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The potential attenuation of lead immuno toxicity in Wistar rats consuming *Opuntia ficus indica*

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ABSTRACT

Male Wistar rats have been given either Pb alone or combined with *Opuntia ficus indica* (OFI) for a period of 4 weeks. Red blood cells (RBC), haemoglobin (Hb), leucocytes, Serum albumin, proteins, Ca, Fe, IgM, and histological study were evaluated. The most important results have showed a significant decrease in RBC, Hb, biochemical parameters and neutrophils with an increase in the rest of leukocyte subpopulations and IgM in Pb exposed group. However, the combined treatment resulted in a significant inverse finding. Concerning eosinophils there were no significant differences between all groups compared to control and positive control. The histological study of spleen have been affected by lead treatment, whereas, those of combined treatment group were significantly very close to both the controls. The results, thus, indicate a significant positive action of flowers of *OFI* against the toxic effects of Pb on protein renal filtration and the immune reactions. To conclude, water contaminated by Pb and containing sufficient amount of OFI, could participate in reducing metal toxicities.

Key words: *Lead, Opuntia ficus indica, IgM, Biochemical parameters, Spleen, rats.*

Introduction

Heavy metal pollution is still a serious environmental problem that reduces the qualityof life in many parts of the world (Moustafa, 2018). Heavy metals generally refer to natural metal elements, metals or in some metalloid cases, characterized by a higher density (Adriano, 2001). Under physiological conditions, heavy metals are classified as essential to organisms (Asati *et al*., 2016). Essential metals such as zinc (Zn), copper (Cu), cobalt (Co), manganese (Mn) and molybdenum (Mo) have an important role in the physiological processes of living organisms (Tchounwou *et al.*, 2012). Other kinds of heavy metals or non-essential metalloids such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg) are not required by organisms, but they affect physiological processes by disrupting enzymatic reactions because their reactivity with thiols or other groups (Tchounwou *et al*., 2012). Among the pollutants involved, lead occupies a special place, since of the abundance its sources and its distribution (water pipes, industrial pollution, automobile transport, etc.), and the possible bioconcentration of this metal in different body systems, because it is not biodegradable (Seregin and Ivanov, 2001; Boujelbenea *et al*., 2002). One of the particularitès of this pollutant is that it induces a wide range of dysfunctions on the central and peripheral nervous systems and on the hemopoietic system. The study by Burroughs and Rollins (2017) showed that lead is responsible for several cardio-

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vascular diseases such as high blood pressure (Burroughs and Rollins, 2017). Liver and kidney are preferred targets for lead, which bioaccumulates in the liver in a very significant way (Cicho¿-Lach, Michalak, 2014). So all toxic metals, lead increases the production of free radicals and reduces the availability of antioxidant reserves to respond to the stress of damage (Valko *et al*., 2007). In addition, 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, 1974) or a no change (Rott and Charles, 1976) on immune function in experimental systems.To address lead poisoning, lots of studies have tested the detoxifying potential of certain plants, drawing inspiration from traditional medicine from different cultures (Rakotoarivelo *et al.,* 2015). Thus, some species of cactus have been the subject of many scientific researches for several decades, and especially the prickly fig *Opuntia ficus* indica. Originally from Mexico, it is currently cultivated in the different countries of the world (Chougui *et al*., 2015) and exploited for its many pharmacological virtues, including its high antioxidant activity that could affect aspects related to oxidative stress, inflammatory states and chondro degeneration (Panico *et al.,* 2007). Following this information, our objective is to assess the attenuation potential of *Opuntia ficus* indica on alterations caused by lead acetate in Wistar rats.

Materials and methods

Ethical approval

The Ethical Committee of Animal Sciences at the University of Badji Mokhtar-Annaba has given the authorization to realize the Ph.D. research program.

Plantmaterial

The flowers of Opuntia. *Ficus indica* were collected in May 2019, in the region of Ben Azouz, an area located to the North Est of Algeria about a hundred kilometers from the citie of Skikda. The dried flowers were then ground into powder and filtered through a fine mesh fabric and kept in paper bags away from light to prevent the photo-oxidation of the active substances contained in the powder. Distilled water was added to the powder to obtain a homogeneous suspension at 500 mg/kg body weight) according to the protocol of (Alimi *et al.,* 2011).

Chemical Product

In this study, we followed the protocol of (Hussein AL-Azawi, 2015). Lead acetate mixed in distilled water at a rate of 50 mg/kg body weight.

