différence significative n'a été observée entre les deux groupes traités, qui ont diminué significativement par rapport au témoin. Le poids des reins et la rate ont été

augmentés de manière significative par rapport au témoin. Au cours de la deuxième période, une diminution significative (albumine et lymphocytes) et une augmentation significative (les Igs, les neutrophiles et les monocytes) ont été observés dans le groupe Pb. En comparant les deux périodes de traitement, de l'albumine, Igs, Ca et Fe ont été retournés à la normale dans le C Pb-Vit, accompagnée d'une diminution (albumine), une augmentation (Igs, Ca et Fe) ou aucun changement (neutrophiles) chez le groupe Pb.

Le poids relatif des organes des reins et la rate ont été plus élevés dans les deux groupes par les deux traitements par rapport au témoin. Résultats, par conséquent, indiquent une importante action de la vitamine C contre la toxicité du plomb, sur les taux d'albumine et de l'Igs.

***D*euxième protocole expérimental :**

On ce qui concerne cette période du traitement qui a enregistrer la supplémentassions de la vitamine C et l'huile d'olive ensemble ou chacun séparer chez les rats soumis a un régime contaminés par le plomb pendant une période de 4 semaines concernant le cholestérol total, HDL-cholestérol, LDL-cholestérol, T triglycérides, Ca^{++}, Fe^{++}, Immunoglobulines totales, globules rouges, hémoglobine et les leucocytes ont été comme suite :

Concernant les paramètres biochimiques du groupe traité par le Pb observé une diminution de la concentration du HDL, cholestérol et Fe^{++}

accompagnée d'une augmentation significative du taux de LDL. Toute fois les groupes traités par le ou et la vitamine C\ extra Huile d'olive les résultats ont de complément inverser surtout au niveau de la concentration du LDL et HDL. Par contre, chez le groupe traité par Pb + vitamine C + l'huile d'olive tous ces paramètres été presque identique au groupe témoin. Les résultats les plus importants ont révélés une augmentation des concentrations des globules blancs et les Igs chez le groupe traité par le plomb. Cependant, tous les groupes supplémenter par les produits curatifs ont révèles des résultats totalement inverse.

L'examen hématologique du groupe Pb a montré une diminution du nombre des globules rouges et de taux l'hémoglobuline. La vitamine C retourné les globules rouge a leurs valeurs normales. Toutefois, l'huile d'olive a été positivement efficace avec ces deux paramètres hématologiques.

On ce qui concerne l'étude histologique du foie et des reins nous avons marque un gonflement avec une more cellulaire accidentelle (nécrose) concernant les hepatocytes pour le groupe traité par le plomb. Ainsi l'intoxication par le plomb à causée une dégénérescence et une nécrose rénale. Plus spécifiquement au niveau des tubes rénal proximal. Donc la présence de la vitamine C a réduit cette nécrose au niveau des deux organes, mais l'huile d'olive été plus efficace que la vitamine C a ce niveau.

Pour conclure, l'alimentation Pb contaminés contenant une quantité suffisante de vitamine C, pourrait contribuer à réduire la toxicité des métaux pour une certaine mesure, si sa supplémentation a été donnée au début de l'intoxication. En outre, lorsque la suppression de la vitamine C dans le second traitement, la toxicité du Pb était évidente dans certains cas.

Cette étude suggère également que l'huile d'olive vierge et également de la vitamine C sont utiles dans la protection des rats d'une intoxication plombique en renforçant le système immunitaire. La conclusion suggère également l'avantage nutritionnel et le rôle antioxydant de l'huile d'olive vierge et de la vitamine C pour préserver la santé des animaux.

الملخص

محاولة منا دراسة إزالة التسمم بالرصاص اللاعضوي باستعمال مضادات أكسدة مثل الفيتامين ج و زيت الزيتون اتبعنا هذان المنهجان (البروتوكولان):

البروتوكول التجريبي الأول:

من أجل دراسة الدور المضاد للأكسدة الذي يلعبه الفيتامين ج على فئران Wistar خضعت لنظام غذائي ملوث بالرصاص, تناولت خلاله الإناث إما الرصاص لوحده أو مجتمعا مع الفيتامين ج في مياه الشرب للفترة الأولى لمدة 4 أسابيع, أين تم ذبح نصف الحيوانات. في الفترة الثانية, عكس أسلوب المعاملة للحيوانات المتبقية و استمر لأسبوعين آخرين (6 أسابيع في المجموع). و جرى تقييم كل من ألبومين المصل, الغلوبيلينات المناعية الكلية, الكالسيوم, الحديد, كريات الدم البيضاء و الوزن النسبي للأعضاء.

بعد 4 أسابيع أظهرت أهم النتائج انخفاض معنوي في تركيز الألبومين, نسبة الخلايا المتعادلة مصحوبة بارتفاع معنوي في تركيز الأجسام المضادة لدى مجموعة الرصاص. في حين الفوج المعامل بالرصاص و الفيتامين ج فقد جاءت نتائجه معاكسة بشكل معنوي واضح.

بالنظر إلى مستويات كل من الكالسيوم و الحديد فإنها تم تسجل فورقات ذات دلالة إحصائية بين المجموعتين المعالجتين و التي انخفضت بشكل ملحوظ مقارنة بمجموعة الشاهد. في حين زاد الوزن النسبي لكل من الكلى و الطحال بالمقارنة مع الشاهد.

خلال الفترة الثانية, انخفاض معنوي (الألبومين و الخلايا اللمفاوية) و ارتفاع معنوي (الغلوبيلينات المناعية, الخلايا المتعادلة و وحيدة النواة) لوحظ لدى المجموعة المعاملة بالرصاص لوحده.

عند مقارنة فترتي العلاج, مستويات كل من الألبومين, الغلوبيلينات المناعية الكلية , الحديد و الكالسيوم عادت إلى المعدل الطبيعي لدى مجموعة الرصاص-فيتامين ج, مرفوقة بانخفاض (الألبومين), ارتفاع (الغلوبيلينات المناعية, الكالسيوم و الحديد) أو دون تغيير (الخلايا المتعادلة) في مجموعة الرصاص.

كان الوزن النسبي للكلى و الطحال أعلى في كلا المجموعتين خلال فترتي العلاج مقارنة بمجموعة الشاهد. بناءا عليه تشير النتائج إلى التأثير الملحوظ للفيتامين ج ضد سمية الرصاص على تركيز الألبومين و الغلوبيلينات المناعية.

البروتوكول التجريبي الثاني:

أما فيما يخص المرحلة الثانية من العلاج و التي تضمنت إضافة كل من الفيتامين ج و زيت الزيتون معا أو كل واحد على حدى في غذاء الفئران الملوث بالرصاص و ذلك لمدة

4 أسابيع متتالية, و قد تمت معايرة المؤشرات التالية: الكولسترول, LDH, LDL, الغليسيريدات الثلاثية, الكالسيوم, الحديد, الغلوبيلينات المناعية, كريات الدم الحمراء, الهيموغلوبين و كريات الدم البيضاء. كما قمنا بالدراسة النسيجية لكل من الكبد و الكلى.

فيما يتعلق بالمؤشرات البيوكيميائية, فقد سجلنا انخفاض في تركيز LDH و الحديد مرفقا بارتفاع معنوي في تركيز LDL. لكن عندما أضيف كل من الفيتامين ج أو زيت الزيتون البكر فقد جاءت النتائج عكسية تماما خاصة فيما يخص تركيز كل من LDL و LDH. في حين كل هذه المؤشرات فقد كانت في أغلبها متقاربة جدا مع مجموعة الشاهد و طلك بالنسبة للفوج المعامل بالرصاص-الفيتامين ج-زيت الزيتون البكر.

كما أظهرت أهم النتائج ارتفاع في مستوى كريات الدم البيضاء و الغلوبيلينات المناعية الكلية لدى مجموعة الرصاص. لكن جميع المجموعات التي أضيفة لها المواد الدوائية المضادة للأكسدة فقد أعطت نتائج عكسية واضحة.

أظهر فحص الدم لمجموعة الرصاص انخفاض في تركيز كريات الدم الحمراء و الهيموغلوبين, التي رجعت إلى تركيزها الطبيعية و ذلك بعد إضافة كل من الفيتامين ج و/أو زيت الزيتون البكر.

فيما يخص الدراسة النسيجية لكل من الكبد و الكلى فقد سجل تضخم مع موت خلوي مفاجئ فيما يخص الخلايا الكبدية في الفوج المعامل بالرصاص. كما تسبب الرصاص كذلك في تلف و موت خلوي للنسيج الكلوي, خصوصا في منطقة الملتوي القريب أين تتم على مستواه أكبر عمليات إعادة الامتصاص. في حين أدخلت إضافة الفيتامين ج نقص إلى حد ما الاضطرابات الحادثة في كلا العضوين , لكن وجود زيت الزيتون كانت له فاعلية أكبر في تحسين البنية النسيجية للكبد و الكلى على حد سواء.

في الختام, فإن الغداء الملوث بالرصاص و المحتوي على كمية كافية من الفيتامين ج, يمكنه الحد من سمية المعادن لحد ما سواء أعطي في بداية التسمم أو في آخره. علاوة على ذلك, فإنه عند إزالة الفيتامين ج أثناء مدة العلاج الثانية, ظهرت سمية الرصاص بوضوح في بعض المؤشرات.كما تشير هذه الدراسة أيضا إلى أن زيت الزيتون البكر مادة مفيدة في حماية الفران من التسمم بالرصاص من خلال تعزيز النظم المناعي.

نتائج هذا البحث تشير أيضا إلى القيمة و الفائدة الغذائية و الدور الفعال المضاد للأكسدة لكل من الفيتامين ج و زيت الزيتون البكر في الحفاظ على الصحة الحيوانية.

LIST OF FIGURES

Summary

السلام عليكم ورحمة الله وبركاته

The pollution is an unfavourable modification of the natural environment, which can affect the man directly or through his agricultural resources in water and the other organic products or by distorting the physical objects which he possesses.

Metals, particularly heavy metals such as lead, mercury, cadmium and arsenic constitute a significant potential threat to human health, both occupational and environmental (Halliwell, 2000). The environmental persistence of metal in concert with their intensive use by modern society has, over the years, created a concentration of metal in the biosphere. Thus, there is ample opportunity for exposure to toxic metal both in and outside the workplace.

According to a comparative study (Borella and Giardino, 1990) was built on some of the above-mentioned metals, lead was found to be the most toxic. In view of its wild spread on one hand and in its flexibility and resistance to corrosion and rust on the other hand.

Lead is used during the Bronze Age with the antimony and the arsenic .We finds it mentioned by the Sumerian cuneiforms about 5000 years ago, or still in the Exodus, drafted more than 2500 years ago.

It is interesting to notice that in the middle Ages the alchemists believed that the lead was the oldest metal and associated it with the planet “Saturn”. Doubtless from there that we find nowadays the notion of "Saturnism" which is a disease bound to the Pb intoxication.

Lead does not have a distinct function in human body. In general, the uptake of lead from various sources suggests a three compartmental tool for lead metabolisms; namely blood lead, lead accumulated in soft tissues and lead of the skeleton, where it appears to compete with calcium for binding sites and acts as a calcium substitute in second messenger metabolism (Goldstein, 1993). In other experimental animal methods, absorption of lead from the gastrointestinal tract has been shown to be enhanced by dietary calcium depletion or administration of vitamin D (Mykkanen and Wasserman, 1981, 1982).

Lead competes also with iron for erythrocytes binding sites (Moor, 1988). However, some increase in lead absorption may be found in iron deficiency states in humans (Watson and Hume, 1983) and in animals (Flamagan et al., 1979). In rats, iron deficiency increase the gastrointestinal absorption of lead, possibly by enhancing binding of lead to iron binding proteins in the intestine (Bannon et al., 2003; Barton et al., 1978; Morrison and Quarteman, 1987). Thus, supplementation of diet of lead-poisoned rats with iron and ascorbic acid prevent the growth depression and anaemia associated with lead (Moor, 1988).

Lead exposure also has been shown to be associated with DNA damage, for example, battery plant workers have significantly elevated levels of DNA breaks in lymphocytes compared to unexposed subjects (Fracasso et al., 2002). The DNA damage has been observed also in a mice model of lead inhalation (Valverde et al., 2002). Reports have appeared in recent literature, which link autoimmune diseases to environmental factors (Hengstler et al., 2003). In many exposed populations, some individuals are extra sensitive while some extra tolerant; children are susceptible to lead exposure (Needleman et al., 1992).

In addition to the well-documented and numerous toxic effects of lead on various targets organs, a number of studies have shown that acute and chronic exposure to inorganic lead may result at high level in an immediate suppressive effect (Koller and Kavavic, 1974, Luster et al., 1978). But at low levels, it has been reported an increase (Borella and Giardino, 1990), a decrease (Koller, 1973) or a no change (Rott and Charles, 1979) on immune function in experimental systems. Many factors could account for such effects, including dose, metal chemical forms, animal strain and/or species. Thus, these controversies regarding the influence of Pb on immune system give a very vast possible domain of research.

The mean possible mechanisms of lead toxicity are two firstly, generation of reactive oxygen species (ROC) secondly disruption of tissue oxidant/antioxidant balance. Free radicals or ROC attack lipids, proteins, enzymes and DNA to cause pathological events and cancer.

The current therapeutic approach to lead toxicity is to increase the excretion of lead by chelation. Nutritional factors are often mentioned as important modifier of the metabolism and toxicity of lead. Ascorbic acid is one of the strongest reductants and radical scavengers and reduces stable oxygen, nitrogen, and thyl radical and acts as a primary defense against aqueous radicals in the blood. Animal experiments have demonstrated that essential elements, such as calcium, zinc, iron, selenium, and various vitamins can counteract the toxic effects of lead (Miller., 1990, Patra et al., 2001, Panda and Flora, 2002). In humans, particularly children low dietary intakes of iron, zinc, and calcium have been associated with increased blood lead levels (Osman et al., 1998, Ahamed et al., 2007). Zinc supplementation was overturning the inhibited activity of blood

delta-aminolevulinic acid dehydratase (δ-ALAD) (2nd enzyme in heam biosynthetic pathway) (Singh et al., 1994). Low iron intake enhances the impairment of iron utilization for heam biosynthesis due to lead (Labbe, 1990). Rats raised on a low-calcium diet have much higher blood lead concentrations among the calcium-deficient animals (Miller et al., 1990), although lead ingestion did not differ. Similar differences have also been observed in tissue lead concentrations.

The extents of understanding how nutritional factors affect susceptibility to lead vary from nutrient to nutrient. Techniques used to investigate nutrient/lead interactions include isolated cells studied *in vitro, in situ–in vivo* methods, whole animal studies, cross sectional and longitudinal studies with humans, and clinical trial with nutrient therapy. The latter have typically been poorly controlled with respect to other variables. Adequate nutrition may be a key factor in reducing the risk of lead exposure. Nutrients supplementation modifies the process of absorption, transport, storage, and inactivation of lead, thus reducing its toxicity (Dorea and Donangelo, 2006).

To explain the importance of using exogenous nutrients in treating environmental lead toxicity the following topics are addressed: (**Figure 1**) different sources of lead exposure influencing the blood lead levels and (**Figure 2**) possible protective effects of nutrients supplementation (some essential metals and vitamins) in lead toxicity.

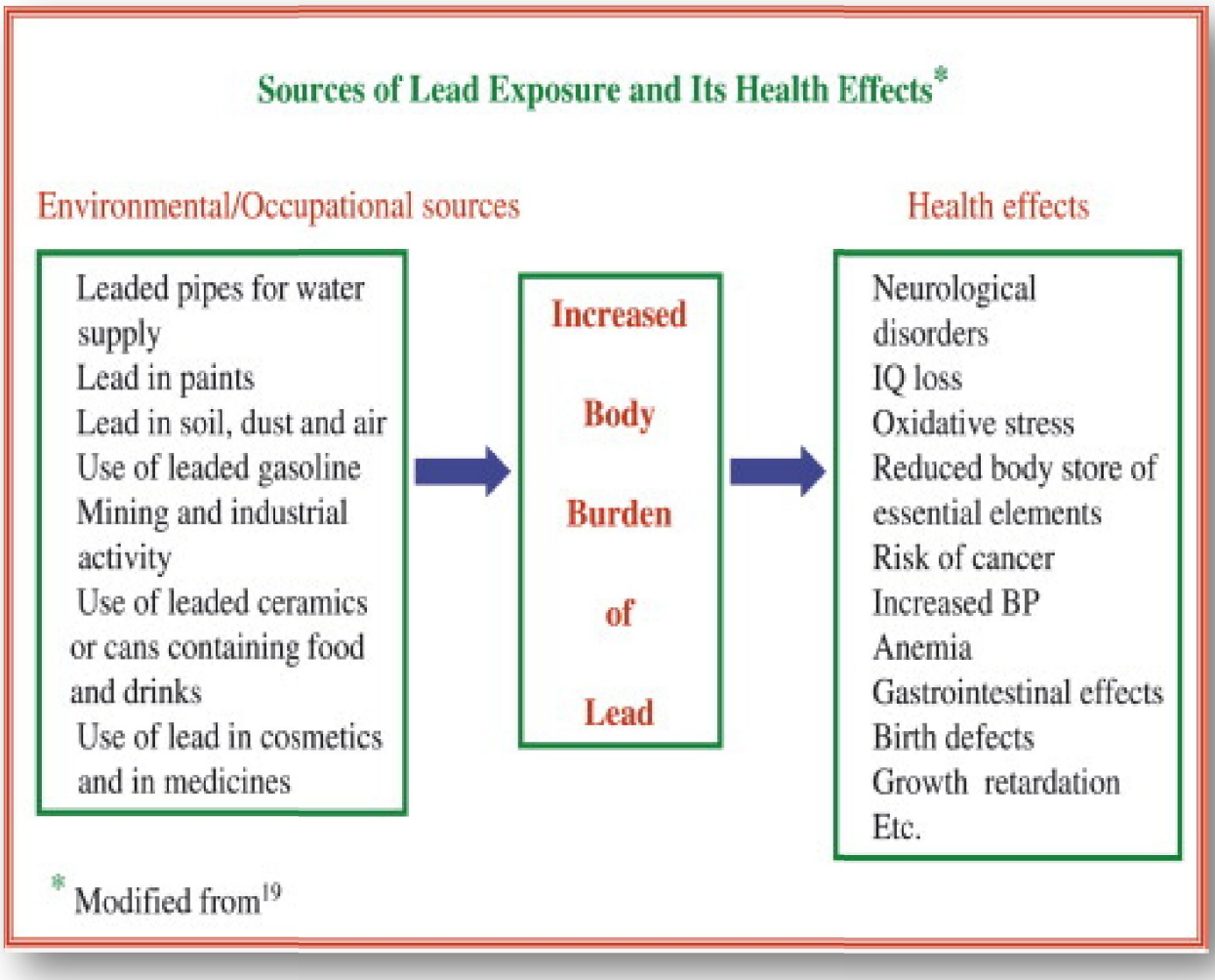


Figure 1: Sources of Pb exposure and its health effects (from Fewtrell et al., 2004).

*N*utritional factors and lead toxicity

*N*utrients and their effects on lead toxicity can be viewed from a number of perspectives. If one is seeking strategies to reduce the toxicity of lead exposure, followings are very common nutritional factors that can reduce lead toxicity(**Figure2).**

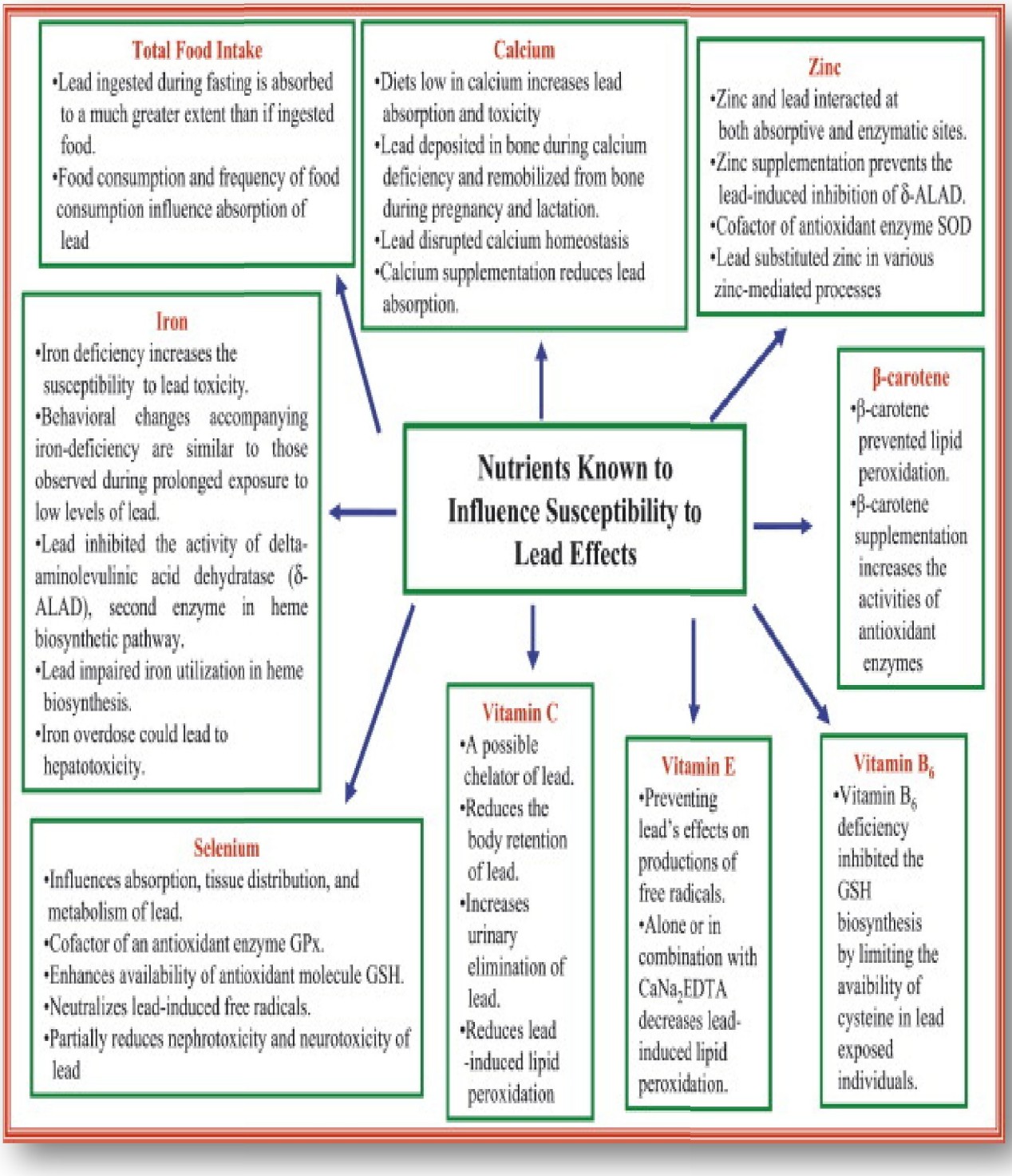


Figure 2: Nutritional factors known to influence susceptibility to lead effects.

***T*otal food intake:** Overall patterns of food consumption and frequency of food intake influence absorption of lead from the gastrointestinal tract (ATSDR, 1999). Although the mechanisms may be complex, Lead that is ingested during fasting is absorbed to a much greater extent than if ingested with food. For example, Rabinowitz et al., 1980 reported that among adult male subjects, lead without food was 35% absorbed; tracer lead ingested with food was absorbed to the extent of 8.2% and lead in food was 10.3% absorbed. Similar results have been reported by Blake and Mann, 1983 and Flanagan et al., 1982. Inter-individual variability of oral absorption was shown in the study by Heusler-Bitschy et al., 1988 in which gastrointestinal absorption ranged from 10% to 80% in eight fasted volunteers receiving a single exposure of 0.007 or 0.002 mg lead/kg/day in drinking water.

***T*he** practical role of this information depends on the reason people have limited food intake. Frequency of food intake is controlled by a number of cultural and economic factors. Certainly, shortages of food exist in many parts of the world.

***C*alcium:** The experimental literature on this topic is extensive and rather consistently supports the observations that ingestion of diets low in calcium increases lead absorption and toxicity. For example, Mahaffey-Six and Goyer, 1970 observed that lowering dietary calcium from 0.7% to 0.1% significantly enhanced the body lead burden of rats exposed for 10 weeks to 200 ppm lead in their drinking water. Under these conditions, blood, kidney, and femur levels of lead increased significantly as did urinary excretion of delta amino-levulinic acid (δ-ALA). A significant increase in renal intra-nuclear lead inclusion bodies also was observed in lead exposed animals consuming the low calcium diet. In a

similar study, metabolic and morphologic manifestations of lead exposure were compared in adult rats receiving 2, 13, 48, 96, and 200 ppm lead in their drinking water and either a 0.1% or 0.7% calcium diet (Mahaffey et al., 1973). Animals consuming the low calcium diet have increased blood, kidney, and femur lead levels, urinary excretion of δ-ALA, and renal intra-nuclear lead inclusion bodies at lower doses of lead than those on the adequate calcium diet.

However, interpretation of these results is confounded by the fact that the animal receiving the low calcium diet without lead supplementation demonstrated sign of enhanced lead uptake as evidenced by increased levels of lead in femur and kidney, as well as elevated urinary excretion of δ-ALA. This may be result of enhanced uptake of ingested ambient lead that does not bode well for inadequately nourished children. Also, exposure to lead resulted in a decline in rate of weight gain and food consumption in animals receiving the low calcium diet. Decreased lead absorption with increasing dietary intake of calcium also has been observed in humans (Heard and Chamberlain, 1982). In 8 adult male subjects fasted overnight, it was observed that absorption of 100 mg lead as lead chloride containing ^{203}Pb decreased from 60% to approximately 10% when followed by a dose of 200 mg calcium (as calcium carbonate) and 140 mg of phosphate (as sodium diphosphate).

Calcium alone reduced lead uptake by a factor of 1.3, and phosphate alone by a factor of 1.2 whereas both together reduced uptake by a factor of 6. The calcium and vitamin D status of subjects may have influenced these results; however, they were not determined. A similar result has been reported by Blake and Mann, 1983. Recently, Ballew and Bowman, 2001 critically evaluated the

few clinical studies. An inverse relationship between dietary calcium and lead absorption was found. Although calcium glycerophosphate supplement prevented lead absorption (Sargent et al., 1999), another study concluded that calcium supplementation should not be routinely prescribing for mild to moderately lead-poisoned children with adequate calcium intakes(Markowitz et al., 2004).

*A*lthough bone has predominantly been considered a storage site for sequestering absorbed lead, bone is not simply an inert storage site. Once deposited in bone, lead can be remobilized from bone in response to both physiological (e.g. pregnancy or lactation) and pathological (e.g. osteoporosis) conditions (Silbergeld, 1991). It has been shown that during the first few years after onset of menopause, there can be marked mobilization of calcium from bone matrix. Analysis of data from the Second National Health and Nutrition Examination Survey (NHANES II) has demonstrated a highly significant increase in whole blood lead concentration after menopause. Mobilization of long-term stores of lead from the maternal skeleton may be a major determinant in transfer of lead from mother to infant during pregnancy and lactation. Because of concern that maternal blood lead concentrations be maintained as low as possible during pregnancy, this remobilization of lead from bone has substantial public heath interest.

***I*ron:** Iron-deficiency is recognized worldwide as one of the most prevalent nutritional problems (Grigg, 2004). The public health implications for enhanced lead toxicity among iron deficient persons are substantial. Both iron and lead affect the heam biosynthetic process. The cellular basis for greater susceptibility of iron deficient animals to lead is that limited iron in the mitochondria

apparently enhances the impairment by lead of iron utilization for heam synthesis (Labbe, 1990). Lead is known to interfere with mitochondrial energy metabolism that is necessary to reduce ferric iron to ferrous iron before insertion of iron into porphyrin ring. Where there is insufficient ferrous iron for incorporation by ferrochelatase into heam, protoporphyrin accumulates. Ferrochelatase activity is sensitive to both lead and iron. Kappor et al., 1984 have reported that the enzyme kinetics of ferrochelatase in isolated human erythrocytes change with both iron and lead concentrations. When iron deficiency is present, ferrochelatase is more sensitive to lead effects. The cellular basis for greater susceptibility of iron deficient animals to lead is that limited iron in the mitochondria apparently enhances the impairment by lead of iron utilization for heam synthesis.

In an earlier study, blood lead levels were negatively correlated with blood iron level (Ahamed et al., 2007) is in agreement with a study of preschool children; an increase in blood lead with decreasing dietary iron intake was found (Hammad et al., 1996). Finding of Osman et al., 1998 also supports who has reported that children with low serum iron level have a tendency to higher blood lead levels, indicating increased gastrointestinal absorption of lead at reduced iron stores. However, Flanagan and Chamberlain, 1982 found no change in lead absorption with increasing iron absorption due to iron deficiency and inconsistent results regarding lead absorption in adults were observed in a study by Watson and coworkers, 1986. Therefore, there is probably not a single pathway for the absorption of these two elements.

The impairment of cognitive function among iron-deficient children has been recognized (Oski and Hongi, 1978, Lozoff et al., 1982). Behavioral changes

accompanying iron-deficiency are similar to those observed during prolonged exposure to low levels of lead. Interference with iron absorption and/or metabolism by lead exposure could result in a synergism of deleterious effects, especially in young children where iron deficiency is one of the most common nutritional deficiencies (Grigg, 2004).

***V*itamin C:** Vitamin C (ascorbic acid) is a low molecular mass antioxidant that scavenges the aqueous free radicals by very rapid electron transfer that inhibits lipid peroxidation (Halliwell and Gutteridge, 1999). Administration of vitamin C significantly inhibited the lipid peroxidation levels of liver and brain, and increased the catalase levels of kidney in lead-exposed rats (Patra et al., 2001). Lead-induced free radicals indicated by rat sperm chemiluminescence generation were reduced by 40% with supplementation of 500 mg vitamin C/L drinking water (Hsu et al., 1998).

*A*nother beneficial role of ascorbic acid supplementation in lead-exposed rats was associated with serum biochemical alterations in the haemopoietic system and drug metabolizing enzymes. Intraperitoneal administration of lead in rats produced a significant inhibition of heam synthesis in blood and liver, and reduced drug metabolism in liver. Vitamin C supplementation in lead-exposed animals significantly reduced blood, liver, and renal lead levels. This result indicated a significant protective action of vitamin C against toxic effects of lead on heam synthesis and drug metabolism (Vij et al., 1998). A study found the combination of vitamin C and thiamine was effective in reducing lead levels in blood, liver, and kidney. In addition, both lead-induced inhibition in the activity of blood δ-ALAD and elevation in the level of blood zinc protoporphyrin (ZPP) were reversed by such combination (Flora and Tandon, 1989).