Animals thirty seven (37) rats from the Pasteur Institute in Algiers were raised in the pet store of the Badji Mokhtar-Annaba University. They were subjected 15days period of adaptation to the conditions of the pet store (T°22C°+2C°, 40% humidity and a clear and dark photoperiod of 8 hours/12 hours). The rats ahoused in polyethylene cages, lined with a litter composed of wood chips. The cages were cleaned and the litter changed once every two days. The animals were fed a balanced diet in the form of croquettes, from the agro-food complex of Oued Fragha (Citie of Guelma). The drinking water is served in bottles of libitum, and renewed daily.

Experimental Design

Rats were divided into 04 groups, each one containing 09 rats per cage,exceptthe treated group containing 10 rats.

- Group 1 (T), which is the control group, each rat receives 1ml of tap water by gavage.
- Group 2 (F2), which is the positive control group, each rat received 1.5ml of opuntia ficus indica(flower) suspension by gavage at 500mg/kg body weight.
- Group 3 (Pb) which is the lead-treated group, each rat received 1ml of Pb acetate solution(50 mg/ Kgbody weight) by gavage.
- Group 4 (F2/pB), which receives a combination of the two products, each rat received the combination of Pb acetate (50 mg/kg/body weight) with opuntia *Ficus indica* suspension (flower 500 mg/kg bw by gavage.

All groups were treated for 30 days. The animals were weighed once a week throughout thetrial period. At the end of the treatment period, the rats were killed by decapitation.

Studied Parameters

At the time of the sacrifice, the blood was collected in two tubes, one containing the anticoagulant EDTA to perform hematologic parameters (the blood count formula (SNF) and the other in dry

tubes. The latter is centrifuged at 3000 rpm for 10 minutes, the serum of the samples obtained was stored in the freezer at –20 °C until analysis for the following biochemical parameters: calcium, iron, albumin,protein total and immunoglobulin.

Statistical analysis

Statistical analysis of all results was analyzed using the Microsoft Excel program (2013). Data analysis was performed by student's test by comparing the means of each parameter analyzed in pairs between groups, using the softwareprism5.

Results

1. Haematological Parameters: The hematological parameters obtained are illustrated in Table 1, thirty (30) days after sub-chronic oral exposure of male Wistar rats. Results indicate a significant decrease ($p<0.05$) in red blood cell count in the lead acetategroup compared to the positive control, and a significant reduction ($p<0.05$) hemoglobinlevels and neutrophil numbers in the toxic group Pb,Compared to control group, positive control and combined group. On the other hand, a significant increase ($p<0.05$) in the number of white blood cells, lymphocytes and monocytes in the lead acetate-treated group compared to the control group, and the combined group. The numbers of eosinophils were not significant. After the Co-administration of the aqueous extract of Opuntia ficus indica an upgrade of the results and a positive effect was obtained.

Biochemical parameters

Biochemical parameters are presented in Table N°02. The results show a significant increase in Igm in the lead acetate group compared to the control, positive control and combined group. On the other hand, there was a significant decrease ($p<0.05$) in total protein, albumin, calcium and iron levels in the toxic group compared to the positive control and combined group.

The administration of the aqueous extract of the flowers of Opuntia ficus indica with lead acetate in

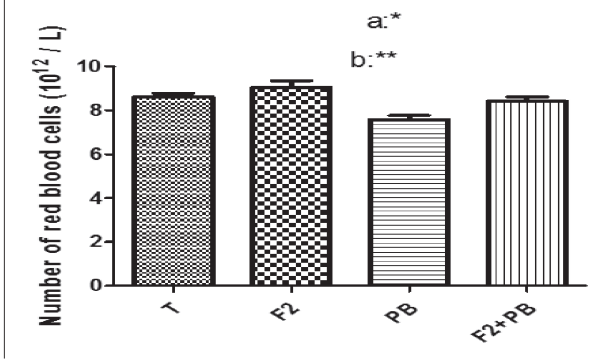

Fig. 1. Variation of red blood cell levels in different groups of rats. (Values are expressed as mean ± standard error)

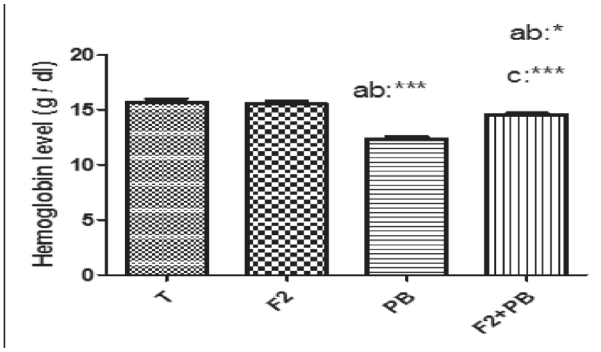

Fig. 2. Variation in Hb levels in different rats Groups (Values are expressed as astandard± mean error)