There has been considerable debate concerning the relationship between vitamin C nutritional status and heavy metal body burden in lead-induced toxic effects. Early reports found that vitamin C might act as a possible chelator of lead, with similar potency to that of EDTA (Goyer and Cherion, 1979). Vitamin–mineral supplementation in African-American women was found to reduce blood lead levels from 5.1 to 1.1 μg/dL, which was negatively correlated with serum levels of vitamin E and C (West et al., 1994). Dhawan et al., 1988 found that ascorbic acid might increase urinary elimination of lead and reduced the hepatic and renal lead burden in rats. A cross-sectional study analyzed 4213 young and 15 365 adult Americans with mean blood lead levels of 2.5–3.5 μg/dL, respectively, and showed an inverse relationship between serum vitamin C and blood lead level (Simon and Hudes, 1999). In another study of 85 volunteers who consumed a lead-containing drink, vitamin C supplementation produced small reductions in lead retention (Dawson and Harris, 1997).

On the other hand, some studies suggested that vitamin C supplementation did not significantly lower blood lead levels. In workers occupationally exposed to lead, and with blood lead levels ranged from 28.9 to 76.4 μg/dL, supplementation of vitamin C and zinc did not significantly reduce blood lead levels (Lauwerys et al., 1983).

***O*live oil:** In the Mediterranean basin, olive oil, along with fruits, vegetables, and fish, is an important constituent of the diet, and is considered a major factor in preserving a healthy and relatively disease-free population. Epidemiological data show that the Mediterranean diet has significant protective effects against cancer and coronary heart disease. We present evidence that it is the unique profile of the phenolic fraction, along with high intakes of squalene

and the monounsaturated fatty acid, oleic acid, which confer its health-promoting properties. The major phenolic compounds identified and quantified in olive oil belong to three different classes: simple phenols (hydroxytyrosol, tyrosol); secoiridoids (oleuropein, the aglycone of ligstroside, and their respective decarboxylated dialdehyde derivatives); and the ligands [(+)-1-acetoxypinoresinol and pinoresinol]. All three classes have potent antioxidant properties. High consumption of extra-virgin olive oils, which are particularly rich in these phenolic antioxidants (as well as squalene and oleic acid), should afford considerable protection against cancer (colon, breast, skin), coronary heart disease, and ageing by inhibiting oxidative stress.

Recent studies (Marubayashi et al., 1985, Bukowska et al., 2000) showed that diets rich in monounsaturated fatty acids, especially oleic acid, produce fapoprotiens. Three papers in the June 1988 issue of the American Journal of chemical Nutrition (Duchnowicz and Koter, 2003, Ozcan and Uner, 2000) suggest that increased consumption of oleic acid in the form of Olive Oil, at the expense of either saturated fatty acids or carbohydrates, is a proper approach to lowering coronary disease risk.

Wiseman et al., 1996 indicated that dietary Olive Oil specifically causes increased concentrations of liver cholesterol in laboratory animals. Although it is not known whether this effect is harmful and whether the animal data can be extrapolated to man.

A study by Lions et al (1991) has suggested that there may be beneficial anti-inflammatory effects of Olive Oil consumption on Rheumatoid Arthritis (RA). This study compared the relation risk of development of RA in relation to

lifelong consumption of Olive Oil in a Greek population and it demonstrated that high consumers of Olive Oil (almost every day throughout life) were four times less likely to develop RA than those who consumed Olive Oil less than six times per month on average throughout their lives (lions et al, 1991).

***V*itamin E:** Vitamin E is the generic term used to describe at least eight natural-occurring compounds that possess the biological activity of α-tocopherol. The group is comprised of α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol. The α-tocopherol has the highest biological activity (Weister & Vecchi, 1983), the other tocopherols and tocotrienols are less biologically active but they are at least as abundant as α-tocopherol in certain foods (Sheppard et al., 1993). Vitamin E is nature's major lipid soluble chain-breaking nutrient that is known to protect biological membranes and lipoproteins from oxidative stress (Packer, 1991). The main biological function of vitamin E is its direct influencing of cellular responses to oxidative stress through modulation of signal-transduction pathways (Azzi et al., 1992). One of the protective roles of vitamin E on lead toxicity was preventing lead's effects on productions of free radicals in liver (Chaurasia and Kar, 1997).

***T*he interaction between vitamin E and other nutrients might have a more efficient protective action against lead toxicity. Vitamin E and C jointly protect lipid structures against peroxidation (Buettner, 1993). Although vitamin E is located in membranes and vitamin C in aqueous phases, vitamin C is able to recycle oxidized vitamin E (Frei, 1991). Vitamin C repairs the tocopherol radical, thus recovering the chain-breaking antioxidant capacity of vitamin E (Buettner, 1993). Vitamin E alone or in combination with conventional chelator,

$CaNa_2EDTA$, was found to decrease the lead-induced lipid peroxide levels of liver and brain in rats (Patra et al., 2001).

A study of the influence of vitamin C on the tissue deposition of lead in rats suggested that it might be useful as a prophylactic agent for lead poisoning (Simon, 2003). Later studies in rats demonstrated that vitamin C decreases Pb intestinal absorption and increases its renal clearance (Fotherby et al., 2000). Further studies indicated that ascorbic acid may chelate lead and decrease the risk of its toxic effects on immune system; it helps this system to fight off foreign invaders and tumour cells possibly by stimulating the production of white blood cells, primarily neutrophiles, which attack foreign antigens. It also boosts the body's production of antibodies and interferon, proteins that helps protect cells from viral invaders (Null, 1994). In addition to its great action against free radical produced by lead, vitamin C works along with vitamin E, a fat-soluble antioxidant, and the enzyme glutathione peroxidase to stop chain reaction of free radicals.

The objectives:

*T*he aim of the present study, however, is to assess the possible protective roles of natural substances (ascorbic acid and local virgin olive oil) in Pb intoxicated rats. The test includes the immune response (leucocytes and total immunoglobulins Igs), liver and kidney function, relative organ weights of kidney, spleen, thymus and adrenal and the level of some biochemical parameters.

*N*atural substances supplementation was given at the beginning of the Pb intoxication or after a while. The suppression of vitamin C from Pb intoxicated rats was also tested.

*A*s part of the Mediterranean basin, Algerians consume much of the virgin olive oil of different strains.

A- What is the effect of this eating habit on exposed people to Pb in the environment?

B- Can they withstand such oxidative stress produced by these pollutant?

C- Can olive oil and Vitamin C be able to protect and/or boost the immune system?

2.1. Biological materials and treatment

*W*istar rats were brought from Pasteur Institute, Algiers, and then reared in the Biology Department rearing house, University of Annaba under standard conditions of temperature, humidity and light. Animals were five week old and weighting an average of 1003g. They were given food and deionised drinking water ad-libitum.

2.1.1The first experimental protocol:

*T*he first experimental protocol is divided into the first and the second treatment:

2.1.1.1. First treatment:

*R*ats were randomly divided into three groups of ten individuals and exposed either to 500mg Pb/l as lead acetate in their drinking water (G2) or 500mg Pb/l combined with 300mg vitamin C/l (G3) for a period of 4 weeks. The control group was given deionized water only (G1).

*A*fter decapitating half of each group, part of peripheral blood was collected in tubes contained EDTA for haematological study. The other part of blood was collected in dry tubes and used for biochemical measurement after obtaining serum, which then frozen immediately at -20°C. Immunoglobulins were separated by cellulose acetate electrophoresis.

The internal organs; Thymus, Adrenal, Kidney and Spleen were removed and their wet weights were obtained.

2.1.1.2.1. Second treatment:

In the second period, the treatment method was inversed for the remaining animals and continued for other two weeks (6 weeks in total). In this period, vitamin C was suppressed from the combined treatment group, and then added to the group which has already received Pb alone.

After decapitation, the same steps of the first period were repeated (**figure 3**).

2.1.2. The second experimental protocol:

Fresh virgin olive oil was obtained from Skikda region at an altitude of 500m. Also vitamin C was obtained from the pharmacy. Both lead and vitamin C were dissolved in deionized water.

Lead was given at 500 mg lead acetate/l, vitamin C was supplemented at 300mg/l and virgin olive oil was supplemented at 5% diet.

Animals were randomly divided into two main groups. The first main group was fed on basal diet as a negative control (G1); the second main group received lead contaminated drinking water ad-libitum for a period of four weeks. This group was divided into the following four subgroups; Pb alone (G2), Pb-vitamin C (Pb-VC (G3)), Pb-virgin olive oil (Pb-OO (G4)) and Pb-vitamin C-virgin olive oil (Pb-VC-OO (G5)).

After decapitation, peripheral blood was collected in tubes contained EDTA, and was used for counting leucocytes subpopulations, red blood cells and haemoglobin using Automatic Coulter Counter Machine (T 540). The other part of blood was collected in dry tubes and used for the measurement of serum HDL, LDL, total cholesterol, triglycerides, Ca^{++} and Fe^{++} using an automat machine. The serum pools sampled for protein measurements were frozen immediately at -20° C until they were separated by cellulose acetate electrophoresis (**figure 4**).

Fig 3 :The first experemental protocol

‼

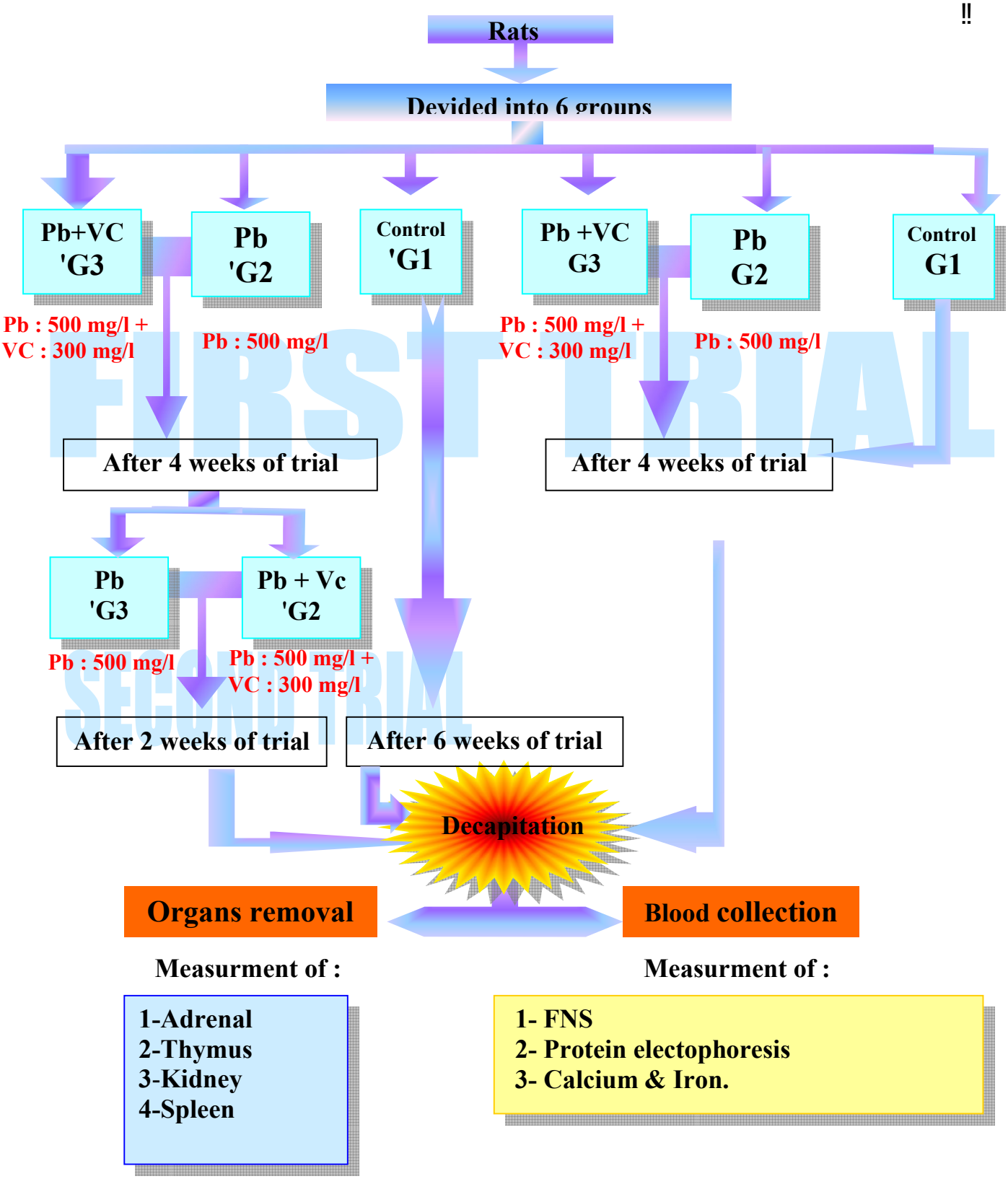
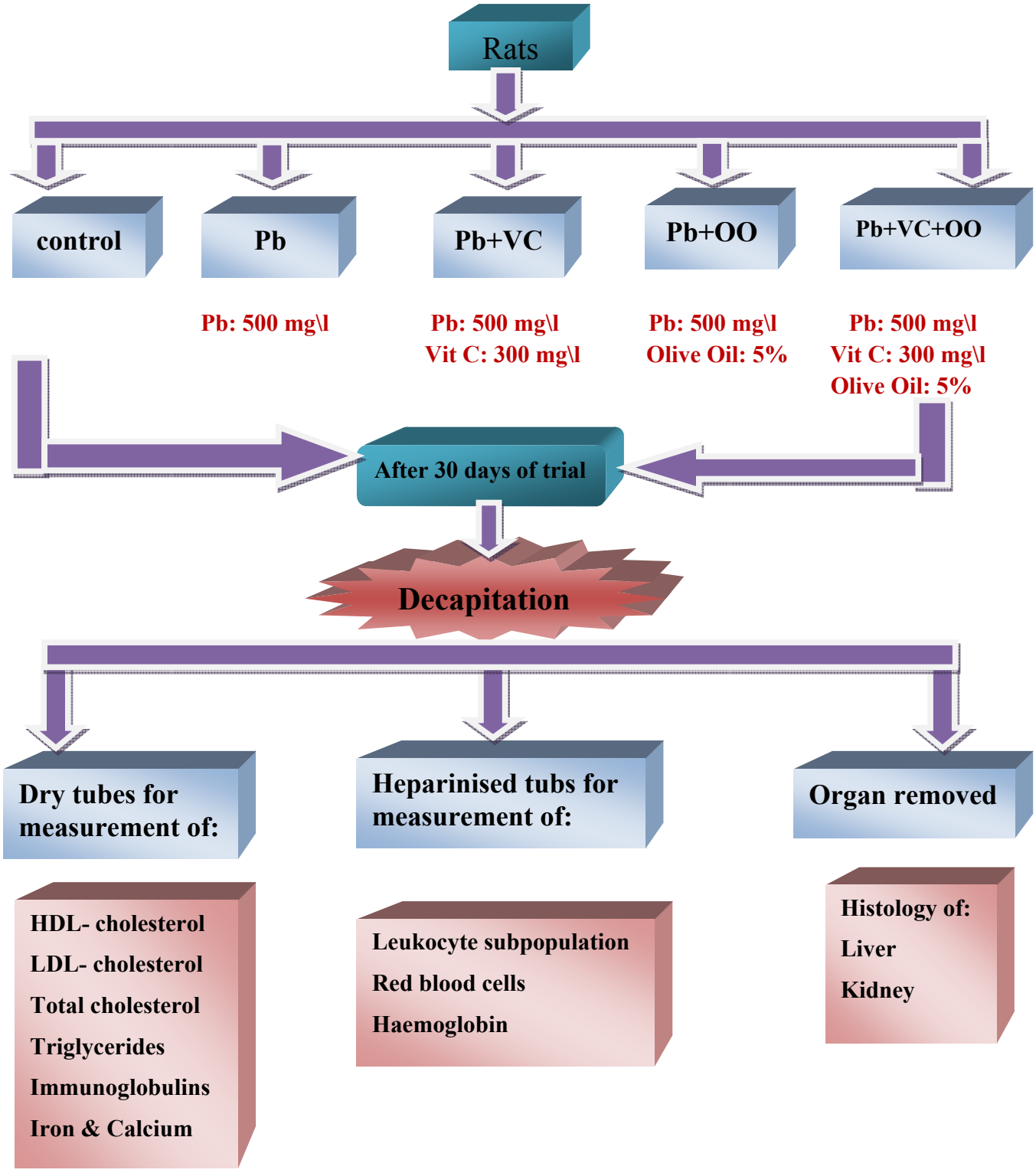


Fig 4: The second experimental protocol



1/ Impact of vitamin C during the first experimental protocol:

Lead exposure altered albumin level compared to the control, whereas vitamin C has returned the albumin level to its normal range in the first period of treatment. When reversing treatment method, the results were also inversed. During the first treatment period, total immunoglobulins were significantly increased by Pb exposed group. However, no significant variation among combined group was observed for this parameter when compared to the control. On the other hand, during the second treatment, immunoglobulin levels after the addition of vitamin C was returned to the normal range, but the suppression of this nutrient has resulted in a significant increase in immunoglobulins when compared to the control.

Neutrophil counts were significantly decreased by Pb and no abnormalities were observed in Pb-Vitamin C group compared to the control during the first treatment period. In addition, monocytes, eosinophils and lymphocyte counts were not influenced by either Pb exposure or combined with vitamin C. During the second treatment period, neutrophil, and lymphocyte counts were significantly influenced by Pb group as well as by vitamin C supplementation, whereas monocyte counts were increased by Pb exposure.

Thymus relative weight was not significantly affected in both groups during the two treatment periods, whereas that of adrenal has recorded a significant change between the control and the combined treatment. However, kidney and spleen relative weights were higher in Pb and Pb-vitamin C treated animals compared to the control and that in both treatment periods.

For the first treatment, calcium and iron were lower in both treated groups compared to the control. However, in the second treatment period, calcium and iron were significantly returned to the normal range in both groups when compared to the control.

Table 1: Summary of the measured values (mean ± SD)) of serum total proteins, calcium, iron, blood leukocyte counts, and organ's relative weights of vitamin C and Pb exposed rats after the first treatment period of 4 weeks.

Groups	**G1** (Control)	**G2** (Pb)	**G3** (Pb+Vit C)	**p**
Parameters				
Albumin (g/l)	39.9(4.77)	32.14(6.71)	40.06(3.88)	**a**
Immunogl. (g/l)	18.6(0.77)	28.12(5.43)	20.52(3.45)	**a**
Lymphocytes (%)	53.2(8.04)	70.8(12.98)	59.4(10.06)	
Neutrophils (%)	39.2(5.63)	15.6(4.61)	33.4(5.59)	**a**
Eosinophils (%)	0.34(0.19)	0.22(0.08)	0.22(0.13)	
Monocytes (%)	1.4(0.39)	4.50(2.05)	6.26(4.82)	
Ca (mmol/l)	2.86(0.17)	1.45(0.76)	1.62(0.78)	**a,b**
Fe (mmol/l)	05.76(3.04)	1.85(0.75)	2.34(0.98)	**a,b**
Adrenal (%)	0.00027 (0.000020)	0.00031 (0.000092)	0.00024 (0.000022)	**b**
Thymus (%)	0.0030 (0.00023)	0.0029 (0.00025)	0.0035 (0.00020)	
Kidney (%)	0.0027 (0.00059)	0.0051 (0.00036)	0.0046 (0.00093)	**a,b**
Spleen (%)	0.0026 (0.00038)	0.0058 (0.0022)	0.0057 (0.0010)	**a,b**

a: G1 vs G2; **b:** G1 vs G3

Table 2: Summary of the measured values (mean ± SD) of serum total proteins, calcium, iron, blood leukocyte counts, and organ's relative weights of vitamin C and Pb exposed rats after the second treatment period of 6 weeks.

Groups	**'G1** (Control)	**'G2** (Pb+Vit C)	**'G3** (Pb)	**p**
Parameters				
Albumin (g/l)	40.78(4.20)	39.46(5.30)	34.97(6.27)	
Immunogl. (g/l)	17.98(1.02)	23.22(1.11)	27.20(4.59)	**b**
Lymphocytes (%)	56.6(12.05)	77(11.89)	46.4(20.59)	**a**
Neutrophils (%)	40.8(12.48)	18.2(11.88)	48.2(23.30)	**a**
Eosinophils (%)	0.38(0.29)	0.38(0.17)	0.36(0.11)	
Monocytes (%)	1.2(0.33)	2.88(0.92)	4.12(2.36)	**a**
Ca (mmol/l)	3.05(0.76)	2.80(0.41)	2.25(0.98)	
Fe (mmol/l)	5.76(2.41)	5.52(2.25)	4.12(2.36)	
Adrenal (%)	0.00029 (0.000016)	0.00030 (0.000085)	0.00026 (0.000026)	
Thymus (%)	0.0027 (0.00041)	0.0024 (0.00024)	0.0025 (0.00069)	
Kidney (%)	0.0030 (0.00039)	0.0040 (0.00040)	0.0042 (0.00060)	**a,b**
Spleen (%)	0.0025 (0.00042)	0.0038 (0.0069)	0.0067 (0.0017)	**a,b**

a: G1 vs G2; **b:** G1 vs G3

The first experimental treatment: after 4 weeks of treatment

Albumin:

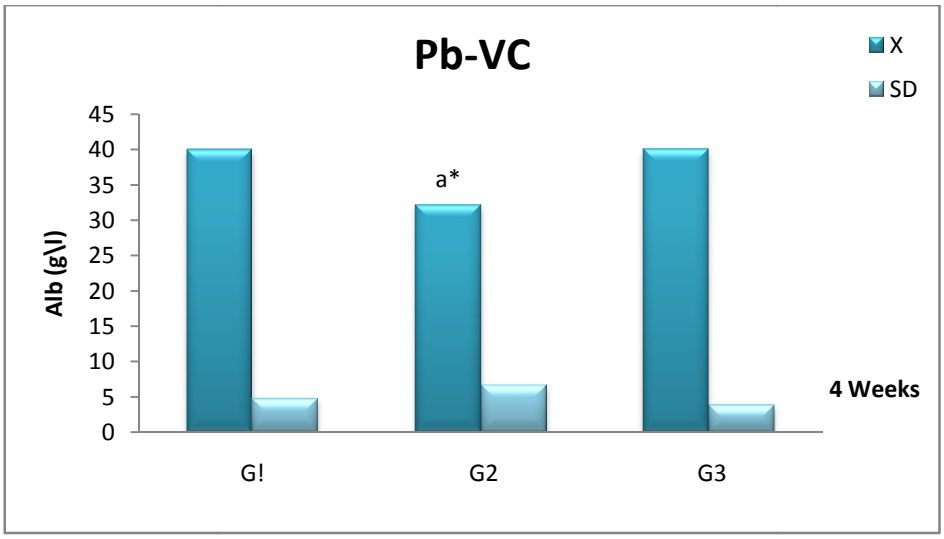


Fig 5: *comparison of albumin (Alb) concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Immunoglobulins:

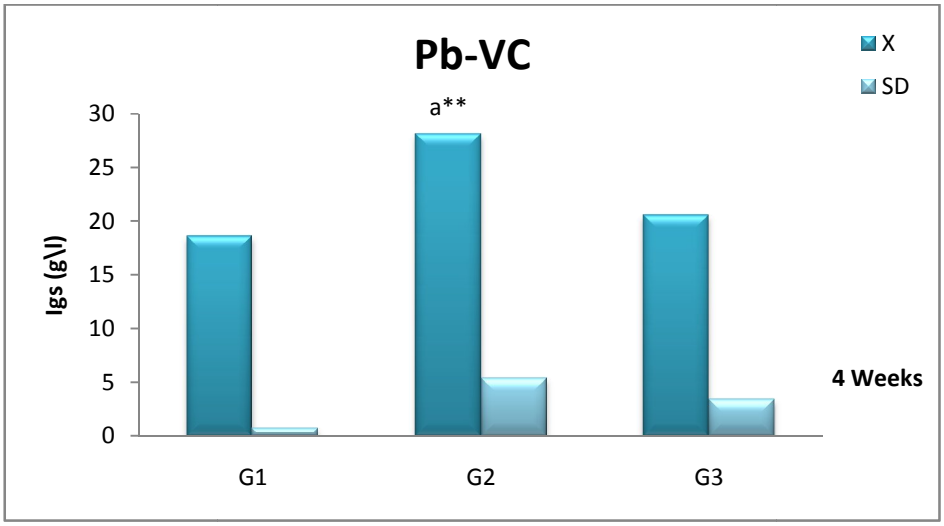


Fig 6: *comparison of immunoglobulins (Igs) concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Lymphocytes:

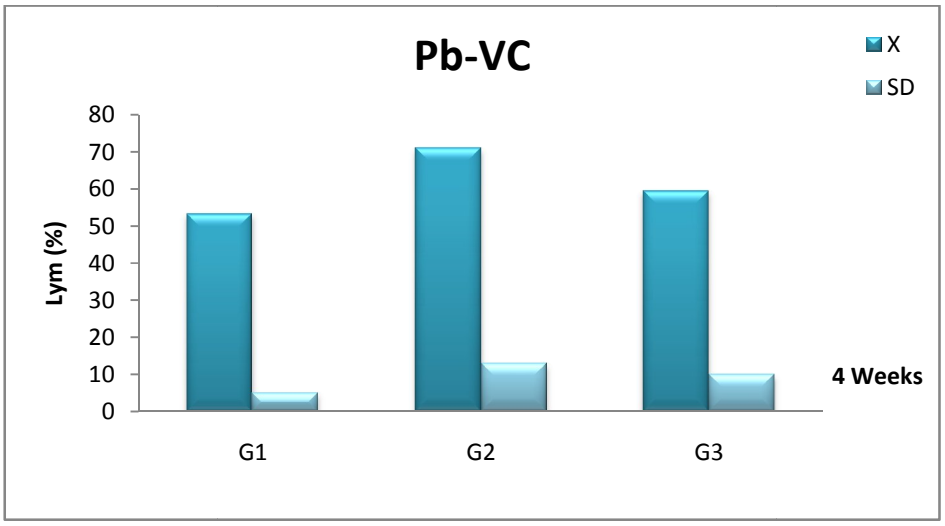


Fig 7: *comparison of white blood cells concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Neutrophils:

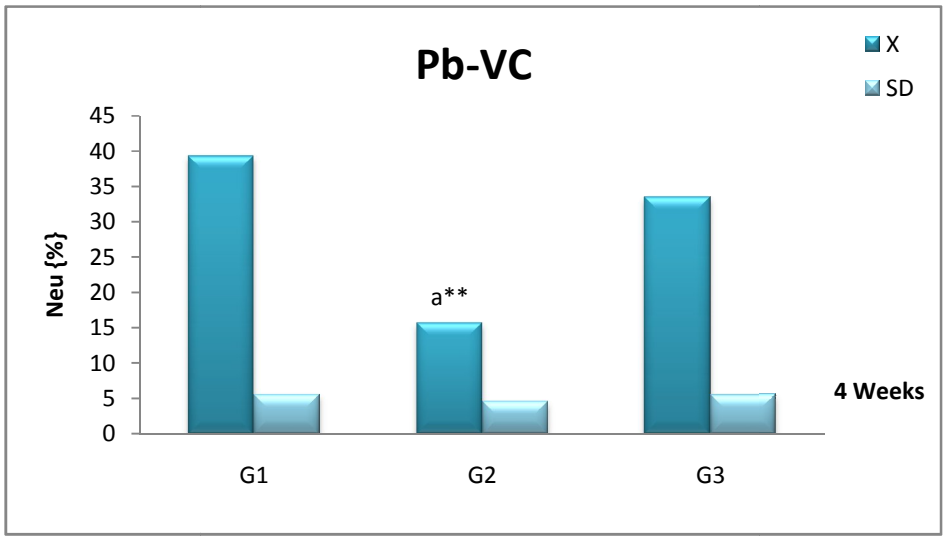


Fig 8: *comparison of Neutrophils (Neu) concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Eosinophils:

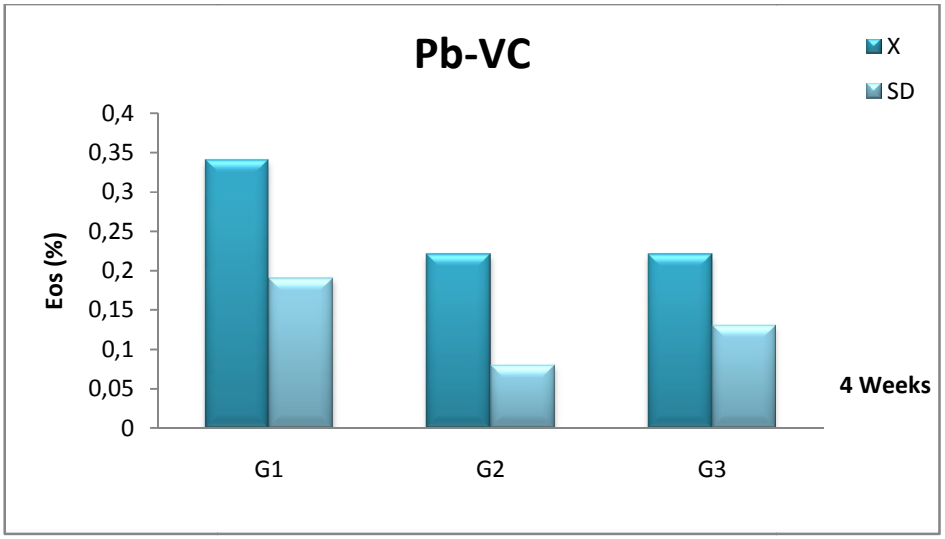


Fig 9: *comparison of eosinophils (Eos) concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Monocytes:

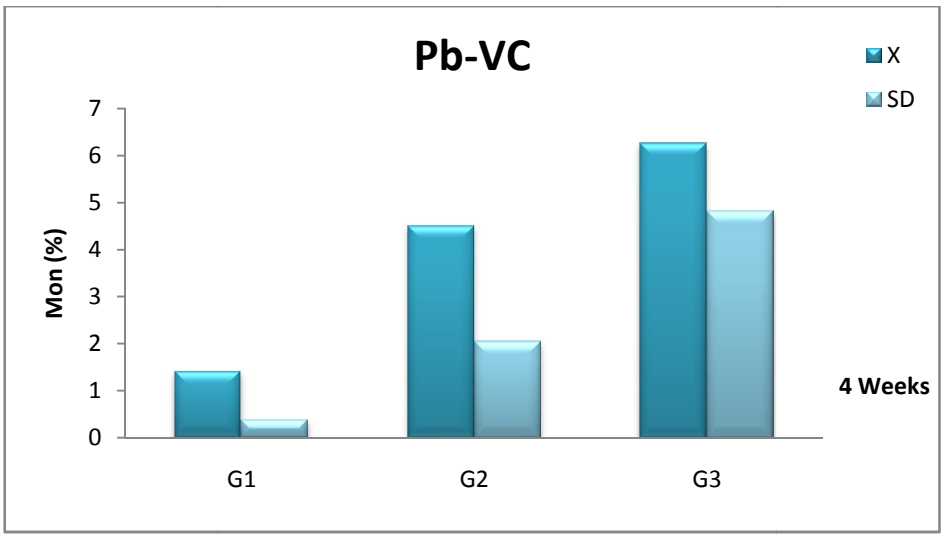


Fig 10: *comparison of monocytes (Mon) concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Minerals: after 4 weeks of treatment

Calcium:

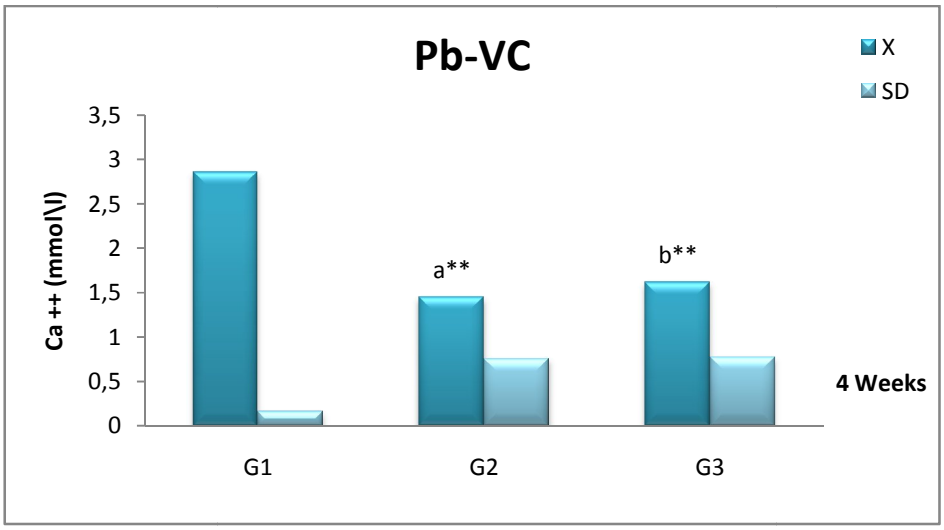


Fig 11: *comparison of calcium (Ca^{++}) concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Iron:

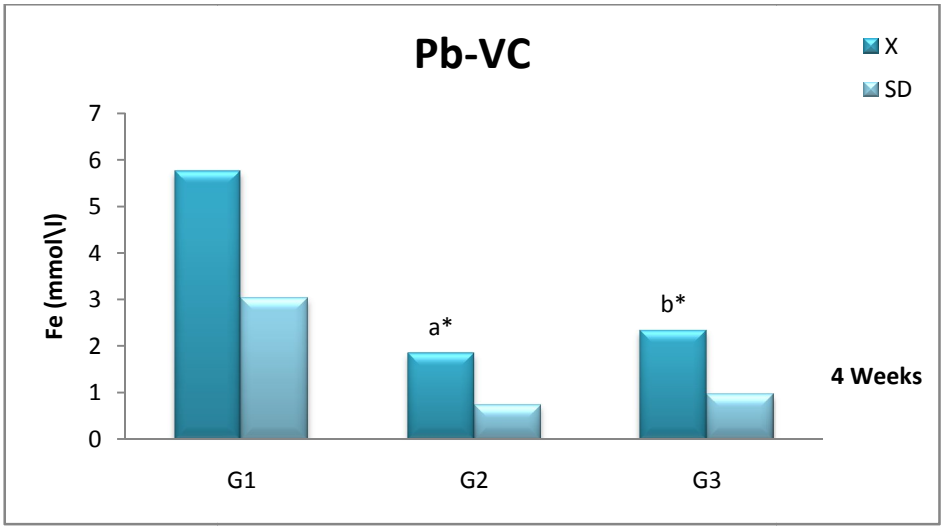


Fig 12: *comparison of iron (Fe^{++}) concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Relative organs weigh: after 4 weeks of treatment

Adrenal:

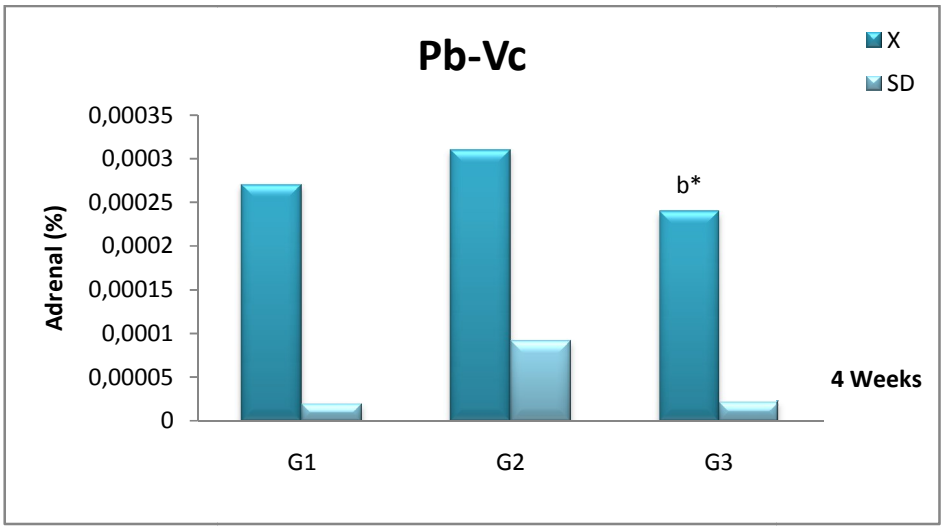


Fig 13: *comparison the relative organ weigh (%) of adrenal female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Thymus:

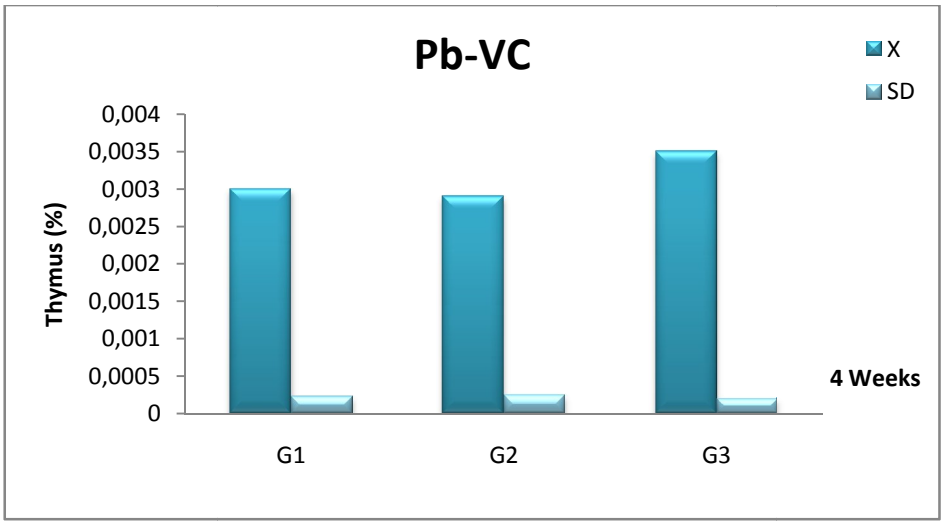


Fig 14: *comparison the relative organ weigh (%) of thymus female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Kidney:

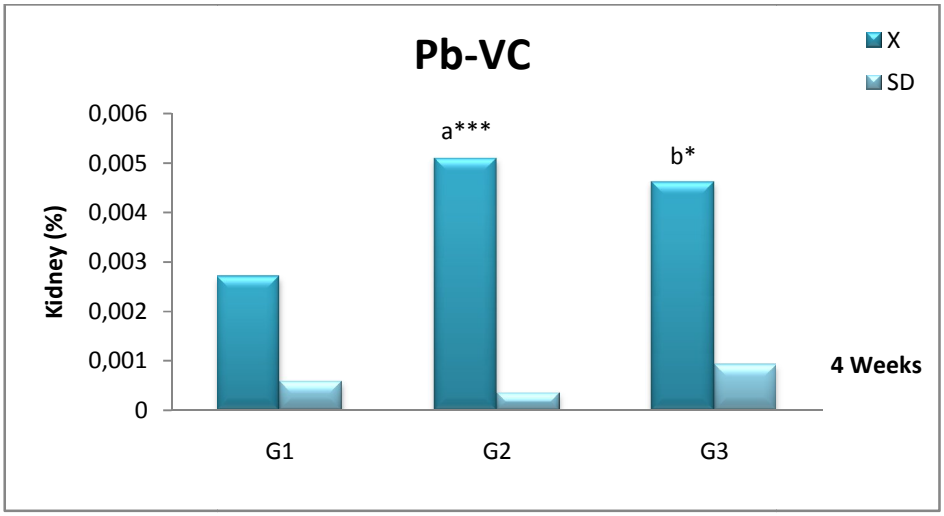


Fig 15: *comparison the relative organ weigh (%) of kidney female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

Spleen:

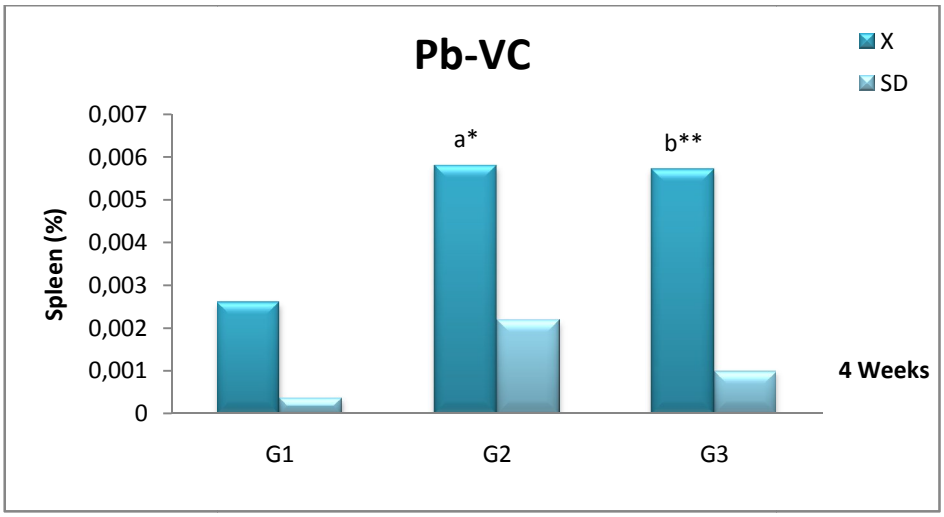


Fig 16: *comparison the relative organ weigh (%) of adrenal female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

The second experimental treatment: after 6 weeks of treatment

Albumin:

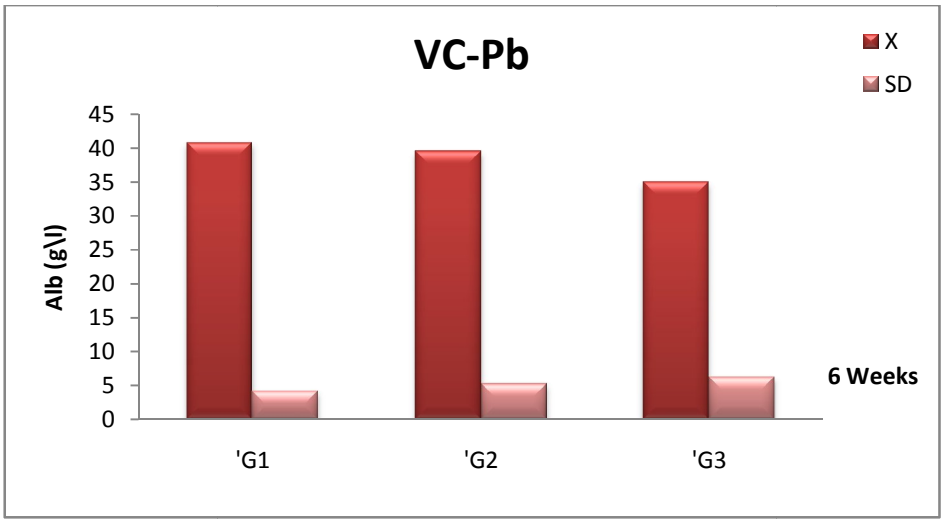


Fig 17: *comparison of albumin (Alb) concentration (X± SD) in the serum of female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Immunoglobulins:

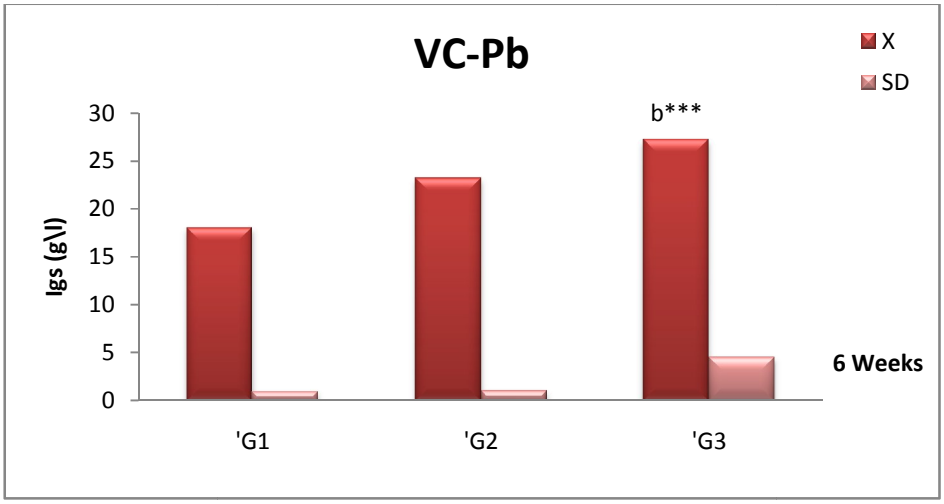


Fig 18: *comparison of immunoglobulins (Igs) concentration (X± SD) in the serum of female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Lymphocytes:

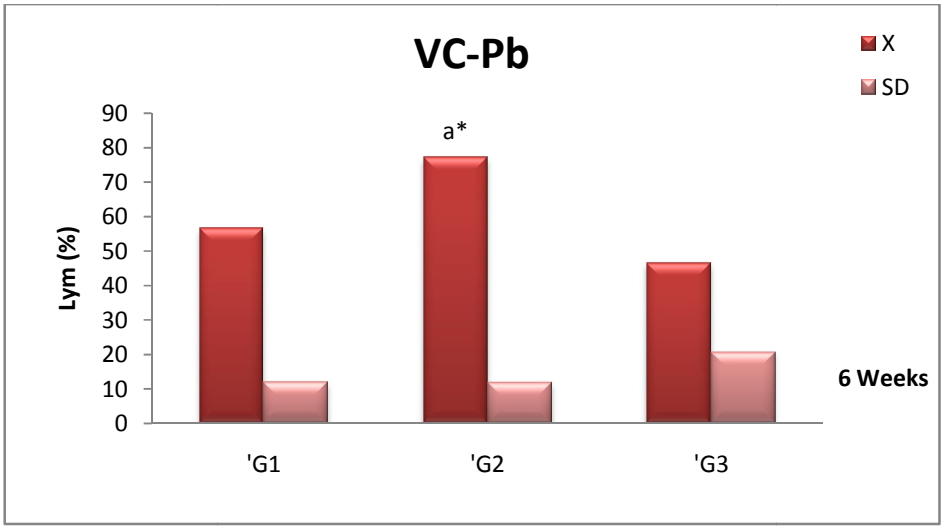


Fig 19: *comparison of lymphocytes (Lym) concentration (X± SD) in the blood of female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Neutrophils:

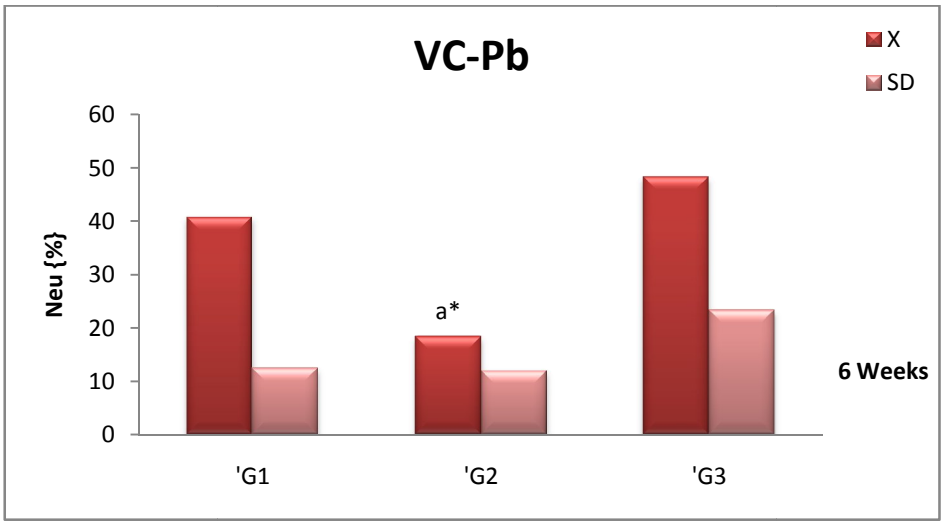


Fig 20: *comparison of Neutrophils (Neu) concentration (X± SD) in the blood of female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Eosinophils:

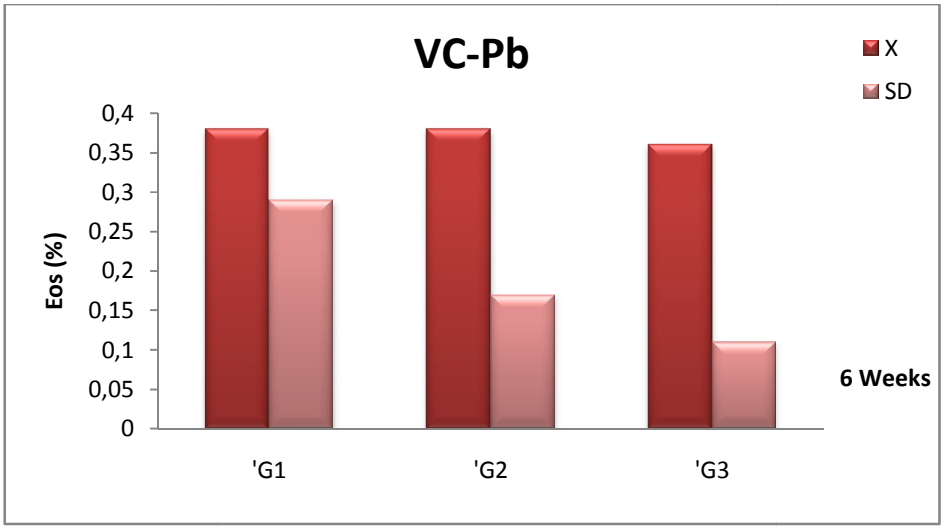


Fig 21: *comparison of eosinophils (Eos) concentration (X± SD) in the blood of female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Monocytes:

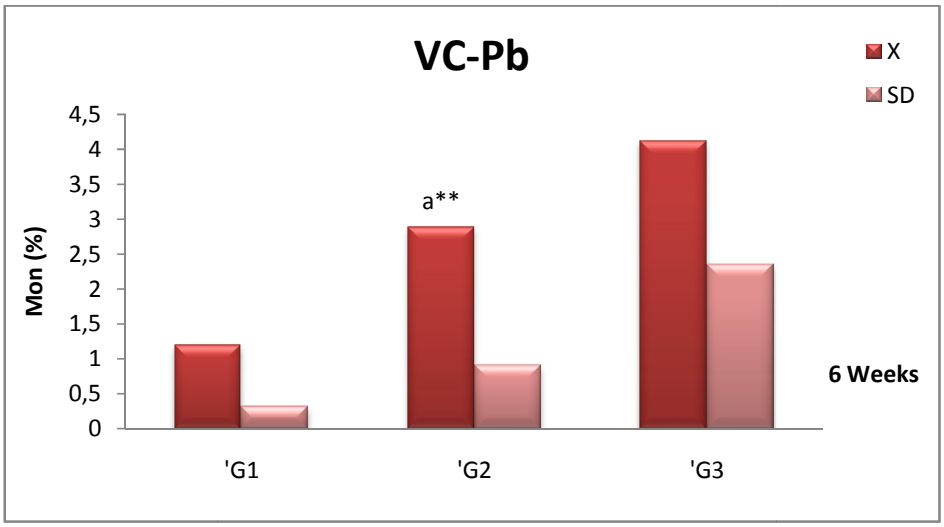


Fig 22: *comparison of monocytes (Mon) concentration (X± SD) in the blood of female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Minerals: after 6 weeks of treatment

Calcium:

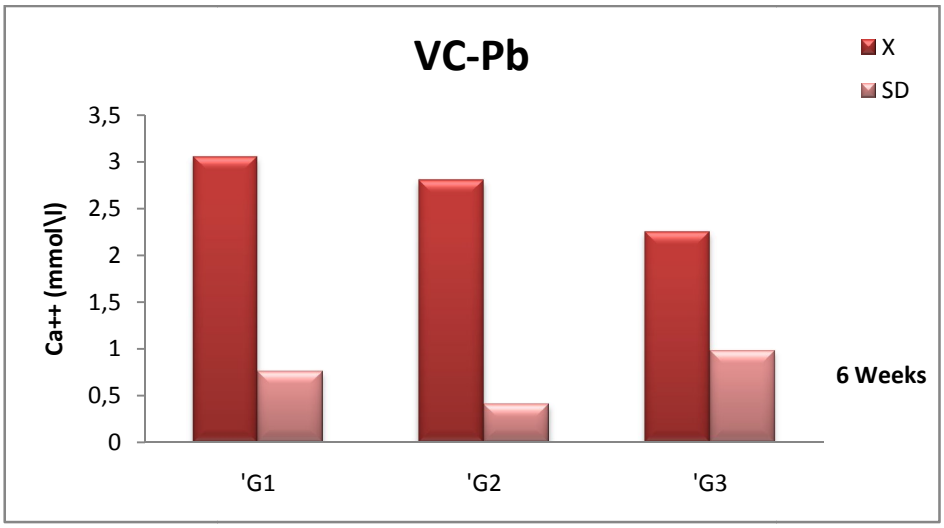


Fig 23: *comparison of calcium (Ca^{++}) concentration (X± SD) in the serum of female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Iron:

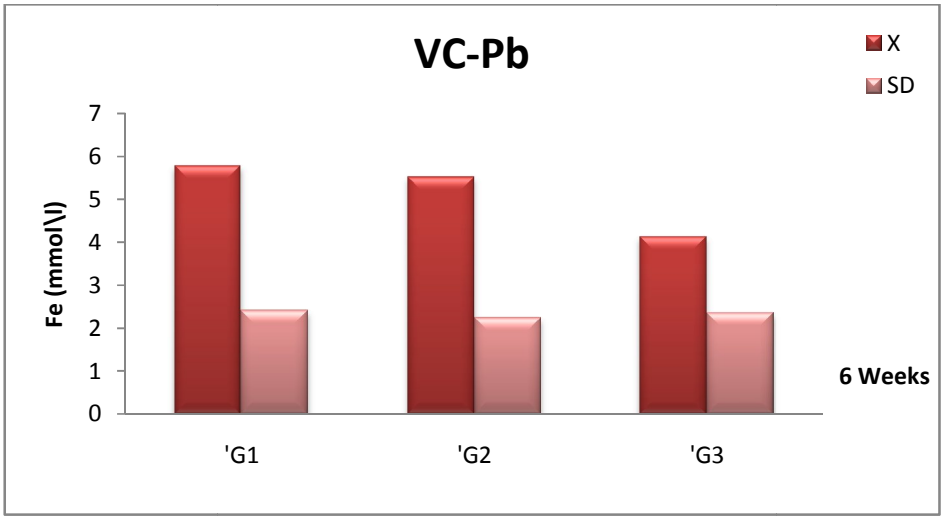


Fig 24: *comparison of iron (Fe^{++}) concentration (X± SD) in the serum of female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Relative organ weigh: after 6 weeks of treatment

Adrenal:

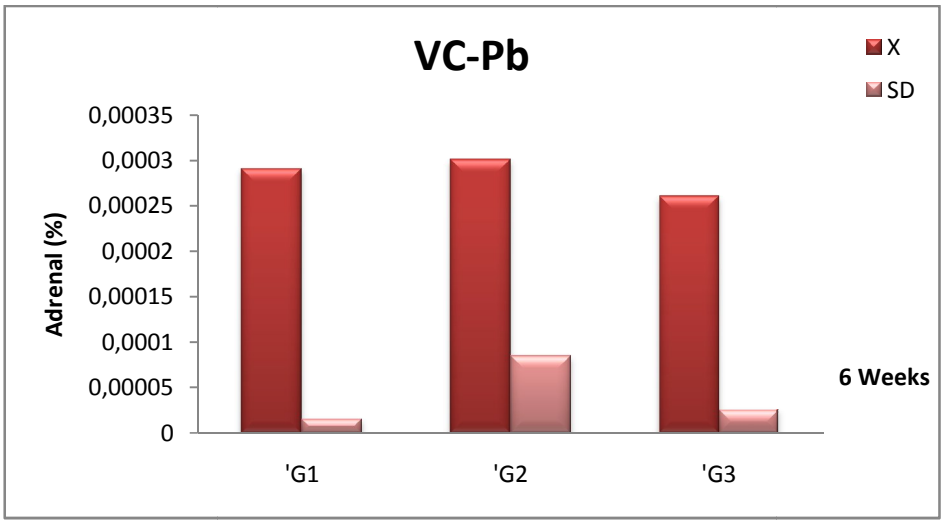


Fig 25: *comparison the relative organ weigh (%) of adrenal female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Thymus:

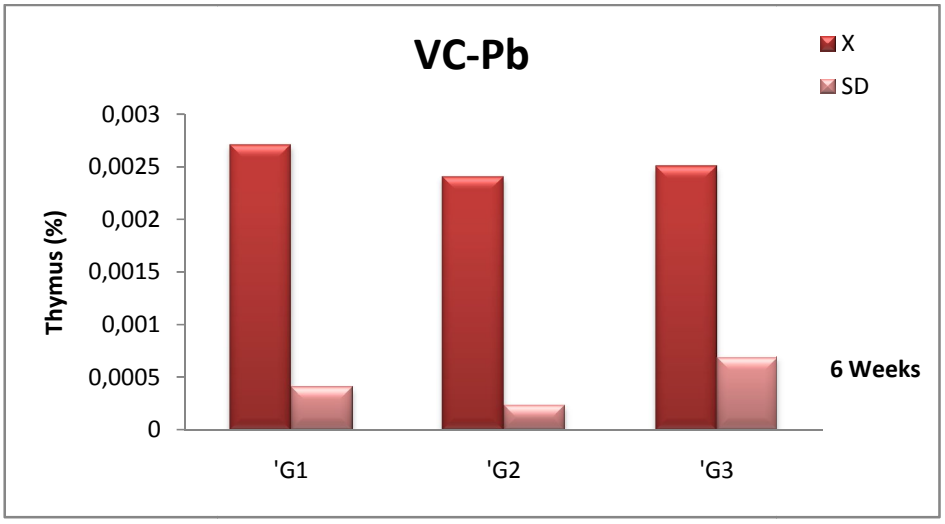


Fig 26: *comparison the relative organ weigh (%) of thymus female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Kidney:

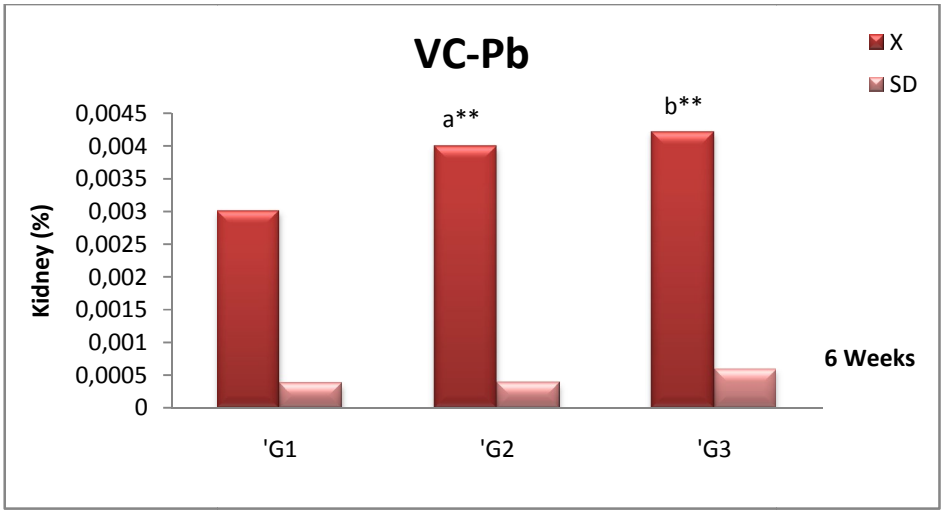


Fig 27: *comparison the relative organ weigh (%) of kidney female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Spleen:

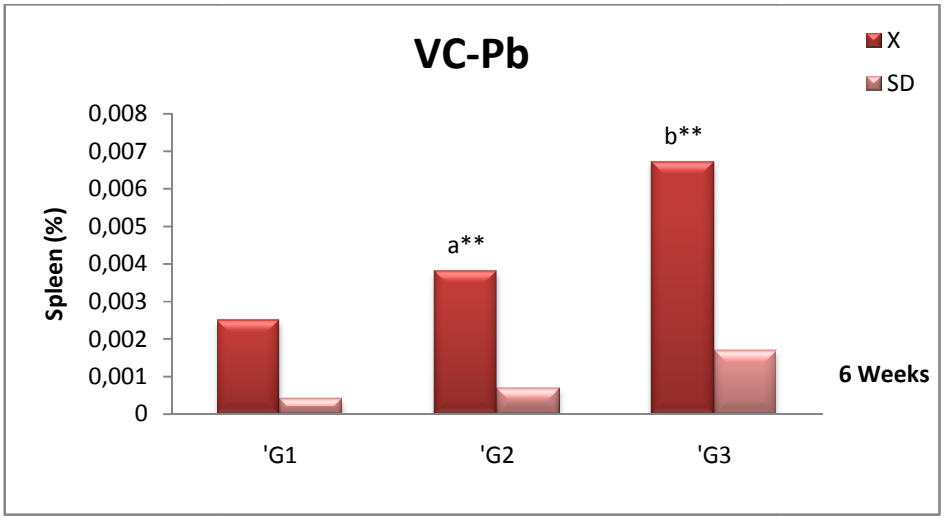


Fig 28: *comparison the relative organ weigh (%) of spleen female Wistar rats in the control ('G1), Pb +Vitamin C ('G2) and Pb ('G3) after 6 weeks.*

Comparison of Immunoglobulins and Albumin after the two treatment periods:

When comparing the two treatment periods we demonstrate that the addition of vitamin C and its elimination have noted a significant differences in both parameters (Albumin & Immunoglobulins) so this antioxidant nutriment is recommended to protect the body from lead intoxication.