Table 1. The results of hematological parameters

Haematological Parameters	Control group (T)1 ml Watern=9	Positive control group (F2) 1,5 ml extrait of flower, 500 mg/kgn=9	Toxic groupe (PB) 1ml 50mg/kg n=10	Combined group (PB+F2) 50mg/kg/ 500 mg/kg n=9
WBC (10^{12}/l)	6.10 ± 0.20	6.43 ± 0.16	9.70 ± 1.3	6.25 ± 0.26
RBC (10^{12}/l)	8.63 ± 0.14	9.03± 0.31	7.57 ± 0.19	8.41 ± 0.20
Hb (g/dL)	15.72 ± 0.32	15.57 ± 0.21	12.35 ± 0.19	14.10 ± 0.23
Lym (%)	49.84 ± 0.98	51.19 ± 0.32	69.24 ± 1.25	54.00 ± 1.12
Monocytes (%)	12.45 ± 0.29	11.57 ± 0.50	16.57 ± 0.59	12.65 ± 0.38
Neutrophiles (%)	15.03 ± 0.60	16.27 ± 1.55	9.767 ± 1.74	15.13 ± 0.54
Eosinophiles (%)	24.97 ± 0.72	25.31 ± 0.92	21.89 ± 1.19	23.29 ± 0.74
Igm	30.53 ± 0.66	31.48 ± 2.208	67.69 ± 1.198	39.28 ± 1.838

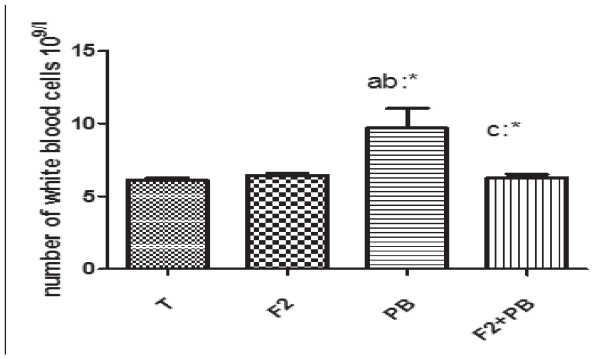

Fig. 3. Variation in white blood cell in different groups of rats. (Values are expressed as means±standard error)

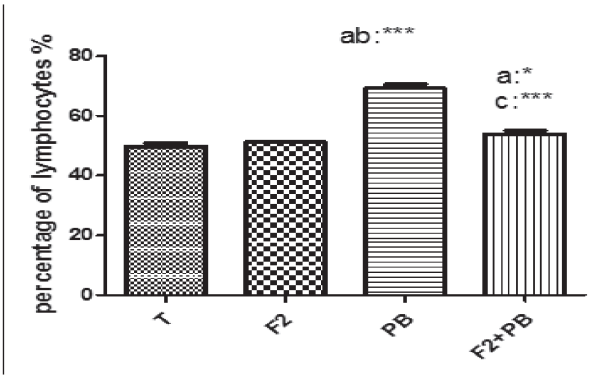

Fig. 4. Variation of lymphocyte levels in different groups of rats (Values are expressed as means ± standard error)

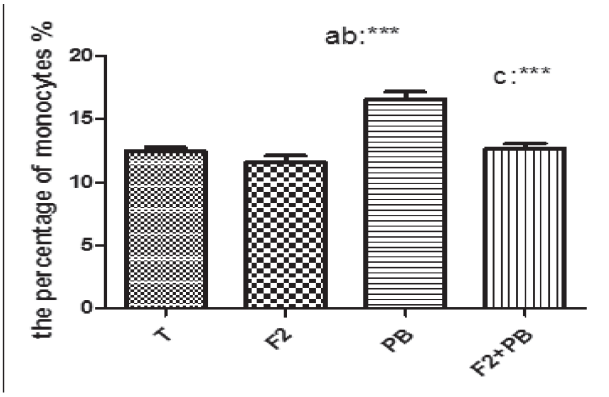

Fig. 5. Variation in monocyte levels in different groups of rats. (Values are expressed as a mean ±standard error)

combined group attenuated the adverse effects induced by lead acetate. And caused registered regulatory changes in hematological and biochemical parameters compared to the control group. Therefore, the aqueous extract producedan effective pro-