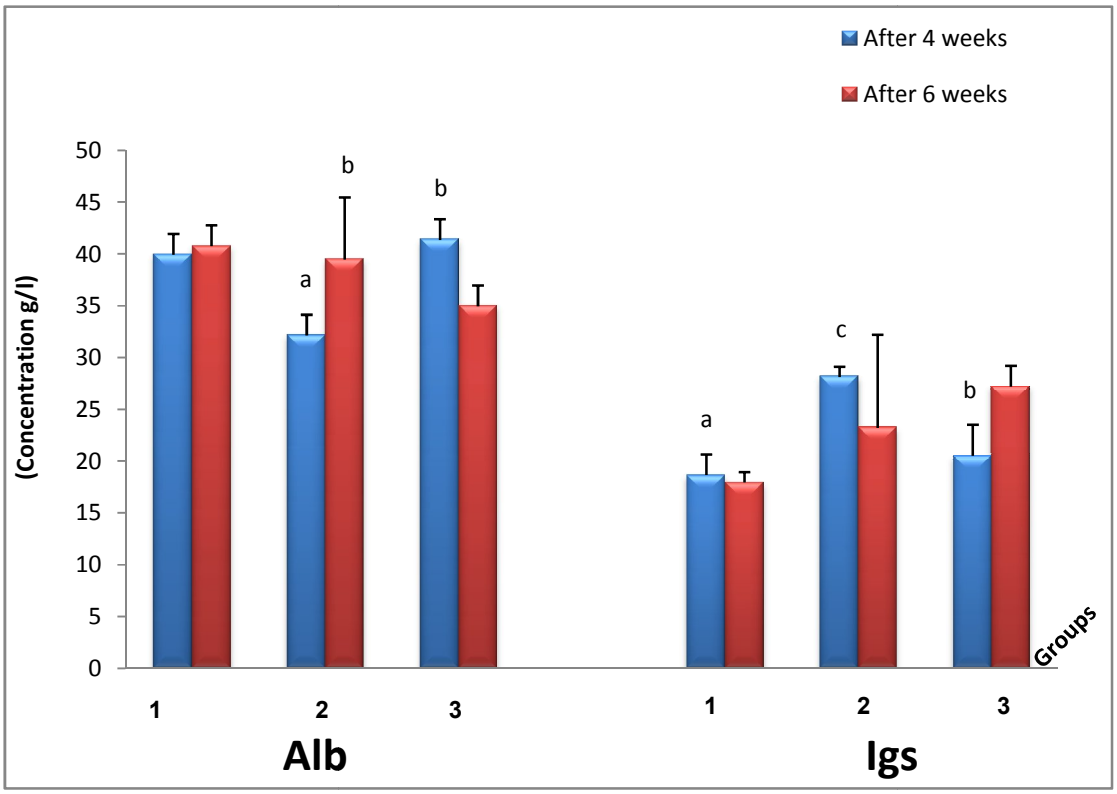


Fig 29: *Comparison of Albumin (Alb) and Immunoglobulins (Igs) concentrations in serum of female Wistar rats after the two treatment periods.*

Comparison of *Lymphocytes and Neutrophils* after the two treatment periods:

These two leucocytes are the indicators of immune system alteration because they represent the first line of defence in the organism specially neutrophiles. We note that Pb and vitamin C engender difference results either an increase or a decrease in there levels either in the first or in the second period treatment.

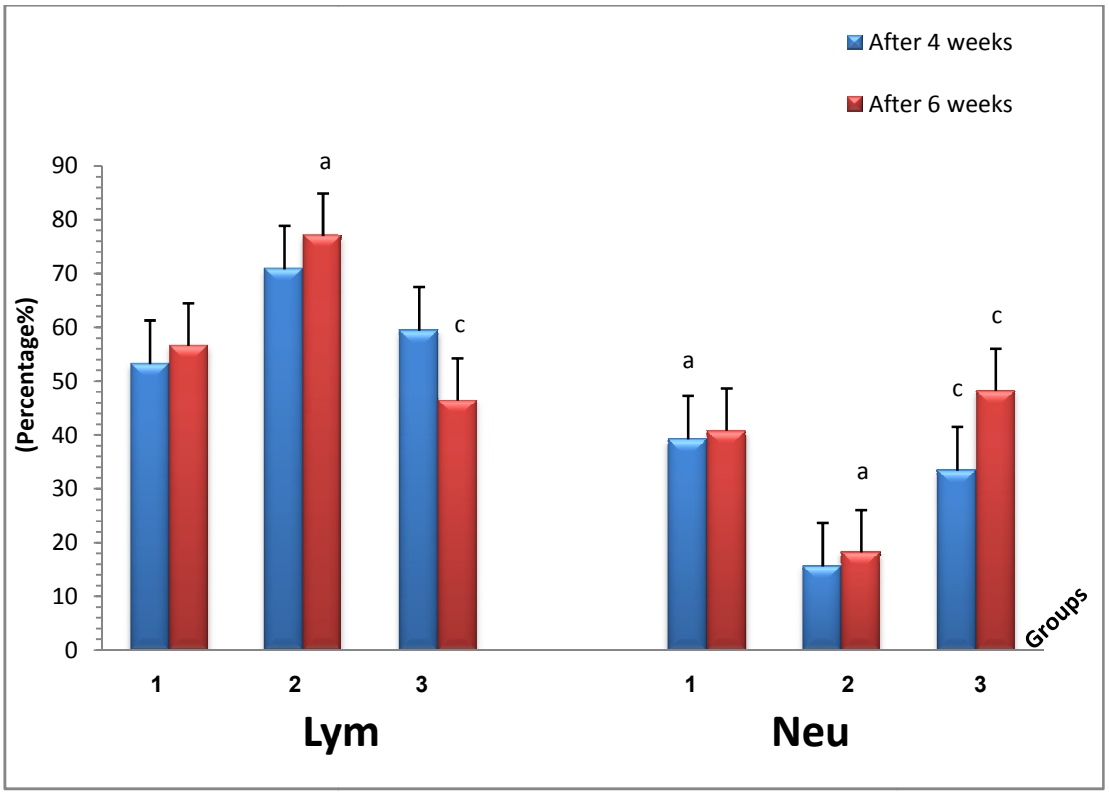


Fig 30: *Comparison of Lymphocytes (Lym) and Neutrophils (Neu) in the blood of female Wistar rats after the two treatment periods.*

Comparison of *Eosinophils and Monocytes* after the two treatment periods:

Supplementation or elimination of vitamin C in both treatment periods induces the same changes in both immune parameters.

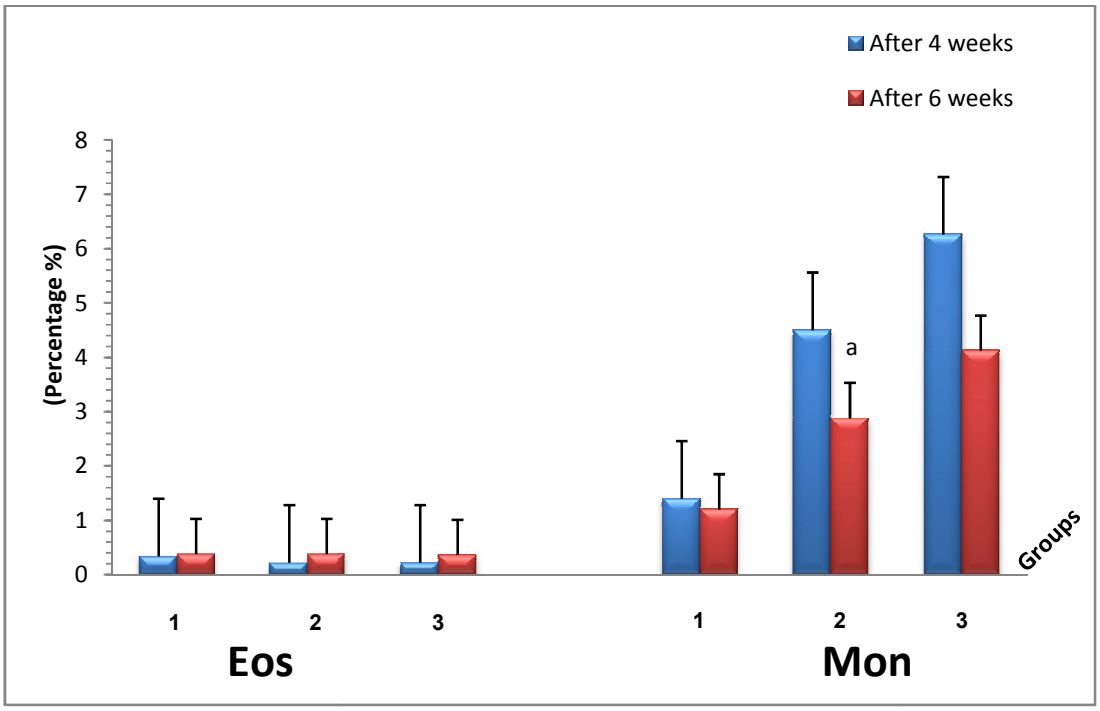


Fig 31: *Comparison of Eosinophils (Eos) and Monocytes (Mon) in the blood of female Wistar rats after the two treatment periods.*

Comparison of *minerals (calcium and iron)* after the two treatment periods:

When comparing these two parameters with control, we observe that lead affects their concentrations by decreased them significantly. But, either supplementation or elimination of vitamin C makes big differences concerning Fe^{++} and Ca^{++}

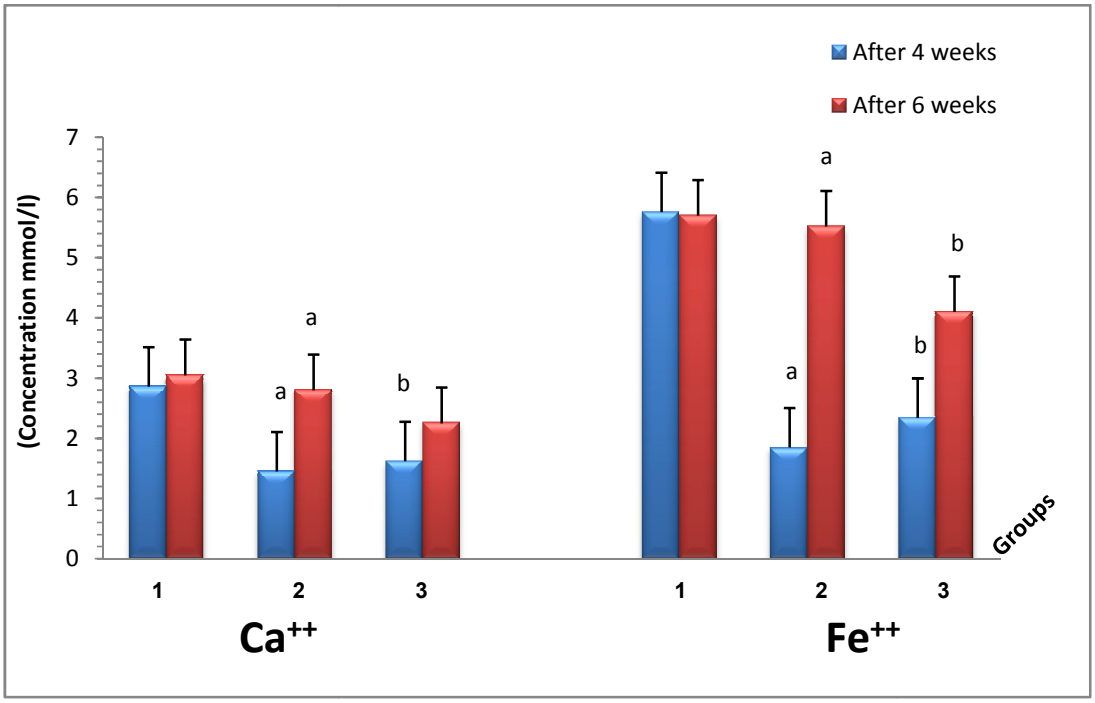


Fig 32: *Comparison of Calcium (Ca^{++}) and Iron (Fe^{++}) concentrations in serum of female Wistar rats after the two treatment periods.*

Comparison of *relative organ weights* after the two treatment periods:

Multiple differences were noted concerning the relative organ weights of these tissues in all treated groups, especially in kidney and spleen ones, were supplementation of vitamin C or its remove keep up their weights over the normal levels, when comparing them with control group, which demonstrate that Pb contaminated diet when it affect tissues there amelioration after that becomes difficult.

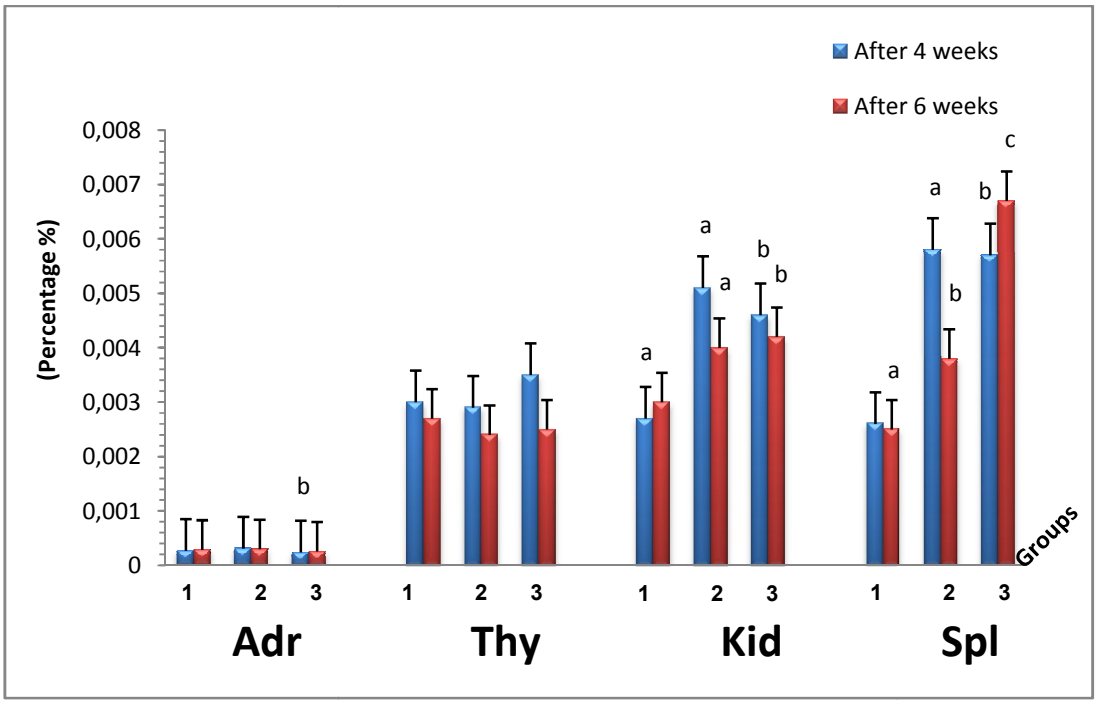


Fig 33: *Comparison of relative weight of Adrenal (Adr), Thymus (Thy), Kidney (Kid) and Spleen (Spl) of female Wistar rats after the two treatment periods.*

The second experimental protocol:

1. White Blood Cells:

Table 3. The measured values (mean ± SD)) of weight blood cell counts (WBC) of rats exposed to Pb, vitamin C and Olive Oil after treatment period of 4 weeks.

WBC 10^3/ul	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	6,6±2,18	11,83±2,22 a**	8,6±2,04	6,72±0,52	7,38±1,02

a:control vs Pb

****: P<0, 01**

WBC were significantly increased (P<0, 01) in Pb treated group when compared to control and Pb-VC group.

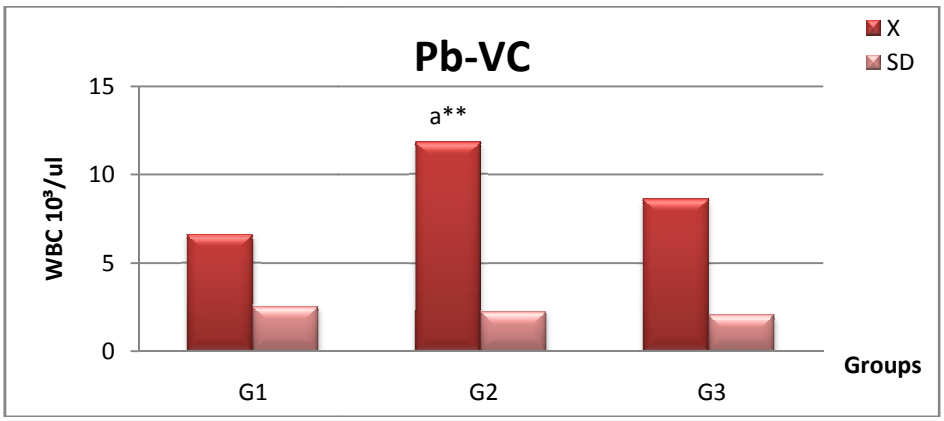


Fig 34: *comparison of white blood cell counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks.*

The same remark was noted in Pb rats fed Pb alone concerning WBC (P<0, 01).

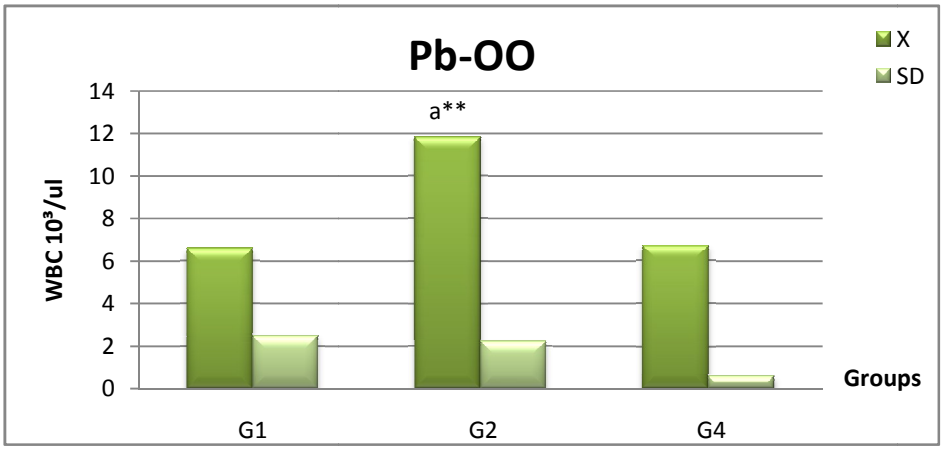


Fig 35: *comparison of white blood cell counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

Both combined groups with VC and OO were not significantly different from control one, in one hand, and high significant difference (P<0, 01) was noted in Pb group concerning white blood cell counts.

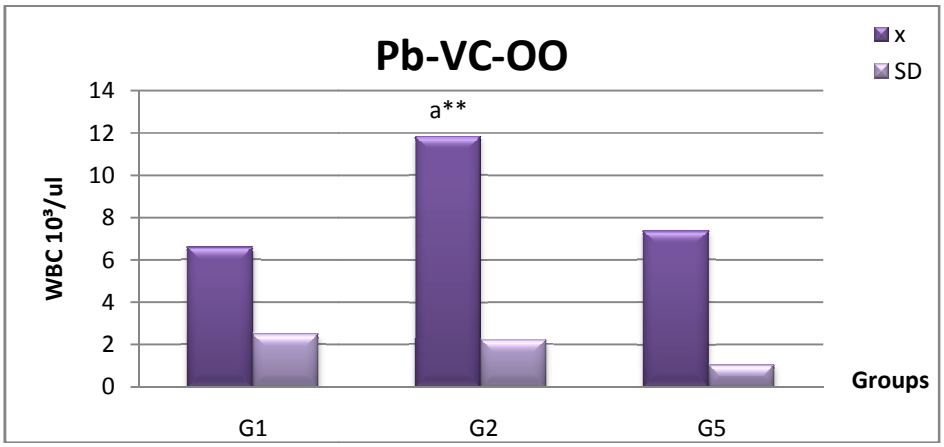


Fig 36: *comparison of white blood cell counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

2. Granulocytes:

Table 4. The measured values (mean ± SD) of blood granulocytes (GRAN) counts of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

GRA 10^3/ul	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	1,08±0,45 NS	2,26±0,86	1,26±0,42	1,48±0,27	1,48±0,27

NS: Non Significant

The statistical analyses revealed no significant differences between Pb or Pb-VC groups when compared to the control.

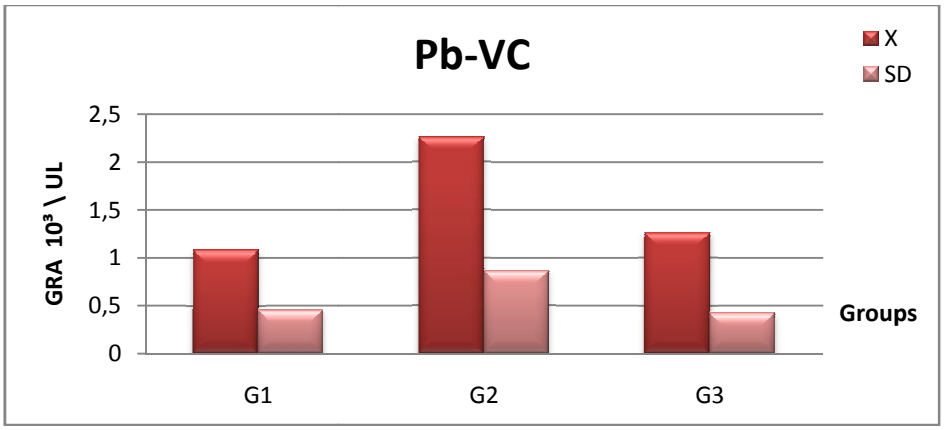


***Fig 37**: comparison of Granulocytes counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

GRAN concentration was not affected by lead intoxication and/or lead-olive oil supplementation comparing to control group.

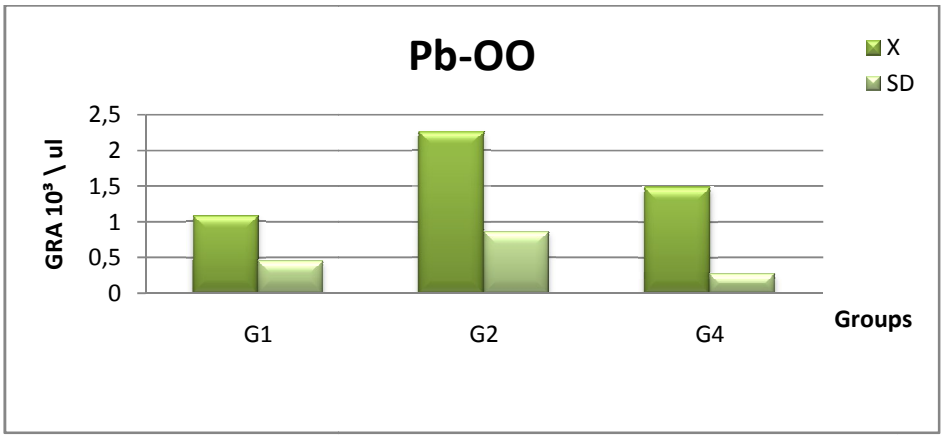


Fig 38: *comparison of white blood cell counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb + Olive Oil (G4) after 4 weeks*

The addition of vitamin C or olive oil in rats Pb contaminated diet does not affect the GRA values.

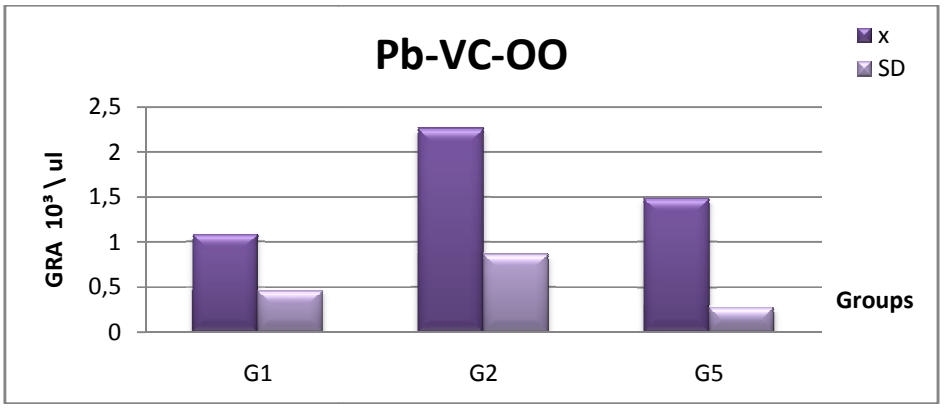


Fig 39*: comparison of white blood cell counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C + Olive Oil (G5) after 4 weeks*

3. Lymphocytes:

Table 5. The measured values (mean ± SD) of blood lymphocytes (Lym) counts of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

Lym 10^3/ul	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	4.84±1,76	7,44±1.71 a*	6.30±1,34	4,56±0,24	4,38±0,99

a: control vs Pb

*: P<0, 05

Concerning lymphocyte count we note a significant increase in Pb treated group when comparing it with control but there were no abnormalities in their values in combined treated group by VC comparing to control.

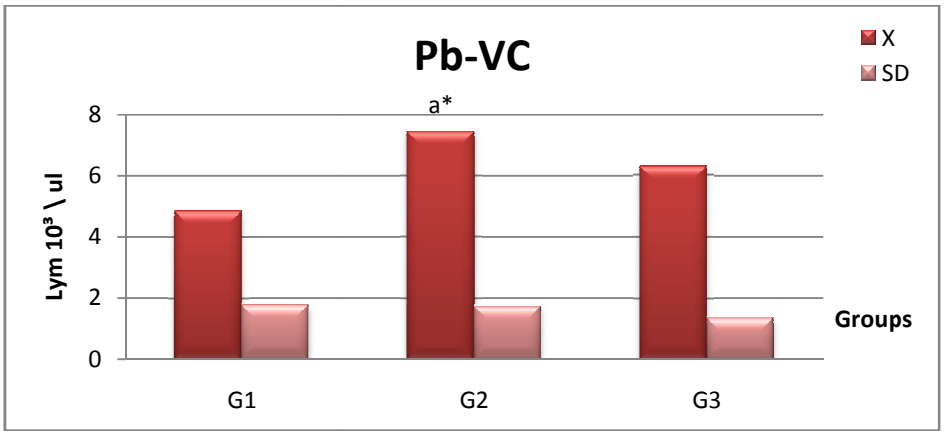


***Fig 40**: comparison of lymphocytes counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

Statistically there were no differences between combined treated group by olive and control one in lymphocyte concentration after 4 weeks trial.

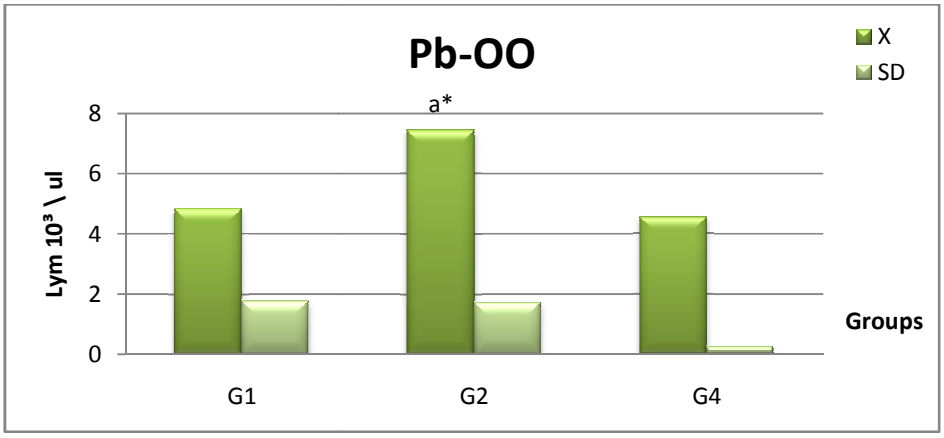


Fig 41: *comparison of lymphocytes counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb + Olive Oil (G4) after 4 weeks*

The addition of both vitamin C and olive oil in rats diet containing heavy metal (Pb) have returned the values of lymphocytes to the normal range and P was >0,05 (NS)

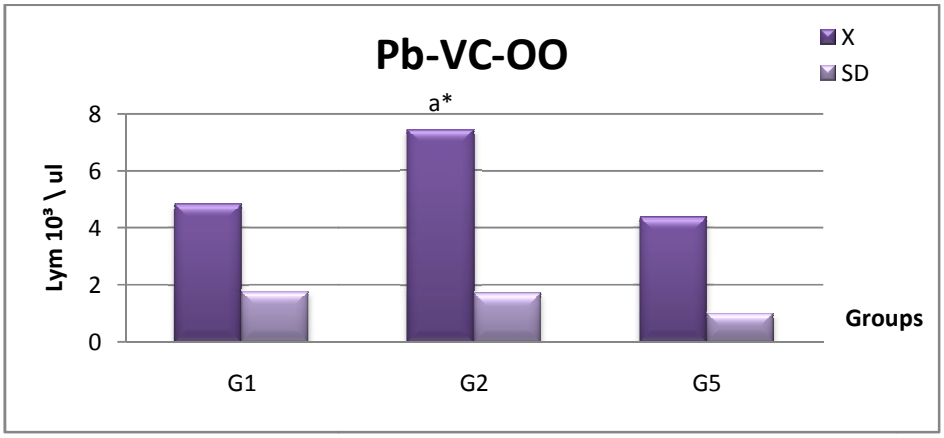


Fig 42*: comparison of lymphocytes counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C + Olive Oil (G5) after 4 weeks*

3. Monocytes:

Table 3. The measured values (mean ± SD) of blood monocytes (Mon) *counts* of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

Mon 10^3/ul	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	0,88±0,19 NS	1,7-±0,41	1,04±0,35	0,92±0,25	0,96±0,23

NS: Non Significant

Monocytes were not affected by the heavy metal (Pb) and no abnormalities were observed in combined treated group.