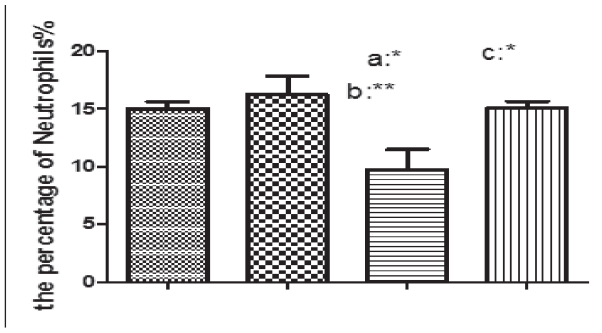

Fig. 6. Variation of neutrophil levels in different groups of rats. (Values are expressed as a mean±standard error)

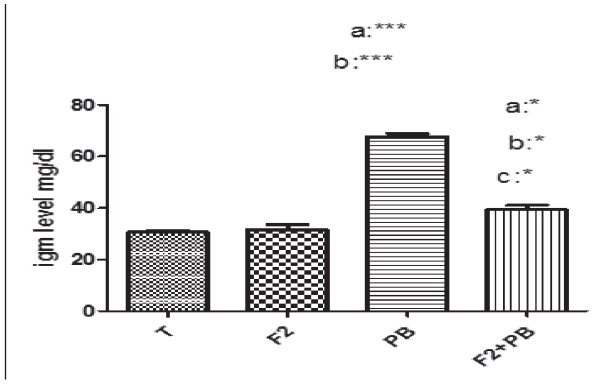

Fig. 7. Variation in Igm levels in different groups of rats. (Values are expressed as a mean± standard error)

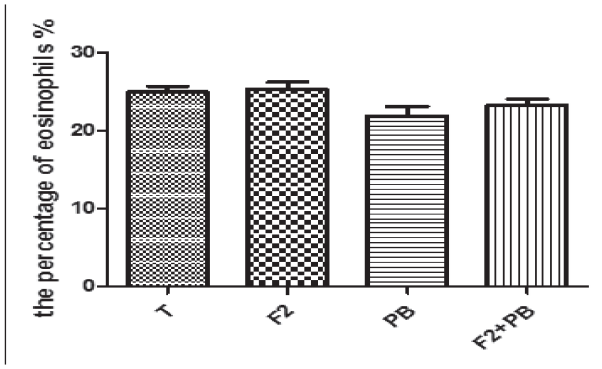

Fig. 8. Variation ofeosinophil levels in different groups of rats. (Values are expressed as a mean ±standard error)

tective action against the haemato-biochemical toxicity induced bylead acetate in male Wistar rats.

Tissue observation

Estimated histological examination of the spleen of rats exposed to lead acetate (50mg/kg) by gavage for four (4) weeks showed some changes that are

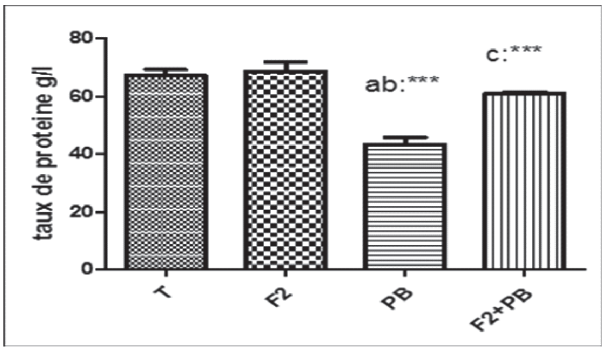

Fig. 9. Variation in total protein levels in different groups of rat. (Values are expressed as standard error averages)

noted according to their severity. Observation by optical microscope showed architectural disorganization, lymphoid hyperplasia, predominance of white pulp, appearance of hyaline fibrosis. The whole is caused by a diffuse necrotic process secondary to the toxicity of lead. Cells are lysed, have eosinophiliccytoplasm or clarified, basophilic nuclei per place are densified. These cellular alterations with necrosis and apoptosis are secondary to the treatment undergone by Pb. In addition, of the presence of vascular alterations represented by a parietal thickening with an obscurevascular lights of the blood dotted with pigment of haemocedrine. Necrotic changes are pronounced, they can lead to esquèmique necrosis (vascular esquèmie). However, the histological examination of the spleen in the control group and the positive control showed no significant difference. For the first group, the spleen is normal reveals red and white pulp, bilroth cords and well-defined blood capillaries. As well as the histological aspect of thesecond group is kept as

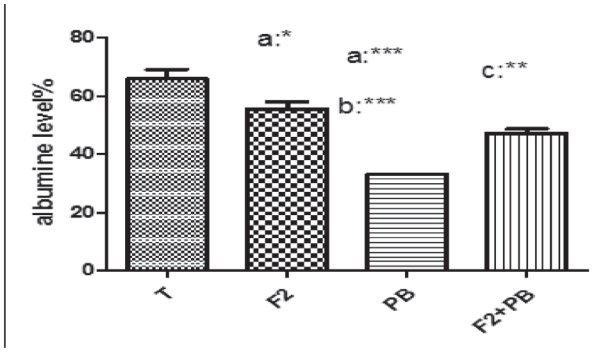

Fig. 10. Variation of albumin levels in different groups of rats. (Values are expressed as mean ± standard error)

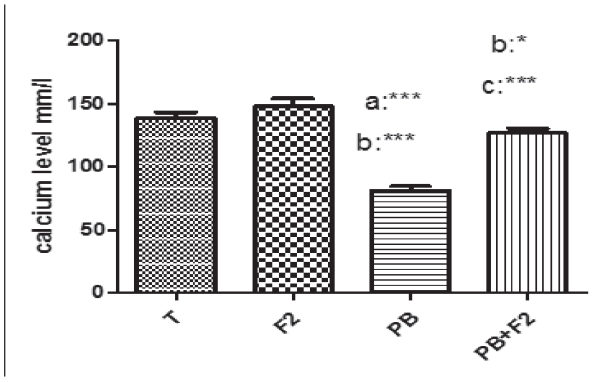

Fig. 11. Variation of calcium levels in different groups of rats. (Values are expressed as mean ± standard error)

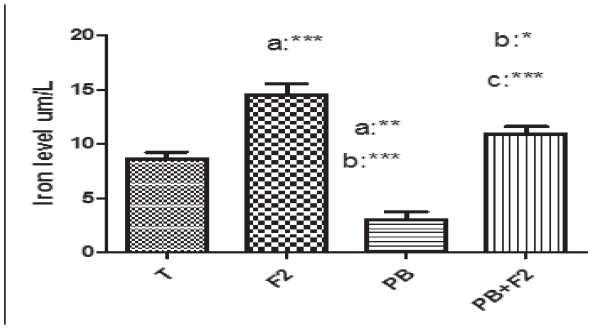

Fig. 12. Variation of iron levels in different groups of rats. (Values are expressed as mean ± standard error)

Table 2. Results of biochemical parameters

The treated groups Parameters biochemical	Control group (T) 1ml Water n=9	Positive control group (F2) 1,5 ml extrait of flower 500 mg/kg n=9	Toxic group (PB) 1ml 50 mg/kg n=10	Combined group (Pb+F2) 50 mg/kg / 500 mg/kg n=9
Protein totals (g/l)	67.12 ± 2.138	68.64 ± 3.241	43.38 ± 2.470	60.84 ± 0.06615
Albumin (g/l)	65.74 ± 3.344	55.40 ± 2.436	33.05 ± 0.2128	47.07 ± 1.567
Globulin (g/l)	7.573 ± 2.643	15.53 ± 6.076	10.80 ± 1.481	11.68 ± 0.8976
Ca^{2+} (mm/l)	138.3 ± 4.780	80.75 ± 3.728	147.5 ± 6.3668	126.6 ± 3.706
Fer (Uml)	8.625 ± 0.6250	133.50 ± 0.6455	3.063 ± 0.6950	10.94 ± 0.6799