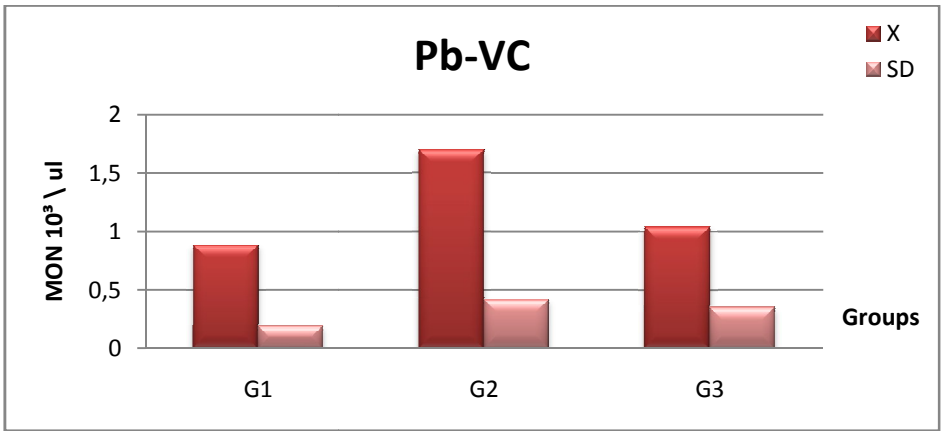


Fig 43: *comparison of monocytes counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

Olive oil supplementation returned the value of monocytes as that of the control group.

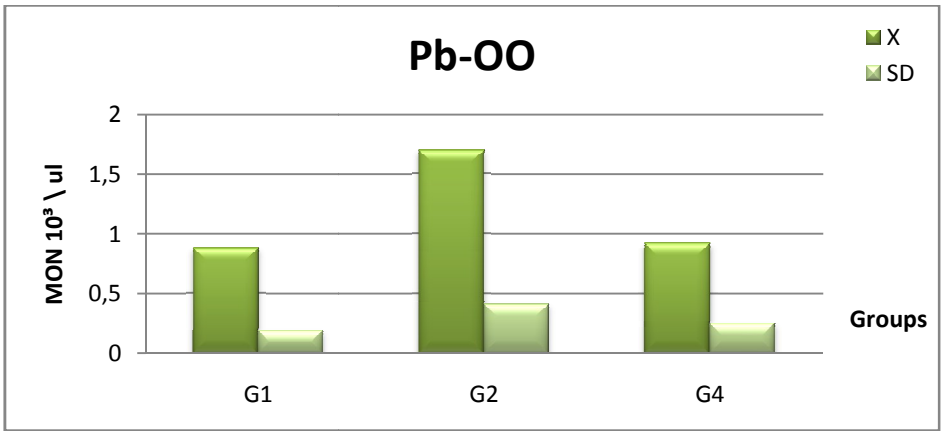


Fig 44: *comparison of monocytes counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

The supplementation diet (olive oil and Vitamin C) in treated group by PB marked no abnormalities in monocyte counts (P>0, 05) when comparing it to the control

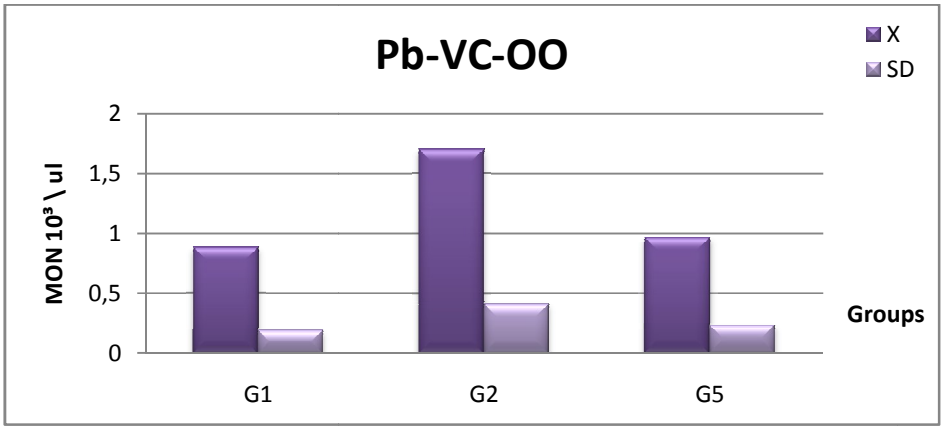


Fig 45*: comparison of monocytes counts (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

3. Immunoglobulins:

Table 6. The measured values (mean ± SD) of serum immunoglobulins (Igs) concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

Igs g/l	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	14,64±0,86	18,7±0,54 a***	16,8±0,76 b**	16,44±1,72	13,96±2,27

a: control vs Pb

b: control vs Pb-VC

****:** P<0, 01

*****:** P<0,001

The immunoglobulins concentration were increased significantly in Pb alone treated group compared to control (P<0,001) and were increased high significantly in Pb-VC treated group when compared to control one (P<0, 01).

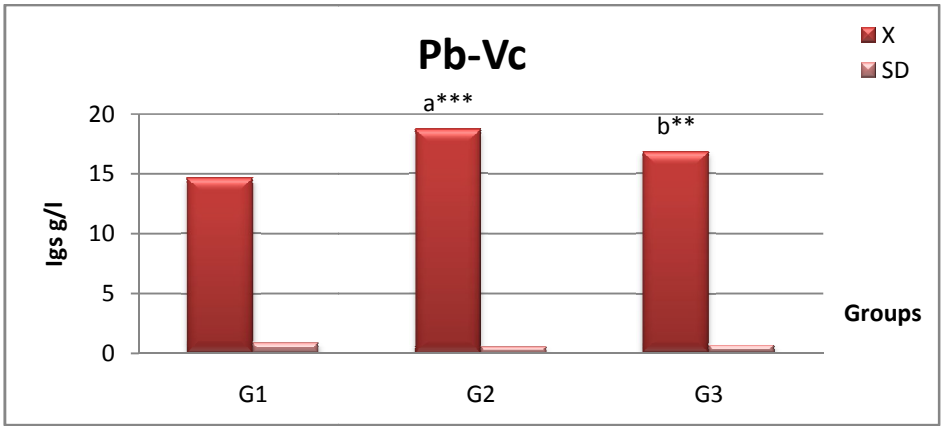


Fig 46: *comparison of immunoglobulins concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

The supplementation of olive oil have ameliorated the concentration of this immunological parameter and returning it across the normal range.

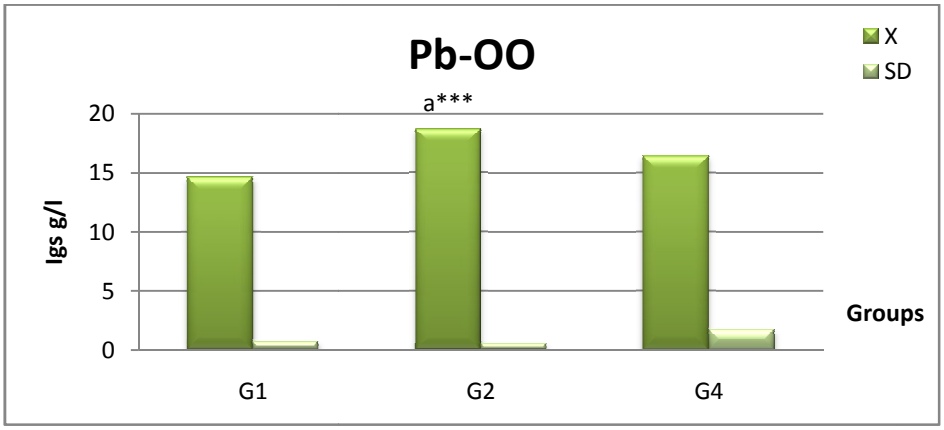


Fig 47: *comparison of immunoglobulins concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

Olive oil and vitamin C supplementation keep down the concentration of Igs which indicate that these two antioxidants protect the immune system from Pb intoxication very well.

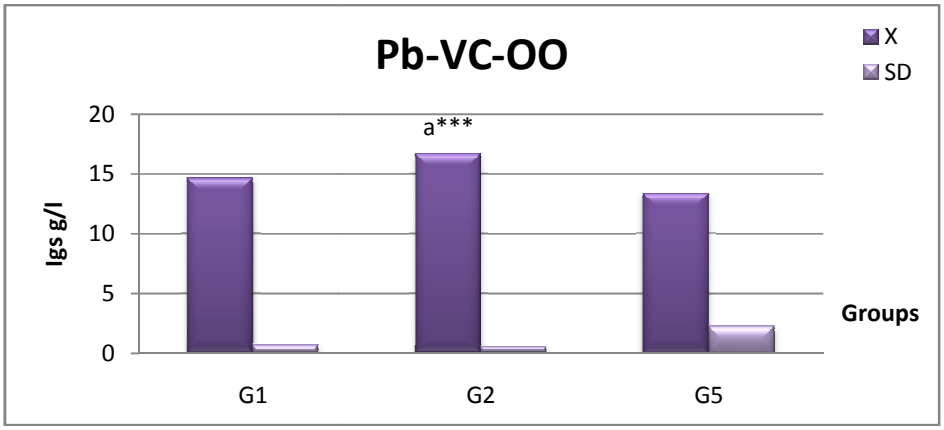


Fig 48: *comparison of immunoglobulins concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

Lipid parameters:

3. Triglycerides:

Table 7. The measured values (mean ± SD) of serum triglycerides (Igs) concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

Triglycerides g/l	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	1,50±0,55	1,58±0,49	0,65±0,17 b*	1,47±0,69	1,63±0,31

b: control vs Pb-VC

*: P<0, 05

The addition of vitamin C in Pb treated rats have decreased significantly the concentration of triglycerides however, its levels in Pb alone contaminated diet revealed no differences when compared to control group.

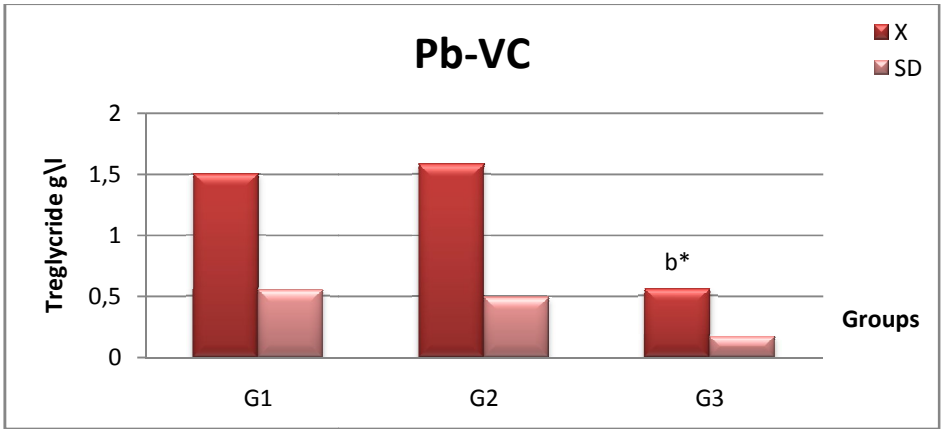


***Fig 49**: comparison of triglycerides concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

When supplementing olive oil to rats fed a Pb contaminated diet we remark no differences between both treated groups either with Pb alone or combined with olive oil (Pb-OO) when compared to the control.

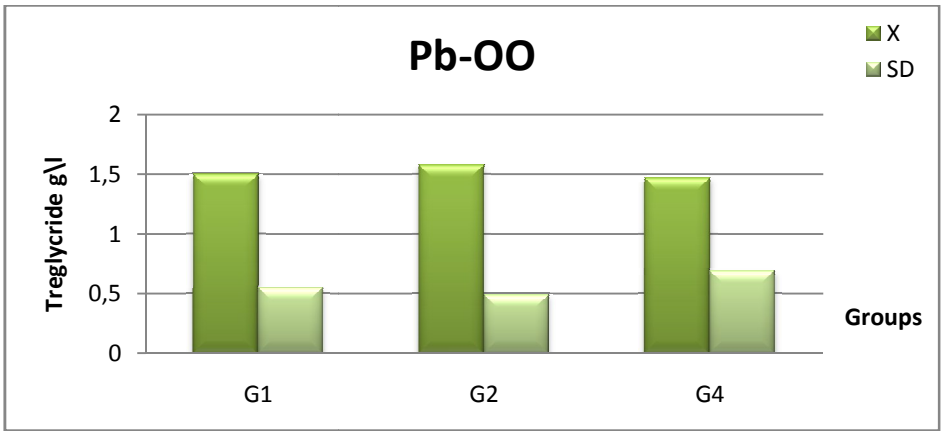


Fig 50: *comparison of triglycerides concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

Statistically non significant differences were marked in Pb-VC-OO compared to control.

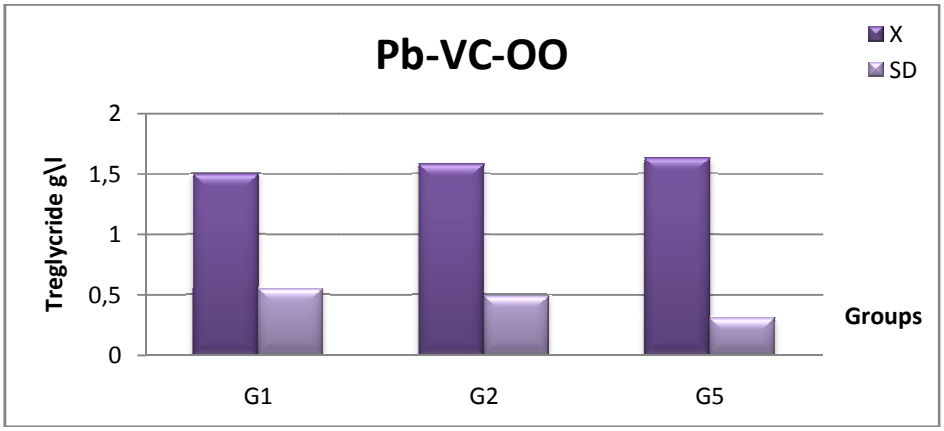


Fig 51: *comparison of triglycerides concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

3. Total cholesterol:

Table 8. The measured values (mean ± SD) of serum total cholesterol concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

T cholesterol g/l	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	0,77±0,20	0,56±0,03 a*	0,66±0,08	0,97±0,23	0,89±0,30

a: control vs Pb

*: P<0, 05

P<0, 05 in Pb treated group was noted in cholesterol parameters comparing to control group and no abnormalities in combined treated group (Pb-VC).

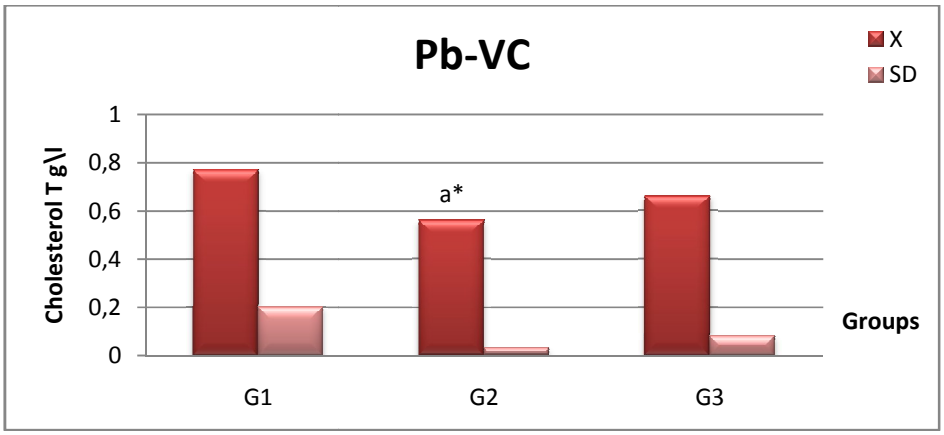


***Fig 52**: comparison of total cholesterol concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

Supplementation of olive oil have increased the level of cholesterol in combined treated group comparing to control but statistically was no significant.

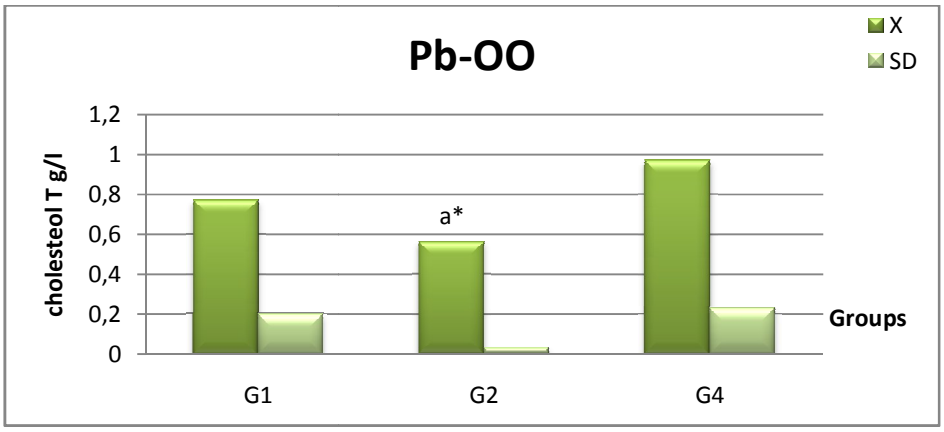


***Fig 53**: comparison of total cholesterol concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

The same remark was noted in combined treated group with both olive oil and vitamin C when compared to the control.

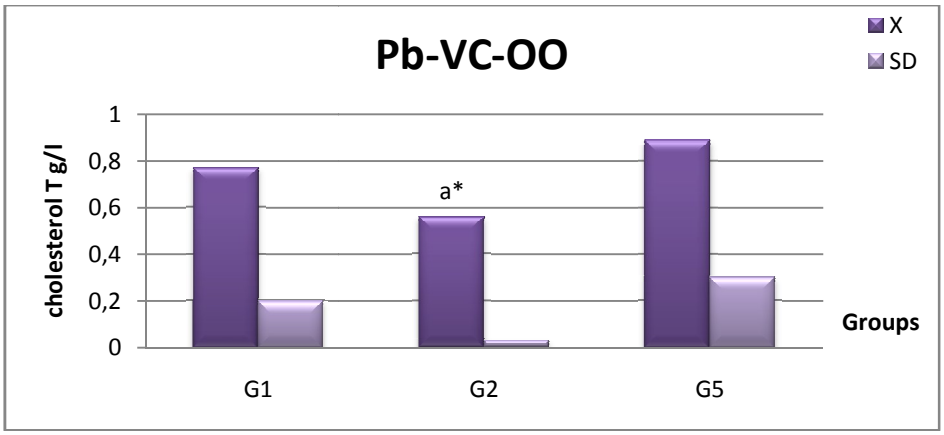


***Fig 54**: comparison of total cholesterol concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

3. High Density Lipoprotein (HDL):

Table 9. The measured values (mean ± SD) of serum high density lipoprotein (HDL) concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

HDL g/l	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	0,26±0,05	0,17±0,03 a***	0,19±0,02	0,23±0,03	0,20±0,05

a: control vs Pb

***: P<0,001

HDL, the good cholesterol was decreased significantly in Pb treated group alone (P<0,001), but the addition of vitamin C has increased it significantly.

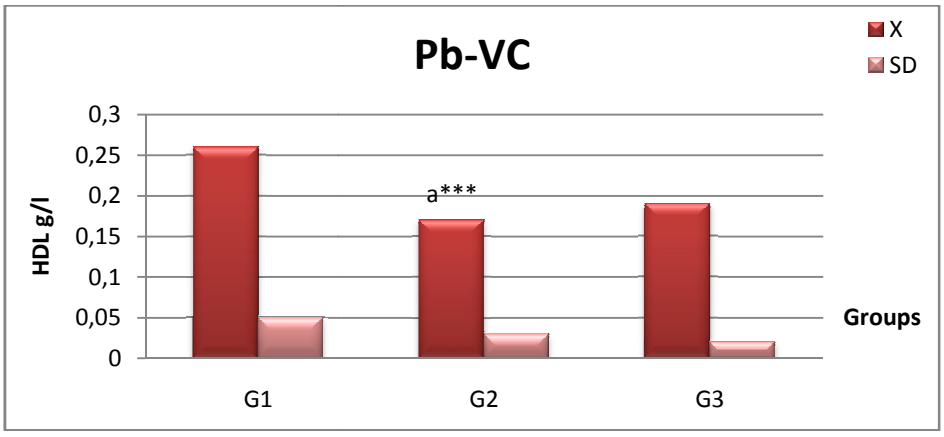


***Fig 55**: comparison of HDL concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

The supplementation of olive oil has ameliorated the concentration of cholesterol-HDL in combined treated group when compared to control one.

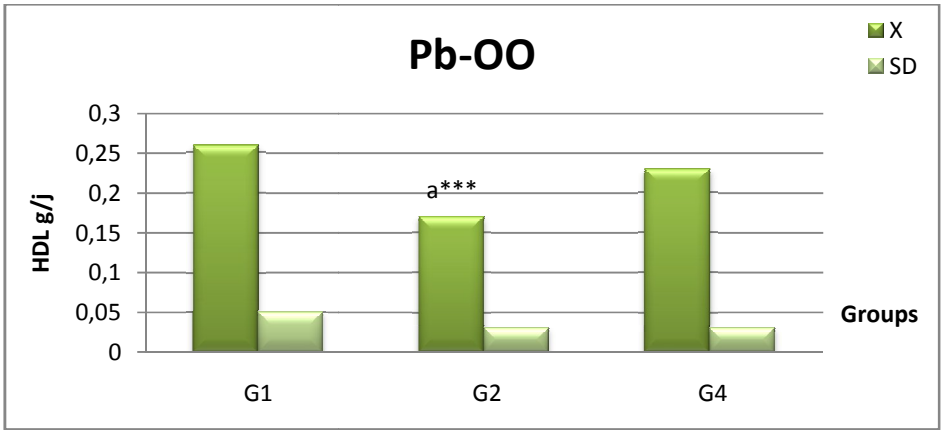


Fig 56: *comparison of HDL concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

No abnormalities were marked in Pb-VC-OO group when compared to control.

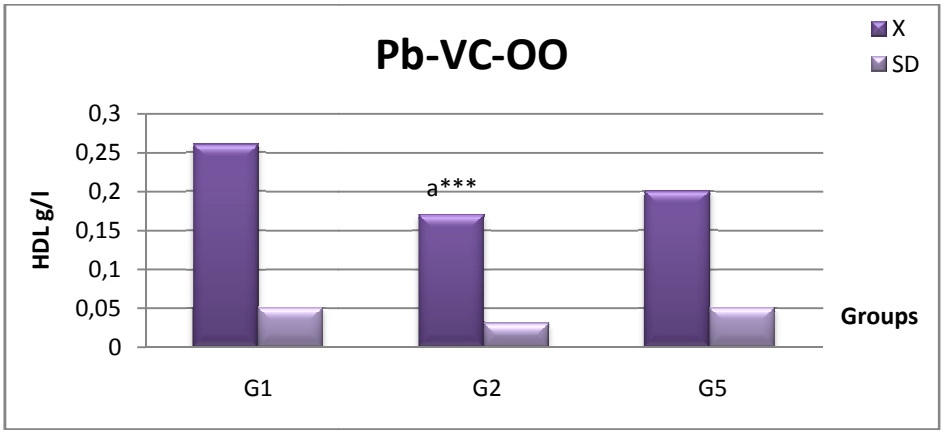


Fig 57: *comparison of HDL concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

4. Low Density Lipoprotein (LDL):

Table 10. The measured values (mean ± SD) of serum low density lipoprotein (LDL) concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

LDL g/l	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	0,20±0,07	0,42±0,02 a**	0,22±0,09 b*	0,19±0,02	0,13±0,02

a: control vs Pb **b:** control vs Pb-VC

*: P<0, 05 **: P<0, 01

Concerning Low Density Lipoprotein levels statistically we noted a high significant differences (P<0, 01) and a significant differences (P<0, 05) in Pb alone treated group and combined treated group (Pb-VC) respectively when compared to control.

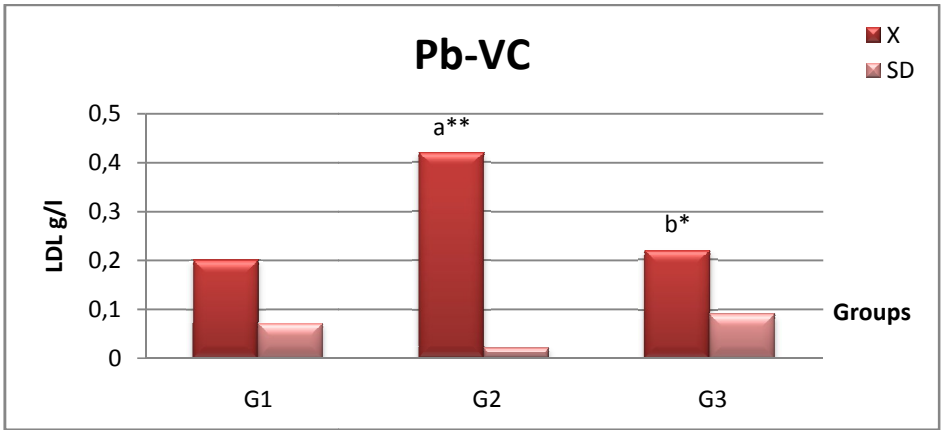


Fig 58: *comparison of LDL concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

We observe that the addition of olive oil in Pb treated group have ameliorated the level of the dab cholesterol (LDL) when compared to the control.

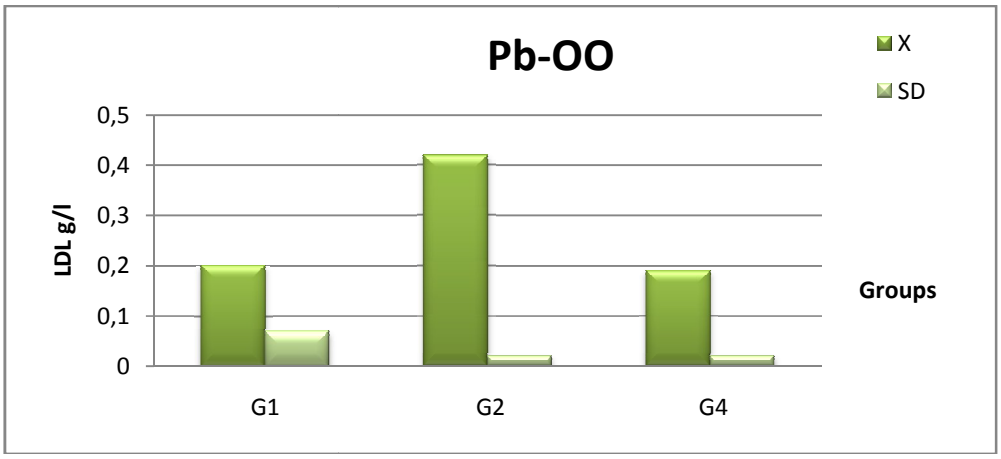


Fig 59: *comparison of LDL concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

The supplementation of both sources of antioxidants (VC and OO) the level of LDL were decreased under the concentration of the control one which prove the beneficial antioxidant role of these antioxidants especially maybe in Atherosclerosis disease.

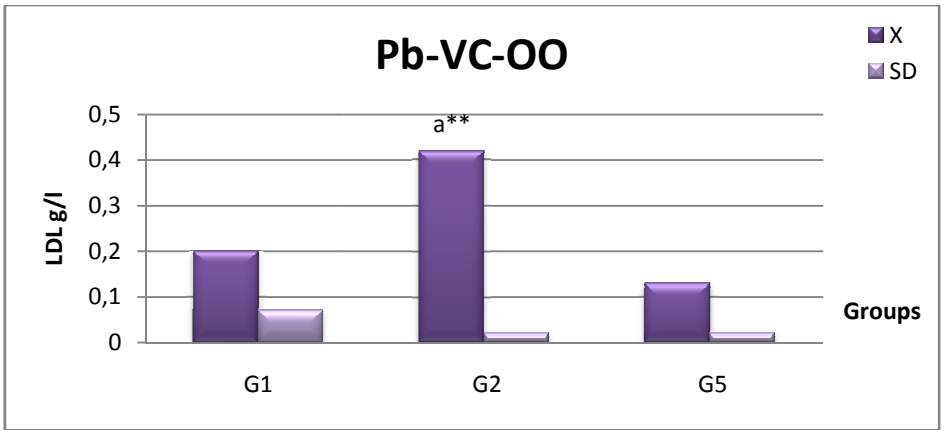


Fig 60: *comparison of LDL concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

Minerals:

1. Calcium:

Table 11. The measured values (mean ± SD) of serum calcium (Ca^{++}) concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

Ca^{++} mmol/l	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	1,92±0,81 NS	0,99±0,13	1,71±0,70	1,11±0,25	1,92±1,12

NS: Non Significant

Although the decreased concentration of Ca^{++} in Pb treated group statistically were not significant and, the supplementation of vitamin C have ameliorated the situation but statistically also non significant.

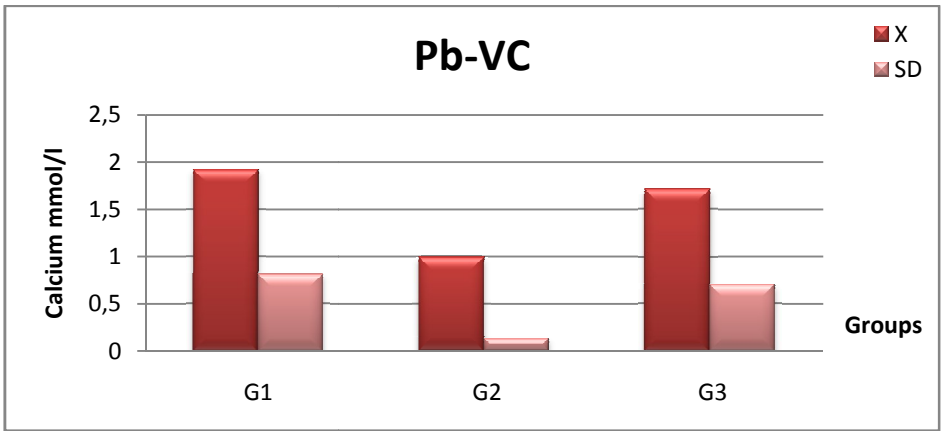


***Fig 61**: comparison of calcium concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

The same remark was noted concerning olive oil supplementation.