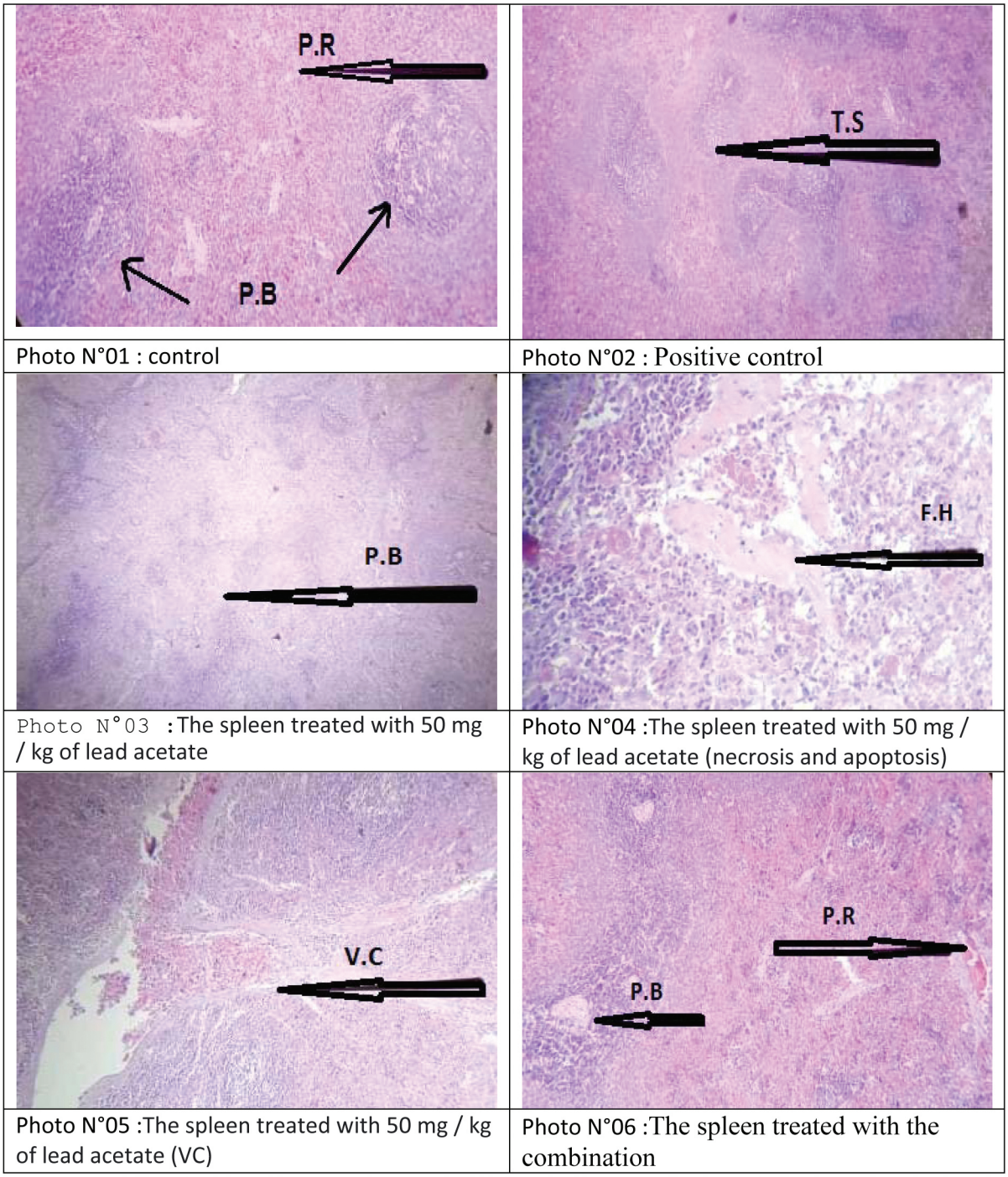

Photo N°01 : control

Photo N°02 : Positive control

Photo N°03 : The spleen treated with 50 mg / kg of lead acetate

Photo N°04 :The spleen treated with 50 mg / kg of lead acetate (necrosis and apoptosis)

Photo N°05 :The spleen treated with 50 mg / kg of lead acetate (VC)

Photo N°06 :The spleen treated with the combination

Plate 1. Histological section of the spleen of the Wistar Rat (Magnification x10). The control spleen composed of two main constituents: the red pulp (P.R) and the white pulp (P.B) (Photo N ° 01). That of the positive control presents a histological appearance like healthy tissue (T.S) (Photo N ° 02). The spleen treated with 50 mg / kg of lead acetate presents an architectural disorganization, lymphoid hyperplasia, predominance of white pulp (Photo N ° 03). The spleen reveals a band of hyaline fibrosis (FH), an inflammatory process by the presence of vascular and cellular alterations with necrosis and apoptosis (Photo N ° 04). Presence of congestive vessels (VC) gorged with blood (Photo N ° 05). The spleen treated with the combination of 500 mg / kg of the suspension of Opuntia ficus indica, and 50 mg / kg of lead acetate revealed normal structures, and cellular distribution as a clear conjunction of red pulp and white pulp. (Photo N ° 06).

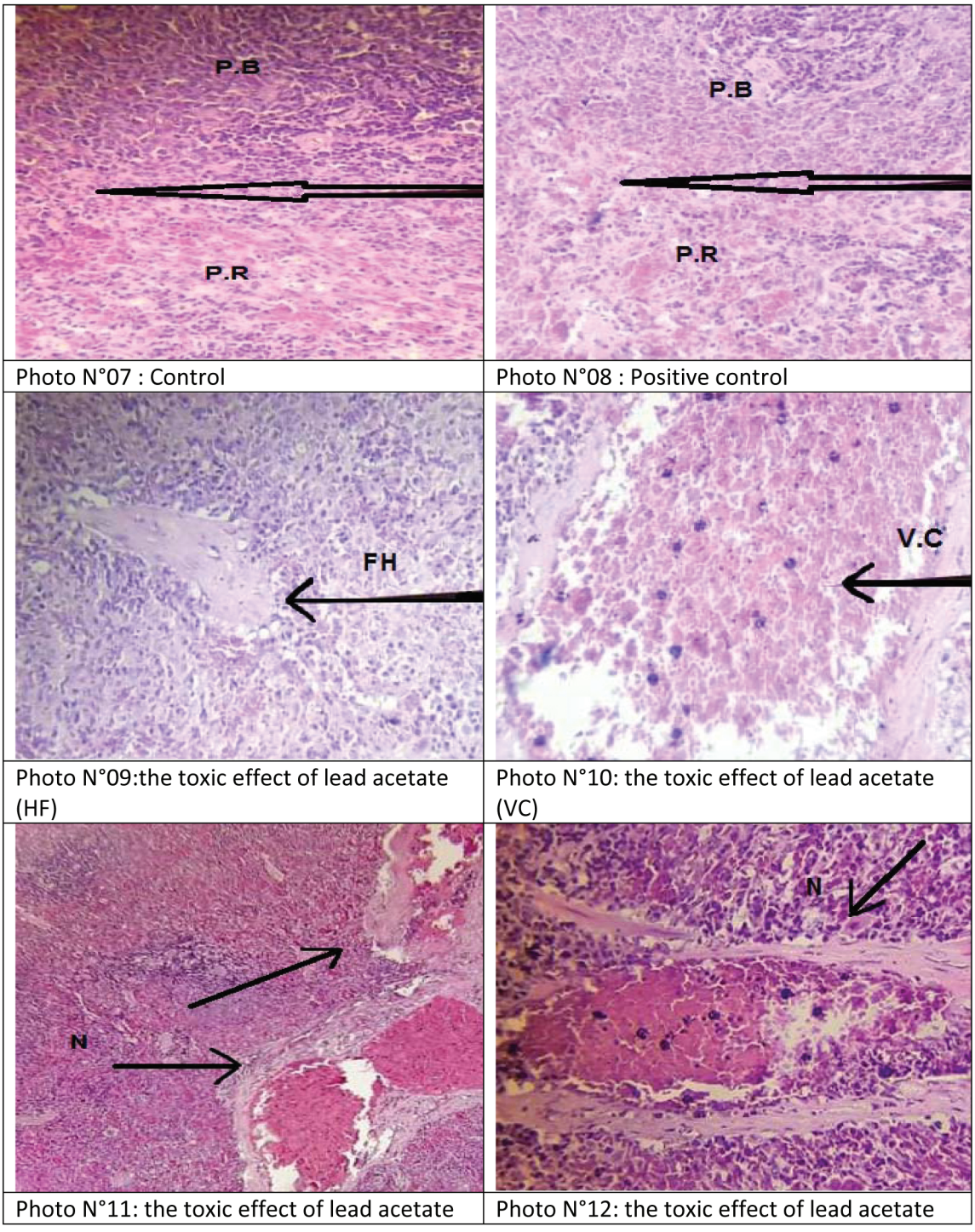

Photo N°07 : Control	Photo N°08 : Positive control
Photo N°09:the toxic effect of lead acetate (HF)	Photo N°10: the toxic effect of lead acetate (VC)
Photo N°11: the toxic effect of lead acetate	Photo N°12: the toxic effect of lead acetate

Plate 2. Histological section of the spleen of the Wistar Rat (Magnification x40). The spleen presents hyaline fibrosis (HF) with lymphocytic inflammatory reaction (photo N ° 09). And presents a congestive vessel (VC) gorged with blood (photo N°10). Necrosis (N) well defined (Photo N ° 11 and 12).

a healthy tissue. Also, examination of the combined group revealed a normal structural architecture and cell distribution, as clear junction of red and white pulp, and numerous lymphatic sheaths around the arteries. As a result, supplementation of theaqueous extract of opuntia ficus indica flowers reduced the Pb content in the combined group spleen and mitigated the induced necrotic damage to splenic cells.