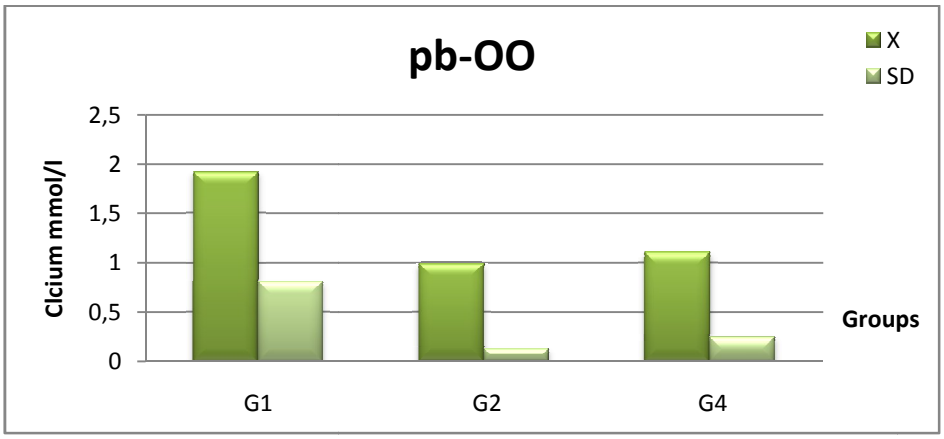


Fig 62: *comparison of calcium concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

We observed that the supplementation of both vitamin C and olive oil have ameliorated very well the concentration of Ca++ combined treated group when comparing with control (the same value)

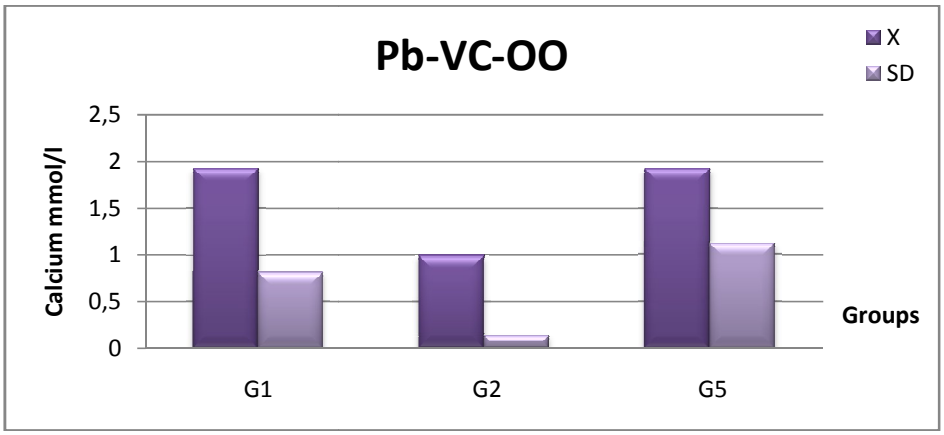


Fig 63: *comparison of calcium concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

2. Iron:

Table 12. The measured values (mean ± SD) of serum iron (Fe^{++}) concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

Fe^{++} umol/l	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	75,9±16	23,82±7,60 a***	33,9±9,1 b**	38,97±8,74 c**	43,1±11,4 d**

a: control vs Pb **b:** control vs Pb-VC

c: control vs Pb-OO **d:** control vs Pb-VC-OO

****:** P<0, 01 *****:** P<0,001

The very high significant decrease in iron level in Pb treated group indicate that the anaemia associated to Pb exposure is the first sign of intoxication, and the supplementation of vitamin C have not ameliorated the situation in the literature VC compete the Fe++ in intestinal absorption and these finding is in accord with our results here.

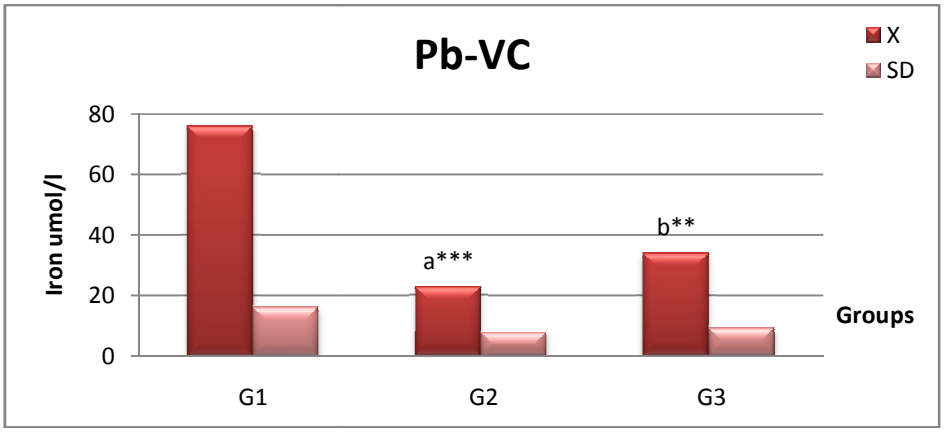


Fig 64: *comparison of iron concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

Also the addition of olive oil in Pb-OO group has not ameliorated the situation.

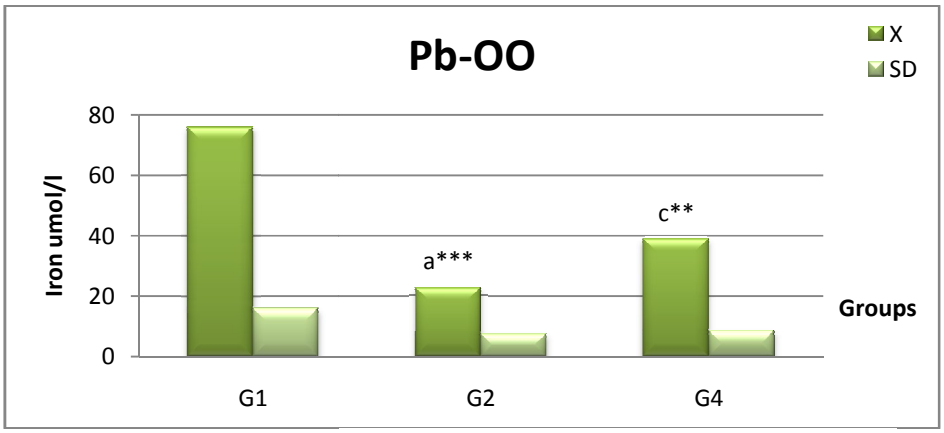


***Fig 65**: comparison of iron concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

Both VC and OO addition statistically mark high significant differences (P<0, 01) which indicated the level of competition observed above and this time 4 parameters entre in this competition and low iron concentration pay the consequences.

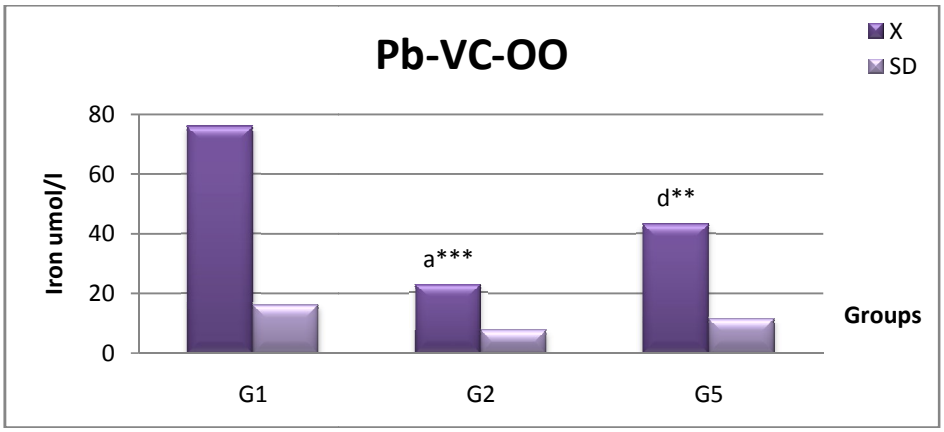


***Fig 66:** comparison of iron concentration (X± SD) in the serum of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

Haematological parameters:

1. Red Blood Cells (RBC):

Table 13. The measured values (mean ± SD) of serum red blood cells (RBC) concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

RBC 10^6/ul	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	9,16±0,62	7,71±0,24 a**	8,75±0,46	7,84±1,15	7,95±1,06

a: control vs Pb ****:** P<0, 01

The count of RBC was noted a high significant decrease in Pb alone treated group when compared to the control. However, no abnormalities were remarked in combined treated group by VC comparing to control.

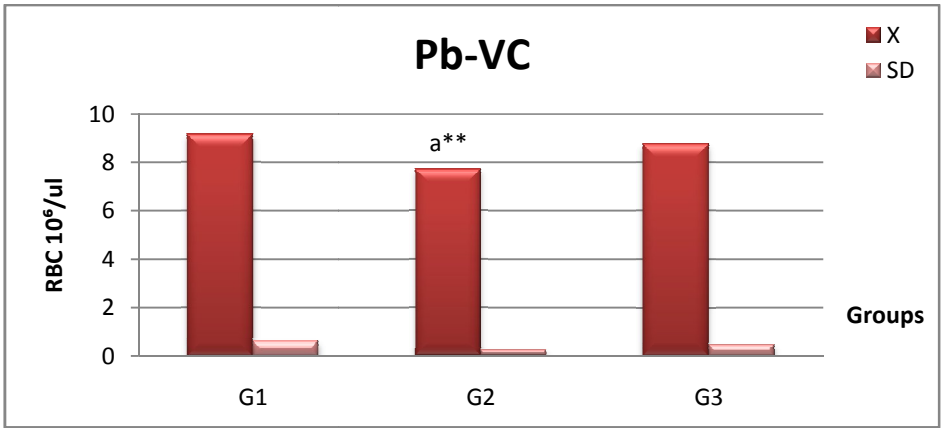


***Fig 67**: comparison of red blood cells concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

The same observation were noted concerning supplementation of olive oil

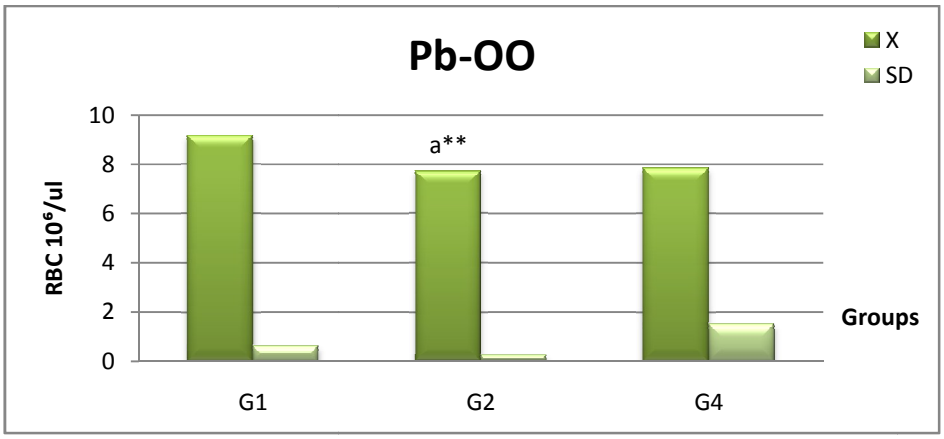


Fig 68: *comparison of red blood cells concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

Statistically no differences in RBC count were observed in Pb-VC-OO group comparing to control.

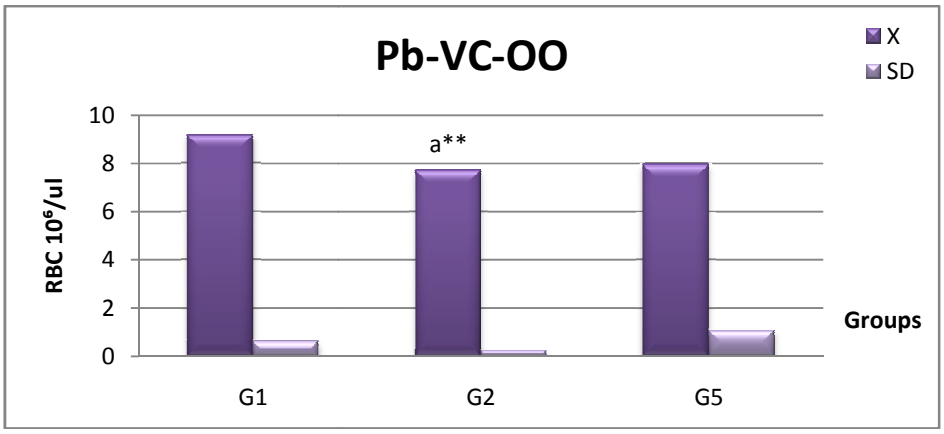


Fig 69: *comparison of red blood cells concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks*

2. Haemoglobin (Hb):

Table 14. The measured values (mean ± SD) of blood haemoglobin (Hb) concentration of vitamin C, Olive Oil and Pb exposed rats after treatment period of 4 weeks.

Hb g/l	Control n= 10	Pb n=10	Pb + Vit C n=10	Pb + OO n=10	Pb+VitC+OO n=10
X± SD	16,72±1,48	12,38±0,79 a**	14,46±0,85 b*	13,1±3,16 c*	14,64±0,74

a: control vs Pb **b:** control vs Pb-VC **c:** control vs Pb-OO

*: P<0, 05 **: P<0, 01

Haemoglobin concentration decreased significantly P<0,001, P<0,05 in Pb treated group and Pb-VC group respectively comparing to control one.

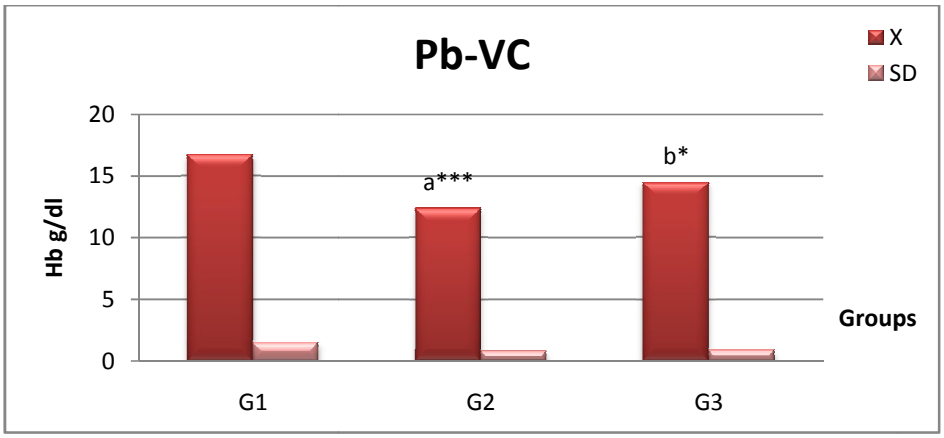


***Fig 70**: comparison of haemoglobin concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C (G3) after 4 weeks*

Olive oil has not returned the concentration of Hb to the normal rang in Pb-OO combined group comparing to the control.

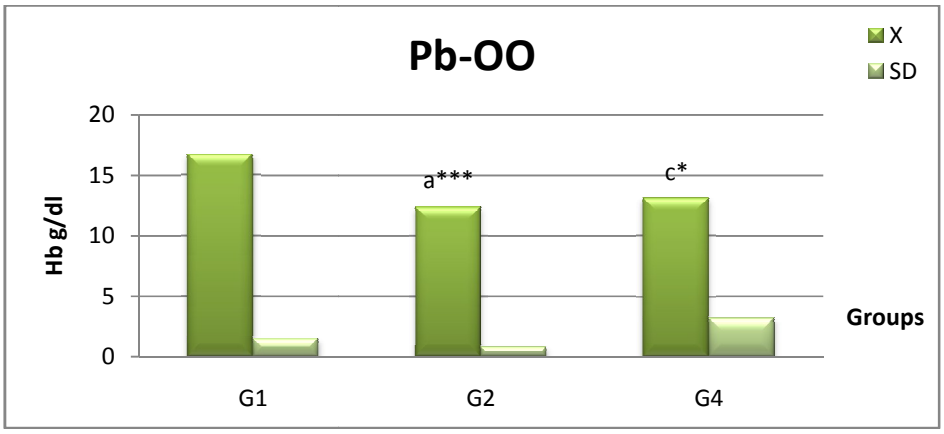


***Fig 71**: comparison of haemoglobin concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Olive Oil (G4) after 4 weeks*

The supplementation of both antioxidants to Pb exposure rats has returned the values of Hb to the normal range.

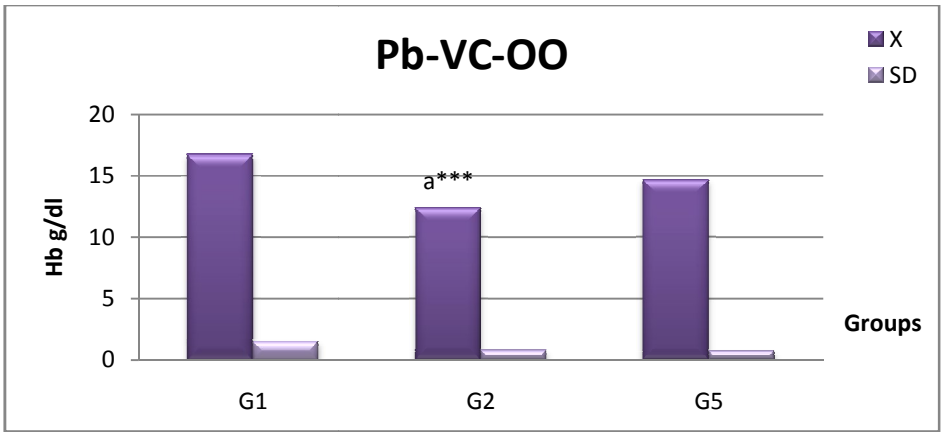


***Fig 72**: comparison of haemoglobin concentration (X± SD) in the blood of female Wistar rats in the control (G1), Pb (G2) and Pb +Vitamin C+ Olive Oil (G5) after 4 weeks.*

Histological study:

Kidney: After treatment by Pb, Vitamin C and Olive Oil.

In **figure 73:** we observe that the kidney tissue in control group was totally normal concerning its architecture (X 400).

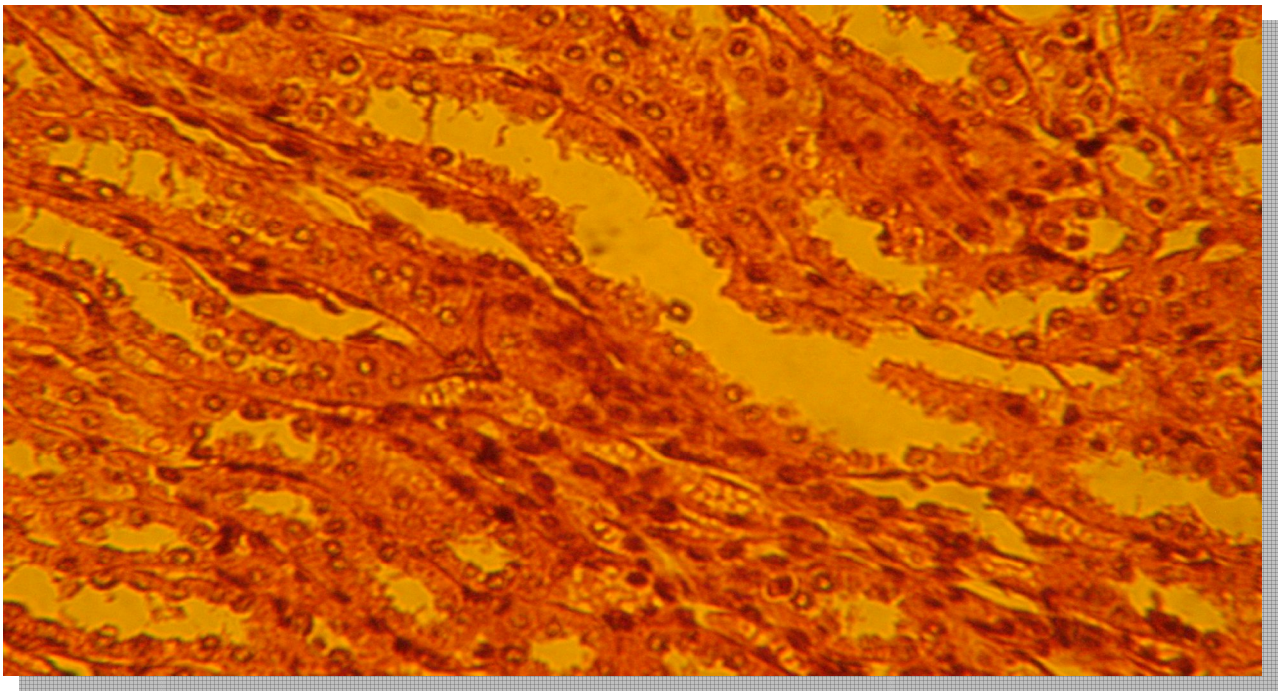


Fig 73: Transversal cross section of rat kidney from the control (X400).

In **figure 74:** in transversal coupe on rats kidney treated by Pb alone, we observed a primary extension in kidney tubes with interstitial nephrite.

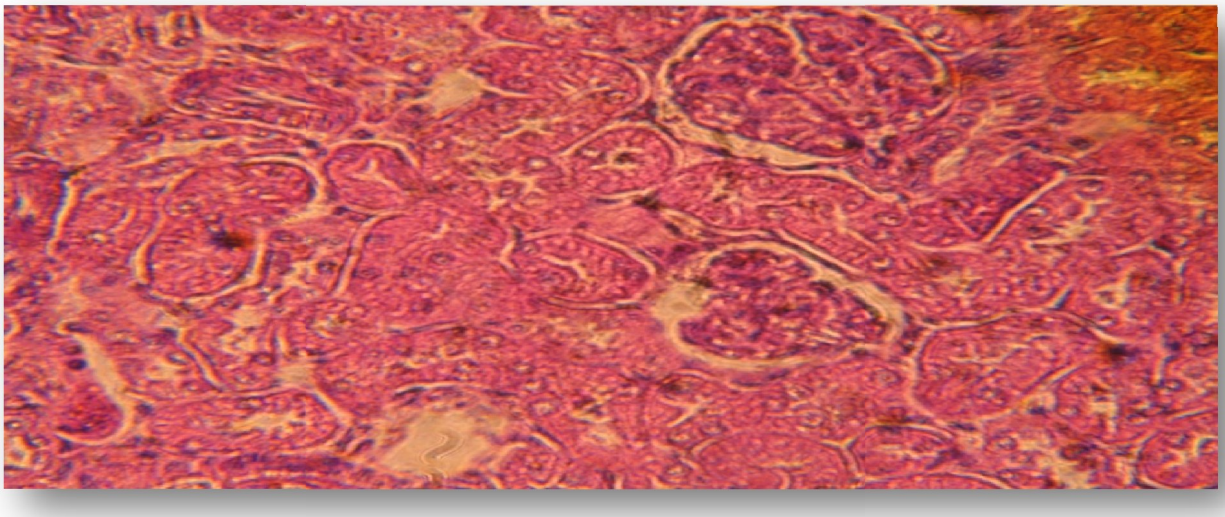


Fig 74: Transversal cross section of rat kidney treated by Pb (X400).

In **figure 75:** the Pb + Vit C treated group there was a decrease in the extension of proximal contoured tubes comparing them with Pb treated group.

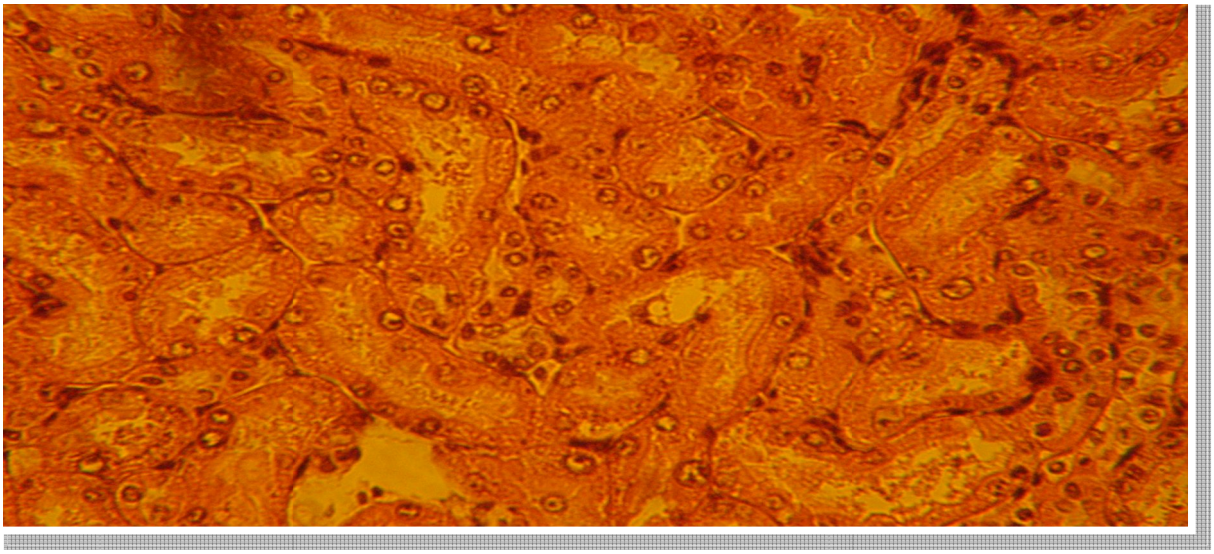


Fig 75: Transversal cross section of rat kidney from group treated by Pb + Vitamin C (X400).

In **figure 76:** in this fig we note amelioration in the kidney tissue architecture after its treatment by olive oil comparing with group treated by Pb alone.

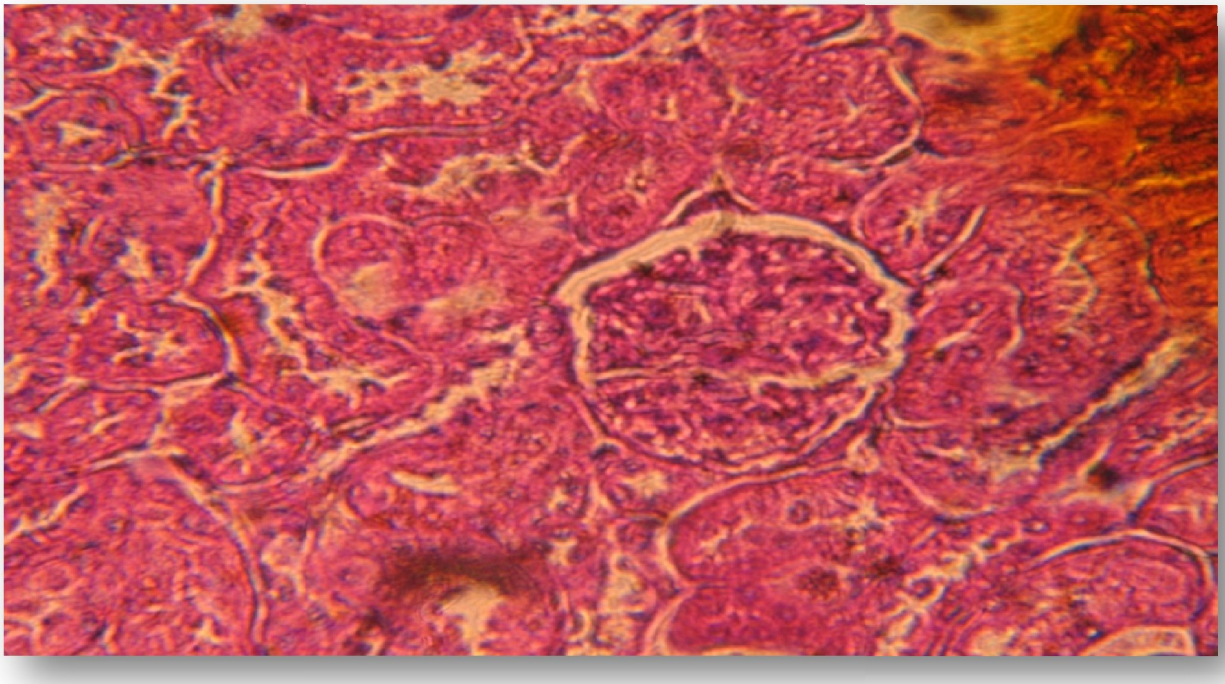


Fig 76: Transversal cross section of rat kidney treated by Pb + Olive Oil (X400).

Liver: After treatment by Pb, Vitamin C and Olive Oil.

In **figure 77:** a control group shows those coherent hepatocytes and no morphological or physiological abnormalities.

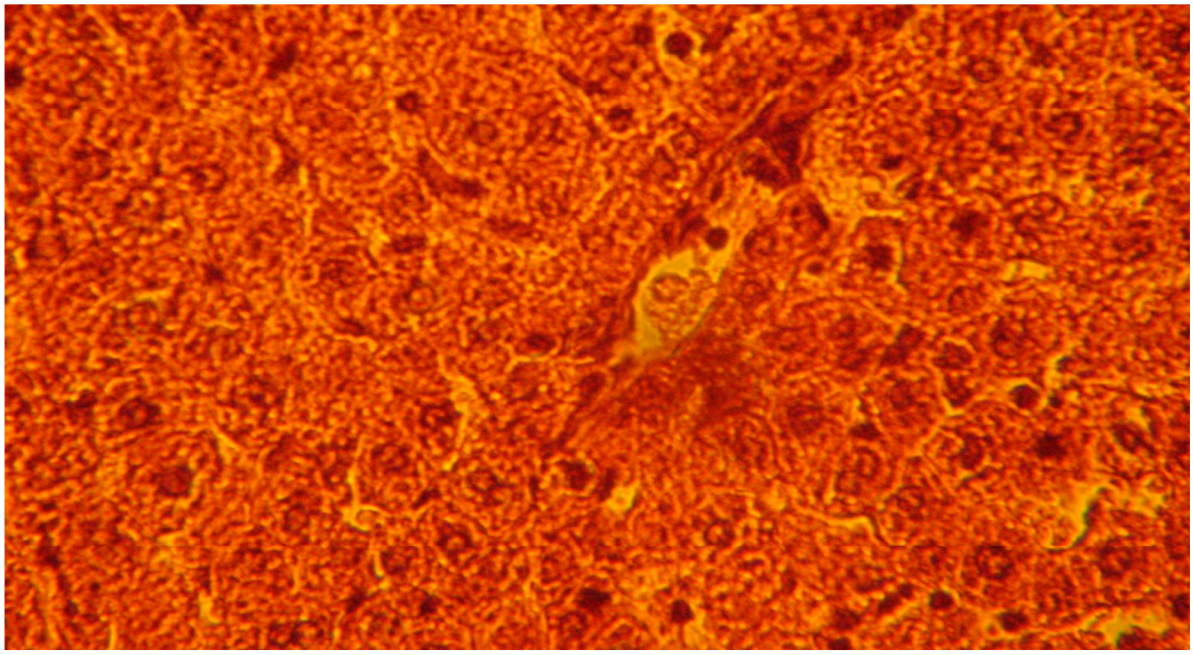


Fig 77: Transversal cross section of rat liver from the control (X400).

In **figure 78:** group treated by Pb has been shown implication of sufferance in the level or cytoplasm of cells which became white or transparent in addition to the emergence of debutant necrosis.

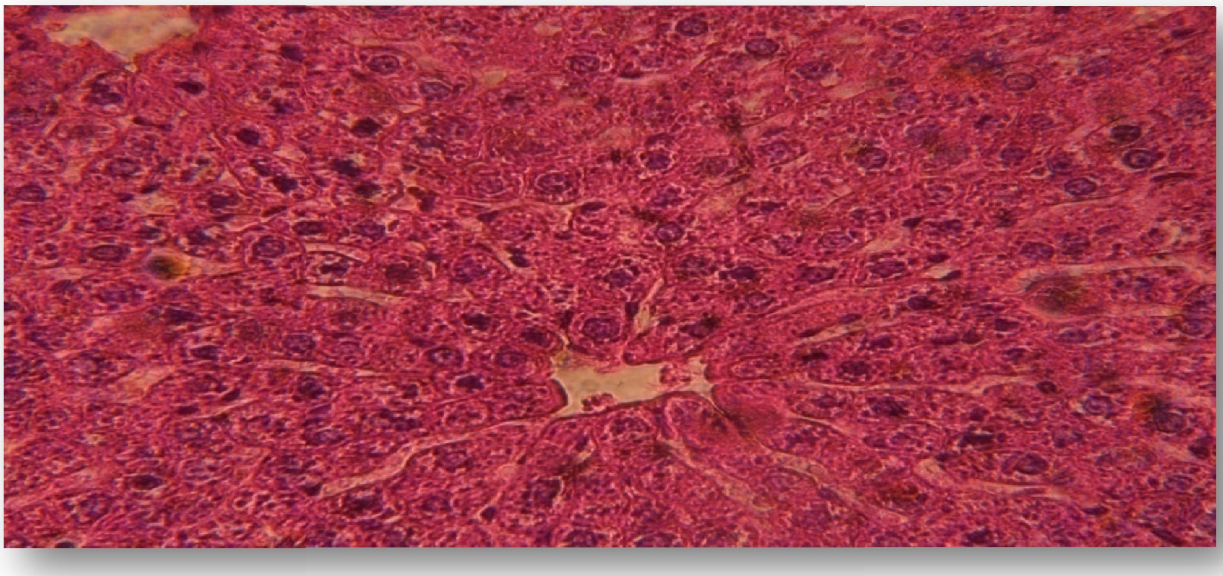


Fig 78: Transversal cross section of rat liver treated by Pb (X400)

In **figure 79:** there were a partially decomposition of liver caused by accumulation of lead which vitamin C was unable to stop the toxic chain reaction of lead cytotoxicity.

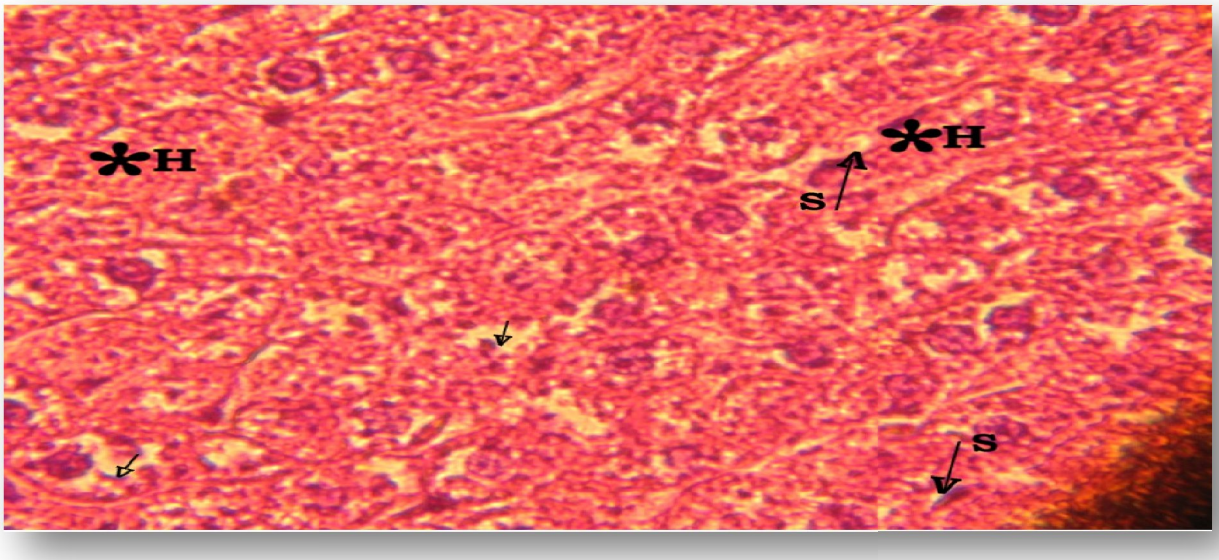


Fig 79: Transversal cross section of rat liver treated by Pb + Vitamin C (X400)

In **figure 80:** but on the treated group by Pb-Olive Oil has been shows an amelioration in total case of tissue.

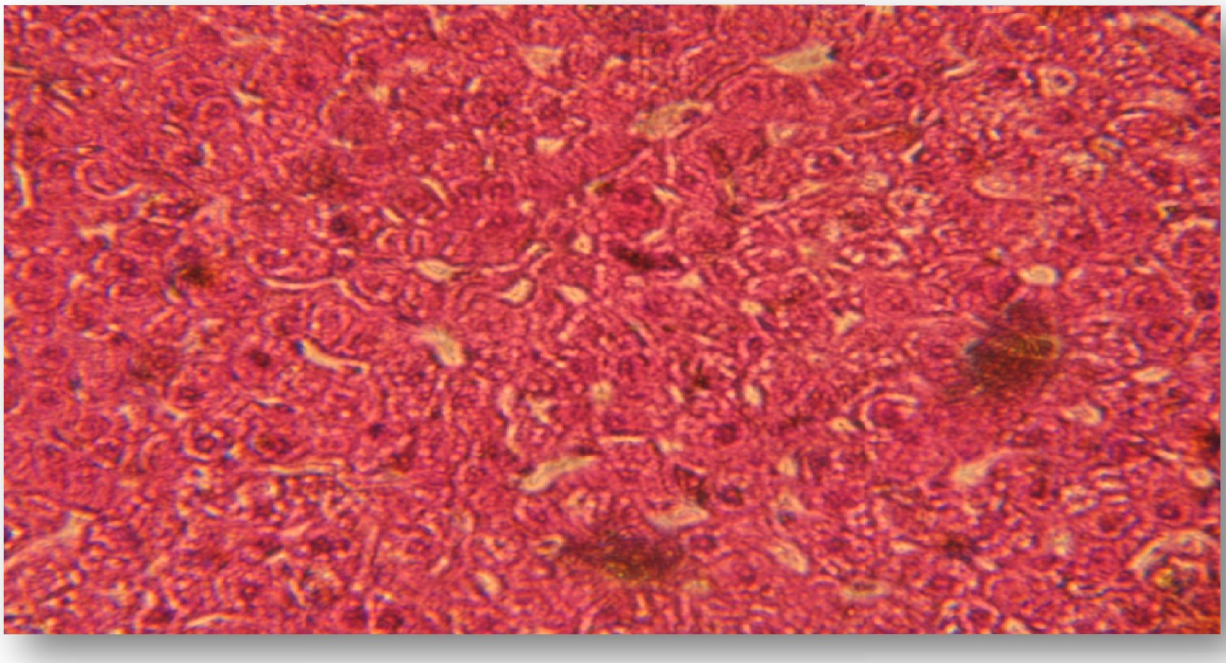


Fig 80: Transversal cross section of rats 'liver treated by Pb + Olive Oil (X400).

Free radicals are atoms or molecules which contain umpired electrons. Since electrons have a very strong tendency to exist in a paired rather than an unpaired state, free radicals indiscriminately pick up electrons from other atoms, which in turn converts those atoms into secondary free radicals, this setting up a chain reaction which can cause substantial biological damage (Halliwell and Gutteridge, 1999).

Heavy metals in our body multiply those free radicals chain reaction several thousands, possibly several million times. When a free radical molecule hits a metal atom in our body, the effect is multiplied many fold. This is partly why it is so important to remove toxic metals from our body through "chelation". Such as vitamin C and vitamin E which prevent free radicals from oxidizing (removing elections from) sensitive biological molecules will slow the again process. So feeding a variety of antioxidants to mammals extended their spans. The above results show that ascorbic acid and olive oil act as radical scavenging antioxidants and suppress lipid peroxidation (Ferni and Bird, 2001; Praksam et al.,2001; Jin et al.,2001., Hogg, 1998)

Although the immunotoxic effects of Pb have been examined in some details (Korzeniewski, and Collewart, 1983), few studies have included the effects of ascorbic acid supplementation on Pb immunotoxicity (Pastoret et al., 1990; Ercal et al., 2000). The present study demonstrates that Pb exposure in rats can modulate the immune system positively, these results are similar to those of Borella and Giardino (1990) who confirmed some results obtained by another study (MaCabe and Lawrence, 1990), that animals were susceptible to the lymphocyte-stimulating characteristics of Pb, translated by a significant increase in immunoglobulin secretion. Unlike Koller (1973) and Koller and Kovavic

(1974) who suggested that Pb is an immunossupresseur. A study of lead-exposed male workers from a large secondary Pb smelter in the United States with a median blood Pb of 39ug/dl found no significant differences in serum immunoglobulin levels between the workers and a group of 84 unexposed workers with a mean blood Pb of <2ug/dl (Pinkerton et al., 1998) In addition, Queiroz et al. (1993) have observed no change in Immunoglobulin levels in individuals with low Pb exposure in one hand , and no correlation between serum Immunoglobulins and blood Pb concentration compared to the control in the other hand, and suggested that chronic exposure to Pb seems to suppress the functional activity of polymorphonuclear cells.

When inversing treatment method in the second period of treatment, the results also were reversed concerning immunoglobulins. It means that the supplementation or the suppression of vitamin C have led to the same concentrations of Immunoglobulins either in the first or in the second treatment. These results are similar with those of Ercal et al., (2000) who mentioned that the administration of 5.5 mmol/kg of N-acetylsysteine (NAC) in the drinking water for 1 week significantly reversed the inhibitory effects of lead on serum immunoglobulin levels. Other comparative result (Gey, 1994) which was done about some known chelators gave the evidence that the effectiveness of vitamin C is similar to those of EDTA, DMSA in protecting cells from the oxidative stress implicating in toxicity during heavy metals intoxication. Meanwhile, the supplementation of ascorbic acid has ameliorated the levels of Immunoglobulins by returning them almost to the normal. That means the beneficial protective action of this antioxidant in suppressing the toxic effects of Pb against immunoglobulins.

The actual results concerning Neutrophils in Pb treated group are in agreement with the previous studies (Queiroz et al., 1993; Queiroz et al., 1994) in which they suggest that the first negative effect of Pb exposure on immune system is based on Neutrophils which are considered as the first line of body defence against invaders.

Earlier, a small study of occupationally-exposed subjects reported that chemotaxis of polymorphonuclear leukocytes (Neutrophils, PMN), stimulated through a specific membrane receptor, was impaired, and compared to a group of unexposed subjects (Valentino et al., 1991). The investigators suggested that the reduction of chemotaxis might be partially due to a Pb related modification of plasma membrane lipids, because PMN locomotion is influenced by fatty acids. Additionally, Neutrophil counts have increased in the presence of Vitamin C, indicating that it accomplishes the vital tasks by stimulating the production of white blood cells, primarily Neutrophils, which are designed to attack antigens (free radicals in the case of Pb) (Dogma, 2002).

Moreover, Simon (2003) has demonstrated that white blood cells have the ability to accumulate vitamin C for about 20-50 times more than any other cell of the organism, in one hand, and also vitamin C prevent body proteins such as albumin, immunoglobulins and Interferon from lead DNA damage in the other hand. The small increase in Neutrophil counts after the second treatment when vitamin C is added explains other protective effects. Thus, the actual results are in line with those of Houston and Johnson (2000), who have suggested the possible detoxifying effects of ascorbic acid against Pb toxicity.

The present results show a significant decrease in the level of serum Ca either in Pb treated group or in combined one, which demonstrate the toxic action of Pb on calcium metabolism, in one hand, and the ineffectiveness role of vitamin C, in the other hand. In these case Goldstein, (1984) investigated the ability of Pb to acts as calcium substitute in the activation of protein kinase C and calmodulin. In addition of the considerable evidence that Pb ions subvert calcium transport systems for absorption from the intestinal, transfer in the blood, deposition into bone, and uptake by specific tissues (Sobel et al., 1939). Furthermore, Goldstein (1993) gives the evidence that Pb acts as a calcium substitute in second messenger metabolism.

However, supplementation of vitamin C in the second treatment period which was treated with Pb alone for 30 days gave good results and this is translated by the comparison of vitamin C suppression animals with the control, in which the return of serum Ca levels to the normal range was noted. It means that vitamin C has a positive role in the absorption, deposition and the intake of Ca by tissues, after the disorders made by Pb. The administration of meso-2-3-demercaptosuccinic acid (DMSA) in the drinking water for 1 week significantly reversed the inhibitory effects of Pb on serum protein levels as well as the return of all oxidative stress parameters to normal levels after 5 weeks of lead poisoning fisher rats (Ercal et al., 2000). Contrary, Jouglard (1987) demonstrated that vitamin C given in chronic Pb exposure has enhanced the intestinal absorption of Pb.

Accordingly, results concerning serum Fe levels were almost in line as that of the calcium, because like calcium, Pb can compete with iron for the intestinal absorption. Consequently, when iron deficiency is happened, erythrocytes

binding sites may be incompletely saturated, making them available for binding of other metal such as Pb in this experiment. This is the case of Pb treated group. Thus, supplementation of the diet of Pb -poisoning rats with ascorbic acid have not showed any differences when compared to Pb group, contrary to Null, (1994) who confirmed previous reports (Watson et al., 1980) that the intestinal ascorbic acid reduces Pb absorption through the reduction of ferric to the ferrous state in which it actively competes with Pb absorption. This information agree with the results obtained from rats in the second treatment period, in which supplementation of vitamin C ameliorated serum Fe level after the recorded significant decrease in the first treatment Pb group. However, early studies (Vij et al., 1998) did not show a beneficial effect of three months vitamin C supplementation on the blood and hair Pb levels. This may be attributed to differences in methodology. Results obtained in the second period of suppressed vitamin C explain those obtained in Pb-vit C first treatment. Thus, after the intestinal absorption competition between Fe, Pb and vitamin C in the first treatment, the lack of vitamin C in the second one gives the chance to Fe to pass through the intestinal mucosa which explains the small increase of serum Fe level (Bartod et al., 1978; Flamagan et al., 1982).

The significant increase in spleen and kidney relative weights in both treated groups of the two experimental periods could explain the inefficiency of vitamin C in all cases. Accordingly, similar response concerning these vital organs has previously been recorded in mice (Zdnek et al., 1991; Mestek et al., 1998).

Moreover, Vaziri et al, (1999) reported that kidney is the essential excretory organ of Pb which may cause functional damage. However, in acute Pb toxicity there is a decrease of renal plasma flow, with a reduction of glomerular filtration

rate accompanied by proximal tubular damage. Lead toxicity result also in an increase of the percentage of Inositol tri-Phosphate in spleen cells by acting as a calcium substitute in second messenger role, which makes G2 and S steps of cell cycle very fast and cause an increase of spleen weight. Vitamin C thus, protects the DNA from damage caused by Pb. Although vitamin C is found in every cell, it is especially useful in key parts of the body this includes glands such as the Thymus and Adrenal (Null, 1994).

When comparing kidney relative weights to those of serum albumin, results show that the proximal tubular damage after Pb accumulation has provoked a decrease in serum albumin concentration. Such result of Pb exposed group were in line with that of Fill (1980), due to the decrease in glomerular clearance rate, especially it has been reported the presence of proteins, composed namely of Albumin, in the urines of exposed workers (Bernard, 1995).

On the other hand, the vitamin C supplementation in the two experimental periods has returned albumin levels to normal range, probably by protecting cells, mainly kidney, from free radicals released by Pb. However, Forherby et al (2000) confirmed that the metabolism of inorganic Pb consists of the formation of complexes with a variety of proteins and non-protein ligands. Thus, the major extracellular ligands include albumin and the non-protein sulfhydryls. Additionally, the effectiveness of vitamin C is proved by many investigators (Jyotika et al., 2003; Crott & Fenech, 1999), without forgetting people who consumed Pb-poisoned diet containing vitamin C (Simon, 2003). In contrast with later studies (Lawerys et al., 1983; Calaberese et al., 1987) which demonstrated that vitamin C is unable to prevent the body form Pb toxicity. Such results have

generated mush controversy about the beneficial role of this essential nutrient towards Pb toxicity.

For most organisms on Earth, life without oxygen is impossible. Animals, plants and microorganisms rely on oxygen for the efficient production of energy. However, the high oxygen concentration in the atmosphere is potentially toxic for living organisms. It is interesting that oxygen toxicity was first described in laboratory animals in 1878 (Knight, 1998). For the last few years' free radical research has generated valuable information far the further understanding of not only determinant, but also beneficial roles of free radicals in cell signalling and other physiological processes. The benefit or harm of free radicals ultimately depends on the level of their production and efficiency of antioxidant defence (Surai, 2007)

Oxidative damage is a major contributor to the development of cardiovascular disease, cancer and neurodegenerative disorders. In healthy individuals, the generation of reactive oxygen species (ROS) is well balanced by the counterbalancing act of antioxidant defences (Marubayashi et al., 1985). ROS are constantly formed as by-products of normal metabolic reactions and their formation is accelerated by accidental exposure to occupational chemicals like lead.

Olive oil is an integral ingredient in the Mediterranean diet. There is growing evidence that it may have great health benefits including the reduction in coronary heart disease risk, the prevention of some cancers and the modification of immune and inflammatory responses (Visioli and Galli C, 2002., Stark and Mader , 2002).

Virgin olive oil appears to be a functional food with various components such as monounsaturated fatty acids that may have nutritional benefits. It is also a good source of phytochemicals, including polyphenolic compounds (Lavelli, 2002., Visioli and Galli , 1998). It is known that an increased consumption of monounsaturated fatty acids (MUFA) instead of polyunsaturated fatty acids (PUFA) reduces the risk of atherosclerosis because it decreases the circulating lipoprotein's sensitivity to peroxidation (Moreno and Mitjavilab , 2003).

Furthermore, the dietary MUFA healthy effects were attributed to decreased endothelial activation (Massaro and De Caterina, 2002), and LDL susceptibility to oxidation (Bonanome et al; 1992). In recent years, scientists have focused on the preventive effects of phenols against degenerative diseases mediated by the ROS. It has been reported that the phenolic compounds are able to interact with the biological systems and act as bioactive molecules. They are particularly important inhibitors of lipid peroxidation (Salah et al; 1995), and are believed to be effective through their free radical scavenging and metal-chelating properties (Kandaswami and Middleton, 1994; Rice-Evans et al ., 1996).

The increasing popularity of olive oil is mainly attributed to its antioxidant and anti-inflammatory effects which may help prevent disease in humans (Tuck and Hayball, 2002; Covasm 2007).

In the current study, an attempt has been made to assess the protective potential of olive oil and it's supplementation in animals subjected to lead acetate was preferred because of its wide use in the industry. However, as a toxicological agent like other heavy metals, it is conceivable that Pb might interact primarily with the liver resulting in structural damage and changes in

enzyme leakage and in the metabolism of the constituents. Some previous studies have looked at the *in vitro* effects of Pb on the generation of oxidative stress, either at the mitochondrial level in hepatocytes or in red blood cells (Palmeira et al., 1995; Duchnowicz and Koter ., 2003). Furthermore, it has been reported that acute exposure to Pb may induce oxidative stress in rats (Celik et al., 2006). The authors found that the administration of Pb induces a decrease in hepatic total proteins.

In fact, they showed that lead induces toxicity which affects energy metabolism, morphological disorders and oxidative stress. These results are in agreement with ours in that the treatment of Pb increased LDL-cholesterol in serum of rats in comparison to controls. In fact, the decreased concentration in iron, red blood cells (RBC) and haemoglobin revealed hepatic and metabolic damage in the lead treated group.

However, the oral administration of Olive oil to Pb treated rats caused a small modification specially in RBC count's and lipid concentration it mean that cholesterol, HDL-cholesterol and triglycerides were returned to the normal range when compared to the control, which may have resulted from the stabilization of plasma membrane as well as the repair of the hepatic tissue damage caused by Pb. This is supported by the view that serum levels of LDL-cholesterol return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew et al., 1987).

This relationship between serum lead and serum lipid levels in the exposed subjects, suggesting an altered lipid metabolism related to lead exposure.

The association between serum lead level and serum total cholesterol and LDL-cholesterol reached statistical significance ($p<0.05$ and $p<0.01$ respectively), but HDL-cholesterol levels significantly decreased in lead exposed animals ($p<0.001$).

Further studies demonstrate that antioxydative activity of olive oil (hydroxyl-asyl) constitutes an effective scavenger for reactive oxygen species (ROS) released from mitochondria. We conclude that the protective effects exerted by phenols and ascorbic acid involved more than one mechanism.

Lead has been recognized as a biological toxicant and different doses have been used to study lead-induced alteration. Absorbed lead following oral ingestion is carried via blood to soft tissues and 95 per cent of blood lead is transported on erythrocytes as lead diphosphate. (Freeman, 1970) this might be the reason of blood concentration increase in the blood following oral exposure to lead.

From our results shown above (second experimental treatment), we noticed a decrease in the level of haemoglobulins and RBCs count, owing to the fact that lead intoxication causes a documented defect in heam synthesis. The results obtained agreed with several authors (Fischman et al., 1981; Serrill et al., 1971) because lead pollution has an inhibitory effect on globin synthesis, inhibits iron to from heam and inhibits delta amino-levulinic acid dehydrates in red cells. Moreover, recent studies have shown that toxicity facilitates conversion of Hb into met-Hb. During Hb oxidation in the presence of lead, H_2O_2 is generated, which may induce lipid peroxidation in the erythrocyte cell membrane (Vargas et al., 2003). As a result, lead might induce generation of ROS by interacting with

oxy-Hb, leading to peroxidative damage of erythrocyte membranes (Ribarov et al., 1981).

Moreover, free radicals produced in the presence of heavy metals contribute to hemoglobin denaturing and precipitation, leading to anaemia translated in our study in all groups shown in second experimental treatment indicates that lead acetate ingestion induces a significant decrease in iron levels at 4 weeks of lead treatment.

Kothapa et al., 2000 have found that Plasma lipid peroxide levels were significantly decreased in olive oil fed rats as compared with controls. There was a significant increase in liver iron concentrations in the olive oil fed groups than in the controls. These findings show that a diet supplemented with olive oil modifies iron concentrations in serum and liver tissues.

Ascorbic acid would affect the stimulation of iron absorption and the main cause of the lead toxicity does not seem to be due to the decreased iron absorption. Therefore, it is considered that the administration of ascorbic acid without iron supplementation did not show the preventive effect on the toxicity of lead (Fox et al., 1971).

Concerning Pb-VC, Pb-OO and Pb-VC-OO groups have significant inverse suppressive effect of the heavy metal, why?

Only because vitamin C is the major water-soluble antioxidant and acts as the first defence against free radicals in whole blood (Niki et al., 1988) and plasma (Frei et al., 1989) in one hand and olive oil rich in vitamin E which is composed of eight different stereoisomer in the side chain and four homolog's on

the chroman ring; RRR-α-tocopherol is known to have the highest biological activity (Machlin, 1991) as the most abundant lipophilic antioxidant (Burton et al., 1983).

A nutritional interaction of vitamin E and ascorbic acid in rats has been reported (Chen and Barnesk, 1976) and dietary vitamin E appears to play a role in hepatic ascorbic acid biosynthesis (Carpenterm, 1959). In PCB-treated rats, dietary vitamin E reduces the elevation of plasma and urinary ascorbic acid (Kobayashki and Yoshida, 1986; Chowc, 1979).

The decreased values of Igs in combined groups and the normal range of all types of leukocytes in cell supplemented vitamin C and/or olive oil indicates direct effect of the phenols and ascorbic acid on DNA transcription of GSH related enzymes and suggests that this is one of the mechanisms that improves antioxydative cellular defence.

To conclude vitamin C and/or Olive Oil supplementation to animals may provide an economical and convenient method of reducing immunotoxic effects of Pb, possibly, firstly by reducing the intestinal absorption of Pb, then by stimulating the production and the chimiotaxic activity of immune cells to fight against foreign invaders and enhance the body's resistance to pollutants by playing the role of antioxidants.

_H_istological finding:

The histological study shows the level of lead poisoning on liver and Kidney function. The current results obtained in the tissue of organs treated with lead

compared with control subjects showed brown pigment in hepatocytes (hemosidersis) and Kupffer cells, congestion, and also express hepatocytes damage by tissue necrosis and dilated blood vessels that alter the architecture of the liver with a suffering cell, swelling of hepatocytes compared to liver of control subjects were we observed a normal architecture of hepatocytes with vascularisation represented by normal sinusoid.

Nephrosis (degeneration, necrosis and detachment of the tubular epithelium), epithelial cells hemosiderosis, intranuclear inclusion bodies mainly at the proximal tubule of the Kidney.

Our results agree with those of (Goyer, 1971) which showed that chronic administration of lead compounds, oral or dermal induced chronic interstitial nephropathy progressing to atrophy and fibrosis. Neoplastic lesions have been shown in rats or mice, and also a reduction in glomerular filtration rate in animals, altered mitochondria of renal tubules can be produced by oral administration of lead (Aliessio et al., 1970), Nephropathy explains that the mitochondria is a place of storage of Pb according to recent studies in rats, it appears that lead is absorbed by endocytosis in the state of a complex with the globulin, at the epithelium of the proximal tubule, this complex can be degraded by lysosomal proteases and migrates up the kernel or it binds to certain regions of DNA, contamination is a change in the nature of proteins that are synthesized (INSERM, 1999).

In rats, during the first 20 weeks of exposure, appears to malfunction of the renal tubule, characterized by aminoaciduria, a phosphaturia, glycosuria and acidosis blood moving thereafter to atrophy of tubule cells (Goyer, 1971). Pb^{+2}

ions bind at the cell membranes of the Kidneys and other organs leading to a total or partial dysfunction of affected organs, as sugars and amino acids across the Kidney barrier while the excretion of uric is strongly reduces (Moor et al., 1988). Sometimes hyperplasia and gradual increase in interstitial fibrosis, over 52 weeks there are few sclerotic glomerular and more than 50% animals have tumors at one or both Kidneys (Goyer, 1971).

The most characteristic cellular effect is the formation of intranuclear inclusion in proximal tubule epithelium, they appear in rats at doses and non-symptomatic, they are farmed of lead-protein complex (about 50µg/mg protein) (Beliles, 1994). It does, however, observed only when Pb exposure is of short duration (<10 years), it disappear when it is prolonged (Gulson et al., 1998), and would reflect a coping mechanism or protection consist in the transcellular transport of Pb. Inclusions are scarce when atrophy and interstitial renal fibrosis worsens (Bililes, 1994).

Although the mechanism of cancer induction by Pb unknown, the presence of nuclear inclusions in the Kidney may be involved, even if their training is supposed to protect the cell, another possible mechanism involving the activation of the Pb protein kinas C (PKC) which phosphorylates cellules proteins, including receptors for growth factors or porto-oncogenesis (Amdur et al., 1996). The synthesis and release of renin are increased after short exposure to moderate Pb and reduced if the exposure is prolonged, these effects on the renin-angiotensin system may be the cause of hypertension associated with exposure (Beliles, 1994).

Pb intoxication is accompanied by an inhibition of liver enzymes; it affects the primary process as the synthesis of protoporphyrin for incorporation into cytochrome required for detoxification in the liver (Hoffman et al., 1981).

The administration of a single dose of Pb in rats reduced the concentration of cytochrome P450 in the liver and thus the oxidative metabolism of various foreign substances (Scoppa et al., 1973)

Pb causes hepatocytes atrophy (Wobeser, 1981) reported atrophy of hepatocytes, the hepatocytes and Kupffer cells (phagocyte cell in liver sinusoids) containing large amounts of iron-containing pigment (haemosiderin).
Hepatocytes necrosis may be present: the mitotic activity of epithelial cells of leaves of provontricule is stopped, with reduction in the height of the papillae (Woberser, 1981).

Lead has a toxic effect of generation of free radicals, they are highly reactive biological molecules important for cell function are prime targets of free radicals, especially unsaturated fatty acids (component of membrane phospholipids) groups, thiols, ribonucleic acid and deoxyribonucleic (Demopoulos, 1973). Indeed, unsaturated fatty acids in the presence of oxygen free radicals and undergo oxidative degradation or lipid peroxidation called lipoperoxidation (Tappel. 1973) causes destruction of biological membranes lipoperoxidation (Quinlan, 1988).

The decrease effects of lead in tissues treated with (lead + olive oil) proves the beneficial effects of olive oil with its minor compound are major agents of detoxification.

Study of (Boscoboini et al., 1991) showed that α-Tocopherol at physiological concentration inhibits proliferation of vascular smooth muscle, a process well known and important in the formation of atherosclerotic lesions. Devaraj et al, 1996 also observed a decrease in the release of reactive oxygen in lipid peroxidation after taking vitamin E.

Tocopherol exerts direct effects on the expression of genes such as adhesive molecules (Islam et al., 1998) or on the activity of enzymes such as 5-lipoxygenase (Devaraj et al., 1999) or protein kinas C (Feedman et al., 1996). Vitamin E is an effective weapon against cancer. In many animal models it has been shown that vitamin E protects against cancers of various locations (Owen et al, 2000b). Owen et al, 2000a evaluated the antioxidant potential of various phenolic compounds of olive oil.

Interesting result, it was observed that extracts olive oil extra virgin (but not refined olive oil) containing a mixture of known and unknown phenolic compounds was effective at concentration much lower than the various compounds studied one by one: this shows that there are between different compounds, synergistic effects that increase the antioxidants potential of the mixture. In addition, extracts of extra virgin olive oil had a major suppressive effect on xanthine oxidase activity; xanthine oxidase gold is an enzyme involved in carcinogenesis and has been shown that its inhibition have an effect chimo-preventive on cancer cells, plus (Manna et al., 1997) showed that DHPE (3, 4-hydroxyphenylethanol) phenolic compound prevents the cytotoxicity effect of reactive oxygen metabolites on cells, thereby preventing cell damage.

Several studies have studied the effect of squalene applied topically or administered systemically on chemically induced cancers of the skin, colon and different tissue in mice, taken together, the results clearly show that dietary squalene has to anti-carcinogenesis undeniable effects (Van Duuren et al,1976; Smith et al., 1999) also several investigations have shown the ability to excrete toxins is increased in animals given brings squalene but some effects are apparent only tall tees (Kamimura et al, 1992; Ritcher et al., 1982)
Klippel et al., 1997; Wilt et al., 1999 show that the B-sitosterol to be effective in the treatment of hyperplasia.

These results justified the stop tumour growth or lack thereof in other tissue, and the cytolysis effect of Pb in the tissue treated with Pb +olive oil.