Discussion

As part of the search for new bioactive molecules extracted from plants, it seemed interesting to us to study the restorative effect of the aqueous extract of the flower of Opuntia ficus indica against the toxicity induced by lead acetate, through the comparative analysis of some hematological, biochemical and tissue parameters. On the hematological level, the results obtained illustrate a significant decrease in the number of red blood cells and the hemoglobin level in the group treated with lead acetate, which is in agreement with many studies (Jennifer, 2010 and Zargar *et al.,* 2016). On the other hand, an attenuating effect was observed on the same haematological parameters, in the batch treated with the aqueous extract of Opuntia ficus indica and previously subjected to intoxication with lead acetate. Few studies have demonstrated this attenuating effect, but many studies have described a similar effect following treatment with ethanol (Alim *et al.,* 2011). If the harmful effects of lead acetate on the hematopoietic system have been widely described in the literature (Sharma *et al.,* 2013), the therapeutic power of the extract of flowers of *Opuntia ficus* indica has been validated and could be explained by several contributing factors. Including phenolic compounds, flavonoids and polysaccharides of plant origin, which act as scavengers and inhibitors of lipid peroxidation of ROS via hydrogen.

Some of the flavonoid compounds in the aqueous extract of Opuntia ficus indica are quercetin, kaempferol and isorhamnetinglycosidederivatives. Quercetin could act as an electron donor of its hydroxide group, and therefore inhibit free radicals (Kahraman *et al.,* 2003). Kaempferol has antiulcerogenic activity and isorhamnetin glycoside derivatives have also been reported to have anti-inflammatory properties. Analysis of the immune response induced by lead acetate poisoningshows a significant increase in monocytes, lymphocytes and plasma levels of IgM in the Pb-exposed group compared to other groups with a highly significant decrease in neutrophils. These results are similar to those reported by Moumen *et al.* (2011) and Loudjani *et al.* (2012), under the same experimental conditions where they have demonstrated that lead has low concentrations can activate the immune response plus it takes the calcium plac e in the role of the second messenger and competes with iron at the level of absorption intestinal. under the same experimental conditions. On the other hand, a leukocyte rebalancing is observed in the batch treated with the aqueous extract of Opuntia ficus indica, which supports the attenuation effect observed for the hematological parameters, as well as the antioxidant and anti-inflammatory potential described in the bibliography for this plant. Biochemically, the results obtained show a significant decrease in the levels of calcium, iron, albumin and total protein in the group treated with lead acetate compared to other groups. Our results are in agreement with numerous studies, which explain this decrease by the inhibitory effect of protein biosynthesis, through specific enzymes in cellular processesand increased renal excretion (Murrey *et al.,* 2006; Saka *et al.,* 2011). In addition, among the key biochemical indicators of lead poisoning, numerous studies describe a destruction of iron and a modification of its metabolism. On the other hand, our results once again show a restorative effect in the batch treated with the aqueous extract of Opuntia ficus indica, which would act as an antioxidant given its richness in vitamin C, betalaine-type pigments, phenolic compounds, reducing sugars and mineral salts, which allows better assimilation of calcium and magnesium (Galati, 2002).

On the tissue level, the histological observations made in the spleen in the lead treated batch show significant histopathological changes and vascular alterations. These observations support the results obtained by who describe the same pathologies in lead poisoned chickens. In the batch treated with the aqueous extract of *Opuntia ficus indica*, the observations show tissue repair, lessening of necrosis, and a decrease in vascular damage compared to the batch treated with lead.

Indeed, *Opuntia ficus indica* flowers exert a wide range of pharmacological activities, including antioxidant, antimicrobial, antiulcerogenic, and wound healing activities (Alim *et al.,* 2011) as well as an anti-inflammatory effect. The plant is rich in vitamins E and allows the body to resist infections and

prevent anemia, and helps fight effectively against free radicals, which are the main responsible for various diseases by reducing the toxic effect of lead (Galati EM, 2002).

Conclusion

This study demonstrated exposure to lead causing altercations and variations in immuno-biochemical and histological parameters in rats. However, treatment via aqueous suspension of the flowers of Opuntia ficus indica induced a significant restoration of the immune and biochemical parameters, this shows the beneficial effect of this therapeutic system against metabolic and physiological dysfunction in the spleen associated with lead toxicity.

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