- **Ahamed M , Singh J R, Behari S, A. Kumar A and Siddiqui MKJ (2007).** Interaction of lead with some essential trace metals in the blood of anemic children from Lucknow,India, *Clin Chim Acta* **377**, pp. 92–97.
- **Alessio L,Gervasini N and Secchi GC (1970).** Ricerche enzimologische sul tessuto renale nella intossicazione saturnine sperimentale in fase acuta ed in fese di remissione ,*Med, lav,* 61,41.
- **Amdur R M O, Doull J and Klaassen C D (1996).** *Lead.* In: Casarrett and Doull's Toxicology 5eéd, New York, McGraw-hill.
- **ATSDR (Agency for Toxic Substances and Disease Registry) (1999),** Toxicological profile for lead, US Department of Health and Human Services, Public Health Service, Atlanta, GA, USA.
- **Azzi A, Boscoboinik D and Hensey C (1992).** The protein kinase C family, *Eur J Biochem* **208** pp. 547–557.
- **Ballew C and Bowman B (2001).** Recommending calcium to reduce lead toxicity in children: a critical review, *Nutr Rev* **59**, pp. 71–79.
- **Bannon DL, Abounader R and Lees PSJ (2003).** Effect of DMT1 knockdown on iron, cadmium and lead uptake in caco-2 cell. *Am. J. Physiol. Cell Physiol*. **284:** c44-c50.
- **Barton JC, Conrad ME and Nuby S (1978).** Effects of iron on the absorption and retention of lead. *J. Lab. Clin. Med*. **92:** 536-547.
- **Beliles R.P (1994) .**The Metals. In: CLAYTON G.D., CLAYTON F.E. (eds.) – *Patty's Industrial hygiene and toxicology*, 4e éd. Vol. II, part C. New York, John Wiley & sons, pp. 2065-2087.

- **Bensalem-Bendjeloul M (1998).** Techniques histologiques theorie et pratique, *Edit. Office des Publication Universitaires*, 109.
- **Bernard AM, Uyskocll A, Roels H, Kriz J, Coll M and Lavwer YSR (1995)**. Renal effects in children living in the vinicity of a lead smelter. *Environ. Res.* **86 :** 91-95.
- **Blake KCH and Mann M (1983).** Effects of calcium and phosphorus on gastro-intestinal absorption of ^{203}Pb in Man, *Environ Res* 30, pp. 188–194.
- **Bauer PG (1981).** Affinity and stoechiometry of calcium binding by arsenazo III. *Anal. Biochem*. **110:** 61.
- **Bonanome A, Pagnan A, Biffanti S, Opportuno A, Sorgato F, Dorella M and Maiorino Ursini F (1992)**. Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. *Arterioscler Thromb*, 12: 529-533.
- **Borella P and Giardino A (1990).** Lead and cadmium at very low doses affect in vitro immune response of human lymphocytes. *Environ. Res*. 55: 165-177.
- **Boscoboinik D,Szewczyk A,Hensey C and Azzi A (1991).** Inhibition of cell proliferation by alpha-tocopherol.Role of protein Kinase C.J Biol.Chem,266 :6188-94.
- **Buccolo G et al. (1973)**. Quantitative diterminationof serum triglycerides by use of enzimes. *Clin Chem* ; 19(**5**) : 476-482.
- **Buettner GR (1993).** The packing order of free radicals and antioxidants: lipid peroxidation, α- tocopherol, and ascorbate, *Arch Biochem Biophys* **300**, pp. 535–543.
- **Bukowska B, Chajdys A, Duda W and Duchnowicz P (2000)**. Catalase activity in human erythrocytes: effect of phenoxy herbicides and their metabolites. *Cell Biol Int*,24: 705–711.

- **Burton GW, Joyce A and Ingold KU (1983).** Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch Biochem Biophys* **221**: 281-90.
- **Calaberese EJ, Stodderd A, Leonard DA and Dinardi SR (1987).** The effects of vitamin C supplementation on blood and hair Levels of cadmium, lead and mercury. *Ann. N Y. A cad. Sci*. **498:** 347-353.
- **Carpenterm P, Kitabchai E, McCay P B and Caputto R (1959)** The activation by tocopherol and other agents of ascorbic acid synthesis by liver homogenates from vitamin E deficient rats. /. Biol. Chem. **234**: 2814-2818.
- **Celik I, Tuluce Y and Isik I (2006)**. Influence of subacute treatment of some plant growth regulators on serum marker enzymes and erythrocyte and tissue antioxidant defence and lipid peroxidation in rats. *J Biochem Mol Toxicol* ; 20(**4**): 174–182.
- **Chaurasia SS and Kar A (1997).** Protective effects of vitamin E against lead induced deterioration of membrane associated type-I iodothyronine 5-monodeiodinase (5D-I) activity in male mice. *Toxicology*, **124**, pp. 203-209.
- **Chen L H and Barnesk f (1976)**. Nutritional relationship of vitamin E and vitamin C in rats. Nutr. Rep. Int. **14**: 699-708.
- **Chowc K and Thacker Rand Gairolac C (1979)**. Increased level of L-ascorbic acid in the plasma of polychlorobiphenylstreated rats and its inhibition by dietary vitamine E. Res. Com *mun. Chem. Pathol. Pharmacol. 26: 605-608.* rat lymphocytes subsets and proliferation. Immunology **82**, 603-610.
- **Covasm MI (2007).** Olive oil and the cardiovascular system. *Pharmacol Res*, **55**: 175-186.

- **Crott JW and Fenech M (1999).** Effects of vitamin C supplementation on chromosome damage, apoptosis and necrosis ex vivo. *Carcinogenesis*. **20:** 1035-1041.
- **Dawson EB and Harris WA (1997).** Effect of ascorbic acid supplementation on blood lead levels, *J Am Coll Nutr* **16**, p. 480.
- **Demopoulos H (1973).** The basis of free radical pathology, *Fed, Proc, 32, 1859.*
- **Devaraj S, Li D and Jialal L (1996).**the effects of alpha tocopherol supplementation on monocyte function. Decreased lipid oxidation, interleukin 1 beta secretion; and monocyte adhesion to endothelium .J clin. Invest; 98:756-63.
- **Devaraj S and Jialal L (1999).**Alpha-tocopherol decreases interleukin-1 beta release from activated human monocytes bu inhibition of 5-lipoxygease .arterioscler.thromb.Vasc.Bio **19**:1125-33.
- **Dhawan M, Kachru DN and Tandon SK (1988).** Influence of thiamine and ascorbic acid supplementation on the antidotal efficacy of thiol chelators in experimental lead intoxication, *Arch Toxicol* **62**, pp. 301–304.
- **Dogma M (2002).** La vitamine C complément alimentaire essentiel. *Extrait du livre Prenez en main votre santé*. p 1-12.
- **Dorea JG and Donangelo CM (2006).** Early (in uterus and infants) exposure to mercury and lead, *Clin Nutr* **25** , pp. 369–376.
- **Duchnowicz P and Koter M (2003).** Damage to the erythrocyte membrane caused by chlorophenoxyacetic herbicides. *Cell Mol Biol Lett,* 88(**1**): 25–30.

- **Ercal R, Treeatphoan P, Lutz PM, Hammond TC, Dennery PA, and Spitz DR (2000).** A role for oxidative stress in suppressing serum immunoglobulin levels in lead-exposed Fisher 344 rats. Arch. Environ. Contam. Toxicol. 39(**2**): 251-256.
- **Fernie KL and Bird DM (2001).** Evidence of oxidative stress in American kestrels exposed to electromagnetic field. *Environmental Research* 86: 198-207.
- **Fewtrell LI, Pruss-Ustan, A, Ladrigan P and Ayuso-Mateos JL (2004).** Estimation of global burden disease of mild mental retardation and cardiovascular diseases from environmental lead exposure, *Environ Res* **94**, pp. 120–133.
- **Fill MD (1980).** A Medcine % 20du % 20 Travail. Htm. Institut Universitaire de Médcine du travail de Rennes. Cours.
- **Fischman CM, Udey MC, Kurtz M and Widner HJ (1981).** Inhibition of lectin-induced lymphocyte activation by 2-cyclohexene-1-one: decreased intracellular glutathione inhibits an early event in the activation sequence. *J Immunol* **127**:2257-62.
- **Flamagan PR, Hamilton DL, Haist J and Valberg LS (1979).** Interrelationship between iron and lead absorption in iron deficient mice. *Gastroenterology*. **77:** 1074-1081.
- **Flanagan PR, Chamberlain MJ and Valberg LS (1982).** The relationship between iron and lead absorption in humans, *Am J Clin Nutr* 38, pp. 334–335.
- **Flora SJS and Tandon SK (1989).** Preventive and therapeutic effects of thiamin, ascorbic acid and their combination in lead intoxication, *Acta Pharmacol Toxicol* **58**, pp. 374– 37 378.

- **Fothreby MD, Williams JC, Forster LA, Craner P and Ferns GA (2000).** Effects of vitamin C on ambulatory blood pressure and plasma lipids in older persons. *J. Hypertens*. **18:** 411-415.
- **Fox M R S, Fry B E J, Harland B F, and Scherte M E and Weeks C E (1971) .Effect** of ascorbic acid on cadmium toxicity in the young coturnix. J. Nutr. **101**, 1295-1306.
- **Fracasso ME, Perbellini L and Solda S (2002).** Lead induced DNA strand breaks in lymphocytes of exposed workers: Role of reaction oxygen species and protein kinase C. *Mutat. Res*. **515:** 159-169.
- **Freeman R (1970).** Chronic lead poisoning in children: a review of 90 children diagnosed in Sydney, 1948-67. II .Clinical features and investigations, *Medical Journal of Australia*, **1**, 648-681.
- **Freedman JE,Farhat JH and Loscalzo J (1996).**Alpha-tocopherol inhibits aggregation of human platelets by a protein Kinase C-dependent mechanism.circulation,**94**:2434-40.
- **Frei B, England L and Ames BN (1989).** Ascorbat is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci* USA **86**: 6377-81.
- **Frei B (1991).** Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage, *Am J Clin Nutr* **54**, pp. 1113–1118.
- **Gey KF (1994).** Optimum plasma levels of antioxidant micronutients: Ten years of antioxidant hypothesis on arterioclerosis.*Bibl. Nutr. Dieta.* **51:** 84-99.
- **Goldstein GW (1984).** Brain capillaries; a target for inorganic lead poisoning. *Neurotoxicology*. **5:** 167-176.

- **Goldstein GW (1993).** Evidence that lead acts as a calcium substitute in second messenger metabolism. *Neurotoxicology*. **14 (2-3):** 97-102.
- **Goyer R.A (1971).** Lead and the kidney. Current Topics in Pathology, 55, pp. 147-176.
- **Goyer RA and Cherion MG (1979).** Ascorbic acid and EDTA treatment of lead toxicity in rats, *Life Sci* **24**, pp. 433–438.
- **Grigg J (2004).** Environment toxins; their impact on children's health, *Arch Dis Child* **89**, pp. 244–250.
- **Gulson BL, Jameson CW, Mahaffey KR, Mizon KJ, Patison N, Law AJ ,Korsch MJ and Salter MA (1998).** Relationships of lead in breast milk to lead in blood, urine, and diet of the infant and mother, environ health perspect, 106(10),667-674.
- **Halliwell B (2000).** *Am. J. Clin. Nutr.* **72,** 1082–1087
- **Halliwell B and Gutteridge JMC (1999).** Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity. In: B. Halliwell and J.M.C. Gutteridge, Editors, *Free radical in biology and medicine*, Clarendon Press, Oxford, pp. 86–123.
- **Hammad TA, Sexton M and Langenberg P (1996).** Relationship between blood lead and dietary iron intake in preschool children: a cross-sectional study, *Ann Epidemiol* **6**, pp. 30–33.
- **Heard MJ and Chamberlain (1982).** Effect of minerals and food on uptake of lead from the gastrointestinal tract in human, *Hum Toxicol* **1**, pp. 411–415.
- **Heusler-Bitschv S, Knutti R and Schiatter C (1988).** Inter-individual variability of the kinetic of lead in man. In: Braetter P, Schramel P, editors. Proceedings of the

international workshop: trace elements analytical chemistry in medicine and biology, vol. 5. Neuherberg, West Germany,. p. 627–34.

- **Hengstler JG, Bolm-Audorff U, Faldum A. et al (2003).** Occupational exposure to heavy metals : DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. Carcinogenesis, 24, pp. 63-73.
- **Hoffman DJ, Pattee OH, Wiemeyer SN and Mulhern B (1981).** Effects of lead shot ingestion on δaminolevulinicacid dehydratase activity, hemoglobin concentration, and serum chemistry inbald eagles, Journal of Wildlife Diseases, Vol **17**, No 3.
- **Hogg N (1998).** Free radicals in disease. *Seminars in reproductive endocrinology* **16**: 241-248.
- **Houston DK and Johnson MA (2000).** Does vitamin C intake protect against lead toxicity?. Nu*tr . Rev. 58 (3pt1): 73-5.*
- **Hsu PC, Hsu CC, Liu MY, Chen Ly and Guo YL (1998).** Lead-induced changes in spermatozoa function and metabolism, *J Toxicol Environ Health* **55**, pp. 45–64
- **INSERM (1999).** Plomb dans l'environnement, quelques risques pour la santé? Les etudions INSERM, 461p.
- **Islam KN, Devaraj S and Jiala L (1998).** Alpha tocopherol enrichment of monocytes decreases agonist-induced adhesion to human endothelial cells. Circulation, **98**:2255-61.
- **Jin X, Kennedy SW, Muccio T and Moon TW (2001).** Role of axidative stress and antioxidant defence in 3.3',4,4',5-pentachlorobiphenyl-induced toxicity and species-

differential sensitivity in chiken and duck embryos. *Toxicology and Applied Pharmacology* **172**: 241-248.

- **Jouglard J, Aquaron R, Arditte J, Jean PH, Bourdon JH and David JM (1987).** Saturnisme et Porphyrisme. *Sem. Hôp , Paris. 63.* **34:** 2767-2772.
- **Jyotika A, Veena D, Safrun M, Promila P, Parwana HK and Ravinder N (2003).** Effects of Vitamin C supplementation on oxidative DNA damage in an experimental model of Lead-induced hypertension. *Am. Nutr. Metab*. **47:** 294-301.
- **Kamimura H, Koga N, Oguri K and Yoshimura H (1992).** Enhanced elimination of theophylline ,Phenobarbital and strychnine from the bodies of rats and mice by squalene treatment pharmacoidyn.**15**:215-21.
- **Kandaswami C and Middleton E Jr (1994)**. Free radical scavenging and antioxidant activity of plant flavonoids. *Adv Exp Med Biol*, **366**: 351–376.
- **Kappor S, Seaman C, Hurst D, Matos S and Piomelli S (1984).** The biochemical basis of the clinical interaction of Fe deficiency and lead intoxication, *Peditr Res* **18**, p. 242A.
- **Klippel KF, Hiltl DM and Scipp B (1997).** A multicentric, placebo-controlled, double-blind clinical trial of beta-sitosterol(phytosterol) for the treatment of bening prostatic hyperplasia.german BPH-Phyto study group.Br.j Urol, 80:427-32.
- **Kobayashki K and YoshidaA A (1986) .**Effect of dietary ascorbic acid and vitamin E on metabolic changes in rats and guinea pigs exposed to PCB. /. Nutr. **116**: 98-106.
- **Koller LD (1973).** Immunosuppression produced by lead, cadmium and mercury. *Am. J. Vet. Res.* **34**, p. 1457.

- **Koller LD and Kovacic S (1974).** Decreased antibody formation in mice exposed to lead. *Nature.* **250,** pp**.** 148-150.
- **Kothapa N. Chetty DA, Ragene Conwaya, Katrina C. Harris T, Waneene C. Dorsey M, Kowluru RA, Engerman RL and Kern TS (2000).** Diabetes-induced metabolic abnormalities in myocardium: effect of antioxidant therapy, *Free Radic Res* **32**, pp. 67–74. **Korzeniewski C, and Collewart DM (1983).** An anzyme-release assay for natural cytotoxicity. *J. Immunol. Methods. (***64**), pp. 313-320.
- **Knight JA (1998).** Free radicals: Their history and current status in aging and diseases. *Annals of the Clinical and Laboratory Science* **28**, pp. 331-346.
- **Labbe R F (1990).** Lead poisoning mechanisms, *Clin Chem* **36**, p. 1970.
- **Lavelli V (2002)**. Comparison of the antioxidant activities of extra virgin olive oils. *J Agric Food Chem* , 50(26): 7704–7708.
- **Lawerys R, Roels H, Buchet JP, Berard AA, Verhoeven L and Konings J (1983).** The influence of orally-administered vitamin C or zinc on the absorption of and the biological response to lead. *J. Occup. Med.* **25:** 668-678.
- **Linos A, Kaklamanis E, Kontomerkos A, Koumantaki Y, Gazi S, Vaiopoulos G, Tsokos GC and Kaklamanis P (1991).** The effect of olive oil and fish consumption on rheumatoid arthritis: a case control study. *Scand J Rheumatol* **20**, pp. 419-426.
- **Lozoff B, Brittenham GM, Viteri FE, Wolg AW and Urruita JL (1982).** The effects ofshort-term oral iron therapy on developmental deficits in iron deficient anemic infants, *J Pediatr* **101**, pp. 351–357.

- **Luster MI, Faith RE and Kimmel CA** (1978). Depression of humoral immunity in rats following chronic developmental lead Exposure. *J. Environ. Pathol. Toxicol.* 1 : 397-402.
- **Manna C, Galletti P, Cucciolla V, Moltedo O, Leone A and Zappia V(1997).** The protective effet of the olive oil polyphenol(3,4-dihydroxyphenyl)-ethanol counteracts reactive oxygene metabolite-induced cytoxicity in caco-2 cells.J Nutr.127 :286-92.
- **Martoja R and Martoja M (1967).**Initiation aux techniques de l'histologie animale *.Edition Masson* .p : 396
- **Marubayashi S, Dohi K, Ochi K and Kawasaki T (1985)**. Role of free radicals in ischemic rat liver cell injury: prevention of damage by alpha-tocopherol administration. *Surgery* 99: 184–191.
- **Massaro M and De Caterina R (2002)**. Vasculoprotective effects of oleic acid: epidemiological background and direct vascular antiatherogenic properties. *Nutr Metab Cardiovasc Dis* , **12**(1): 42-51.
- **Machlin LJ (1991).** Vitamin E in.:Machlin LJ .ed. Handbook of vitamins. Newyork: Marcel Dekker 99-144.
- **Mahaffey-Six MK and Goyer RR (1970).** Experimental enhancement of lead toxicity by low dietary calcium, *J Lab Clin Med* 76, pp. 933–942.
- **Mahaffey KR, Haseman JD and Goyer RA (1973).** Dose-response to lead ingestion in rats on low dietary calcium, *J Lab Clin Med* **83**, pp. 92–100.
- **Markowitz ME, Sinnett M and Rosen JF (2004).** A randomized trial of calcium supplementation for childhood lead poisoning, *Pediatrics* **113**, pp. 34–39.

- **McCabe ML and Lawrence DA** (1990). The heavy metal lead exhibits B cell–stimulatory factor activity by enhancing B Cell IA expression and differentiation. *J. Immunol*. 145: 671-677.
- **Miller GD, Massaro TF and E.J and Massaro EJ (1990).** Interactions between lead and essential elements: a review, *Neurotoxicolgy* **11**, pp. 99–120.
- **Moreno JJ and Mitjavilab MT (2003)**. The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (Review). *J Nutr Biochem*, **14**(4): 182–195.
- **Moore MR (1988).** Hematological effects of lead. *Sci. Total. Environ*. **71***:* 419-431.
- **Moore M R, Campbel B C and Goldberg l,A (1977).** The Chemical Environment (Editeurs: J. Lenihan, W. W. Fletcher), Academic Press, New York, Vol. 6, p. 64.
- **Morrison JN and Quarteman J (1987).** The relationship between iron satatus and lead absorption in rats. *Biol. Tarce Element Res*. **14:** 115-126.
- **Mykkanen HM and Wasserman RH (1981).** Gastro-intestinal absorption of lead (^{203}Pb) in chicks: Influence of lead, calcium and age. *J. Nutr*. **111:** 1757-1765.
- **Mykkanen HM and Wasserman RH (1982).** Effects of vitamin D on the intestinal absorption of ^{203}Pb and ^{47}Ca in chicks. *J. Nutr*. **112:** 520-527.
- **Naito HK (1984).** Cholesterol. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. *Torron. Princeton* ; 1194-11206 and 437.
- **Needleman HL, Schell A ,Bellinger D , Levition A and Allred E (1992) .**The long-term effects of exposure to low doses of lead in childhood.An 11 year flow –up report, *New England journal of medicine* ,**322**, pp. 83-88.

- **Niki E, Yamamoto Y and Takahashi M (1988).** Free-radical mediated damage of blood and its inhibition by antioxydants. *J Nutr Sci Vita-minol* (Tokyo) **34**: 507-12.
- **Null G (1994).** The antioxidant vitamin C. *Townsend Letter for doctors.* P: 1-19.
- **Oski FA and Hongi AS (1978).** The effects of iron therapy on the developmental scores of iron deficient infants, *J Pediatr* **92**, pp. 21–25.
- **Osman K, Schutz A, Akesson B, Maciag A and Vahter M (1998).** Interactions between essential and toxic elements in lead exposed children in Katowice, Poland, *Clin Biochem* **31**, pp. 657–665.
- **Owen RW, Mier W, Giacosa A, Hullwe S,Pigelhalder B and Bartxh H (2000a).** The antioxidant/anticancer potential of phenolic compounds isolated from olive oil.Eur.J cancer,**12**: 35-47.
- **Owen RW, Mier W, Giacosa A, Hullwe S,Pigelhalder B and Bartxh H (2000b).** Phenolic compounds and antioxidant potential of total phenols, simple phenols, secroiridoids , lignaansand squalene *,food chem.,toxicol ,**38**:647-59.*
- **Ozcan E and Uner N (2000).** Combined effects of 2,4-D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of Oreochromis niloticus. *Comp Biochem Phys C*, 127(**3**): 291–296.
- **Packer L (1991).** Protective role of vitamin E in biological systems, *Am J Clin Nutr* **53**, pp. 1050S–1055S.
- **Palmeira CM, Moreno AJ and Madeira VMC (1995)**. Thiols metabolism is altered by theherbicides paraquat, dinoseb and 2,4-D: A study in isolated hepatocytes. *Toxicol Lett*, 81: 115-123.

- **Panda M and Flora SJS (2002).** Lead-induced oxidative damage and its response to combined adminitration of lipoic acid and succimers in rat, *Toxicology* **177**, pp. 187–196.
- **Patra RC, Swarup D and Dwidedi SK (2001).** Antioxidant effects of α-tocopherol, ascorbic acid and L-methionine on lead-induced oxidative stress of the liver, kidney and brain in rats, *Toxicology* **162**, pp. 81–88.
- **Pastoret PP, Govaerts A and Bazin H (1990).** Medecine, Science, Flammarion. pp: 411-695.
- **Pinkerton LE, Biagini RE and Ward EM (1998).** Immunologic finding among lead-exposed workers. *Am. J. Ind. Med*. **33(4):** 400-408.
- **Praksam A, Sethupathy S and Lalitha S (2001).** Plasma and RBCs antioxidant status in occupational male pesticide sprayers. *Clinica and chimica Acta* **310**: 107-112.
- **Queiroz MLS, Almeida M, Gallâo ML and Hochr NF (1993).** Defective neutrophil function in workers occupationaly exposed to lead. *Pharmacol. Toxicol.* **72**: 73.
- **Queiroz MLS, Perlingeiro RCR, Dincoletto C, Almeida M, Cardoso MP & Dantas CM (1994).** Immunoglobulin levels and cellular immun function in lead exposed workers. *Immunopharmacol. immunotoxicol.* 16: 115-128.
- **Quinlan GJ, Halliwell B, Moorhouse CP, Gutteridge JMC (1988).**Action of lead (II) and aluminium(III) ions on iron – stimulated lipid peroxidatio in liposomes, erythrocytes and rat liver microsomal fractions, *Biochim,Biophy,Acta,962,196.*
- **Rabinowitz MB, Kopple JD and Wetherill GW (1980).** Effect of food intake and fasting on gastrointestinal lead absorption in humans, *Am J Clin Nutrition* 33, pp. 1784–1788.

- **Ribarov SR, Binov LC and Benchev IC (1981).** the effect of lead on haemoglobin-catalysed lipid Peroxidation , *Biochem. Biophys. Acta*, **664**, 453-459.
- **Rice-Evans CA, Miller NJ and Paganga G (1996)**. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Bio Med*, 20(**7**): 933–956.
- **Ritchter E, Fichtl B and Schafer SG (1982).** Effets of squalene on hexachlorobenzene (HCB)concentrations in tissues of mice.*J environ.Sci.Health B.***17***:*195-203.
- **Rott RJ and Charles D (1979).** Evolution of the humoral immune response of children with low level lead exposure. *Bulltine. Environ. Contam. toxicol.* 16: 112-117.
- **Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP and Rice-Evans C (1995)**. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breakin antioxidants. *Arch Biochem Biophys* , 322(**2**): 339–346.
- **Sargent JD, Dalton MA, O'Connor GT, Olmstead EM and Klein RZ (1999).** Randomized trial of calcium glycerophosphate-supplemented infant formula to prevent lead absorption, *Am J Clin Nutr* **69**, pp. 1224–1230.
- **Scoppa P, Roumengous M, Penning W (1973) .**Hepatic drug metabolizing activity in lead rats,*Expe-rientia,* 29,970.
- **Serrill A, Jefferson D, Quick J and Mengel CE (1971).** Effect of acetylsalicylic acid and ascorbic acid on oxygen toxicity. *Aerospace Med*: **42**: 436-8.
- **Sheppard AJ, Pennington JAT and Weihrauch JL (1993).** Analysis and distribution of vitamin E in vegetable oils and foods. In: L. Packer and J. Fuchs, Editors, *Vitamin E in health and disease*, Marcel-Dekker, New York, pp. 9–31.

- **Silbergeld EK (1991).** Lead in bones: implications for toxicology during pregnancy and lactation, *Environ Health Perspect* **91**, pp. 63–70.
- **Simon JA (2003).** Relationship of ascorbic acid to blood Pb levels. *JAMA, jun 23-30.* **24:** 281.
- **Simon JA and Hudes ES (1999).** Relationships of ascorbic acid to blood lead levels, *J Am Med Assoc* **281**, pp. 2289–2293.
- **Smith TJ, Kim S, Lee MJ, Yang GY, Newmark HL and yang CS (1999).** Inhibition of 4-(methylnitlosamino)-1-(3-pyridyl)-1-butanone (NKK)-induced lung tumorigenesis and DNA oxidation by dietary squalene.proceedings of the American association for cancer research 40,262.
- **Singh B, Dhawan B and Nehru B (1994).** Impact of lead pollution on the status of other trace metals in blood and alterations in hepatic functions, *Biol Trace Elem Res* **40** (1), pp. 21–29.
- **Sobel AE, Yuska H, Peters DD and Kramer B (1939).** The biochemical behavior of lead: Influence of calcium, phosphorus, and vitamin D on lead in blood and bone. J. Biol. Chem. **128:** 239-265.
- **Stark AH and Mader Z (2002)**. Olive oil as a functional food: epidemiology and nutritionalapproaches. *Nutr Rev* , 60(6): 170– 176.
- **Surai PF (2007).** Natural antioxidants in avian nutrition and reproduction. Nottingham *University Press,* Book second reprint: pp: 1-615.
- **Tappel A (1973).** Lipide peroxidation damage to cell components, *Fed, Proc, 32, 1870.*

- **Thabrew MI, Joice PD and Rajatissa WA (1987)**. Comparative study of efficacy of Paetta Indica and Osbeckia octandra in the treatment of liver dysfunction. *Planta Med* **5**, 53: 239–241.
- **Tsalev DL (1985).** Atomic absorption in occupational and environmental health practice. *246, CRC Press, Florida*.
- **Tuck KL and Hayball PJ (2002)**. Major phenolic compounds in olive oil: metabolism and health effects. *J Nutr Biochem*, **13**: 636-644.
- **Valentino M, Govema M and Marechippe I (1991).** Effects of lead on polymorphonuclear leukocyte (PMN) function in occupationally exposed workers. *Arch. Toxicol.* **65 :** 685-688.
- **Valverde M, Fortoul TI and Diaz-Barrga (2002).** Genotoxicity induced in CD-1 mice by inhaled lead : Differencial organ response. *Mutagenesis*. **17(1) :** 55-61.
- **Van Duuren BL and Goldschmidt BM (1976).** Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis .*J Natl,cancer Inst,56 :1237-42.*
- **Vargas I, Casteno C, Posadas F and Escalante B (2003).** Acute lead exposure induces renal heme oxygenas-1 and decreases urinary Na+ excretion, *Hum. Exp. Toxicol*, **22**, 237-244.
- **Vij AG, Satifa NK and Flora SJ (1998).** Lead induced disorders in hematopoietic and drug metabolizing enzyme system and their protection by ascorbic acid supplementation. *Biomed. Environ. Sci*. **11(1):** 7-14.
- **Visioli F and Galli C (1998)**. The effect of minor constituents of olive oil on cardiovascular disease: new findings. *Nutr Rev* , **56**(5 Pt1): 142–147.

- **Visioli F and Galli C (2002)**. Biological properties of olive oil phytochemicals. *Crit Rev Food Sci Nutr* , 42(3): 209–221.
- **Watson WS and Hume R (1983).** Iron and lead absorption in human. *Am. J. Clini. Nutrition*. **38:** 333-341.
- **Wick M (1998).** Iron metabolism and its disorders, In: Thomas L, editor. Clinical laboratory diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; p. 268-73.
- **Wilt TJ, MacDonald R and Ishani A (1999).** Beta-sitosterol for the treatment of benign prostatic huperplasia: a systematic review .BJU.Int.83:976-83.
- **Weister H and Vecchi M (1982).** Stereoisomers of α-tocopheryl acetate. II. Biopotencies of all eight stereoisomers, individually or in mixtures, as determined by rat resorption–gestation tests, *Int J Vit Nutr Res* **52**, pp. 351–370.
- **West WL, Knight KH and Edwards CH (1994).** Material low level lead and pregnancy outcomes, *J Nutr* **124**, pp. 981–986.
- **Wiseman SA, Mathot JN, de Fouw NJ and Tijburg LB (1996).** Dietary non-tocopherol antioxidants present in extra virgin olive oil increase the resistance of low density lipoproteins to oxidation in rabbits**.** *Atherosclerosis* 1996, **120(1-2)**: 15–23.
- **Wobeser G.A (1981)**. Diseases of wild waterfowl, Plenum Press, 151-159.

Chapter 1:
General Introduction

Chapter 2:
Material & Methods

Chapter 3: Results

Chapter 4: Discussion

Abstracts

References

Scientific Activities