The Attenuating Effect of the Aqueous Extract of the Flower of Opuntia Ficus Indica Against Lead-Induced Reprotoxicity in the Wistar Rat

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Received 17 /08/ 2023; Accepted 11/01/ 2024, Published 19/01/2024

Abstract

The objective of this study is to investigate the beneficial effect of Opuntia ficus indica flower aqueous extract against lead (Pb)-induced reprotoxicity in male Wistar rats administered orally for 4 weeks. These are divided into 6 groups: the control group (C), 2 positive controls treated with the aqueous extract of the flower of Opuntia ficus indica F1 and F2 respectively (250 mg/kg, 500 mg/kg of body weight/day) ;1 group treated with lead (50 mg/kg body weight/ day) and 2 groups of combined treatment treated with lead and aqueous extract of opuntia ficus indica flowers (F1 + Pb), (F2 + Pb).Extracts of Opuntia ficus indica flowers are the subject of a phytochemical study. After 30 days of oral administration, the absolute weight of the testicles and of the epididymis, the plasma levels of testosterone are evaluated; in addition to sperm biology parameters (concentration, motility, vitality, velocity (VCL, VSL and VAP), cross-level (BCF) and DNA fragmentation test are evaluated by the CASA system. Testicular glutathione and epididymal (GSH), Malondialdehyde (MDA) and glutathione peroxidase (GPX) are also evaluated. The results obtained show a significant reduction in testosterone levels, absolute testicular and epididymal weights, live sperm, VCL, VSL, VAP, BCF, GSH and GPX as well as an increase in dead spermatozoa and MDA in the group treated with lead (Pb) compared to the control group (C) and the positive controls, the administration of the aqueous extract of the flowers of opuntia ficus indica in the combination groups (F1+Pb and F2+Pb) caused an attenuating effect against lead-induced toxicity. Even the phytochemical study confirms that the methanolic extract of Opuntia ficus indica extract is rich in tannins and flavonoids. Keywords: Opuntia ficus indica, CASA sperm, lead, DNA, testosterone.

Keywords: Opuntia ficus indica; reprotoxicity; phytochemical study; DNA fragmentation; sperm biology parameters.

Tob Regul Sci.™ 2024;10(1):1555 - 1583

DOI: doi.org/10.18001/TRS.10.1.99

introduction:

Environmental pollution by heavy metals is a global problem that reduces the quality of life and has negative consequences on living organisms (James Daniel et al., 2018). These persistent environmental pollutants have the ability to accumulate in the tissues of living organisms and cause

more or less significant damage (INRS, 2018). Among these pollutants, lead is a very abundant compound with a fairly wide distribution in all types of ecosystems, with a bioconcentrating potential in the different body systems (Drif et al., 2019). Lead is not biodegradable (Seregin and Ivanov, 2001), and therefore can induce a wide range of central, peripheral, and hematopoietic nervous system dysfunctions (Burroughs and Rollins, 2017; Derouiche et al., 2018). Lead enters the body mainly by ingestion or inhalation, it can also pass through the skin (Anomaa et al., 2017). Once absorbed, lead passes into the bloodstream (Andjelkovic et al., 2019). And is then distributed to several tissues such as bones, brain, kidneys and liver. It is also found in the male reproductive system (epididymis, seminal vesicles, testicles and prostate.) (Picot, 2010; Andjelkovic et al., 2019), leading to disruption of male reproduction (Alouche et al; 2009). This negatively affects the number of sperm, their movements and delays their activity (Berredjem et al., 2014). The mechanism of lead-induced oxidative stress involves a balance between the generation and elimination of ROS (reactive oxygen species) in tissues and cellular components causing damage to the membrane, DNA and proteins (Patra et al., 2011; Berrahal et al. 2011; Flora et al. 2012; Abdel-Moneim et al. 2015; Wu et al. 2016; BaSalamah et al. 2018). Lead significantly reduces the antioxidant system).To remedy the poisoning caused by heavy metals and lead in particular, many studies have focused on the therapeutic virtues of the flowers of the prickly pear (Opuntia ficus indica), which is a plant native to Mexico, but cultivated in different countries. of the world (Toure, 2017). This plant is characterized by rapid growth and great adaptation to difficult abiotic conditions (Pèrez-Torrero et al. 2017). The flowers of Opuntia ficus indica are known for their antioxidant properties, helping to protect cells against oxidative damage caused by radicals (Toure, 2017) They are also used as an anti hemorrhagic, antibacterial, antifungal, antiseptic and diuretic (Ammar et al., 2012; Aragona et al., 2017; Keller et al., 2019). Among the indications of Opuntia ficus indica flowers relieve kidney pain and effectively fight against cholesterol, type II diabetes, prostate adenoma, burns, peptic ulcers and even certain types of cancers whose progression it would slow down (ref).Analysis of the physicochemical properties of broths and infusions prepared from Opuntia ficus-indica flowers reveals that these preparations are rich in minerals (potassium and calcium), polyphenols, flavonoids and tannins (Chahdoura et al., 2014 Ammar et al. 2015). The main active compounds in Opuntia ficus indica flowers are polysaccharides which accelerate the repair process throughout the tissue. Glycoproteins, aromatic compounds, in particular betacyanins, betaxanthins, polyphenols, fatty acids, sterols, β-carotene, due to their antioxidant activity and polyphenolic flavonoids are metabolites which have numerous pharmacological properties (Panico et al., 2007). Other studies have shown the beneficial effects of Opuntia ficus indica flowers on sperm DNA fragmentation after sperm cryopreservation in humans (Meamar et al., 2012). Other researchers have also observed a significant reduction in sperm DNA fragmentation as well as improved sperm quality (Ladouali et al., 2022), motility and viability (Allai et al., 2016).

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.

Plant material

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 city 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 250 mg and 500 mg/kg body weight) according to the protocol of (Alimi et al. 2011).

The dosage of phenolic compounds

Preparation of the methanolic extract

The preparation of this extract consists in macerating 1g of vegetable powder in 20ml of absolute methanol for 24 hours at room temperature, the extracts are then filtered and then evaporated under reduced pressure to dryness by a rotary evaporator at T = 65C °. The dry residue is taken up in a few milliliters of methanol and stored in an amber flask at + 4 °C.

Determination of phenolic compounds:

The determination of the total phenolic compounds was carried out by the method using the Folin-Ciocalteu reagent. This method was described in 1965 by Singleton and Rossi.

This assay is based on the quantification of the total concentration of hydroxyl groups present in the extract. A volume of 200 µl of the stock solution of gallic acid and of the methanolic extract of the species studied is introduced into test tubes, 1 ml of the Folin-Ciocalteu reagent diluted 10 times is Added There to. After standing for 5 min, 800 µl of 7.5% sodium carbonate are introduced. The tubes are shaken and stored for30 minutes at room temperature and protected from light. Absorbance is measured at 765 nm against a blank using a SHIMADZU UV-1202 spectrophotometer. A calibration curve is performed in parallel under the same operating conditions using gallic acid as a positive control. The results are expressed in milligram (mg) equivalent of gallic acid per gram of dry plant matter (mg EAG / g of dry matter).

Dosage of tannins:

The total tannin content was evaluated according to the colorimetric method described by Polshettiwar et al. (2007), based on the reduction, in an alkaline medium, of phosphomolybdic and tungstic acids contained in the Folin-Denis reagent (RFD). The reaction gives a blue color, the intensity of which is measured by spectrophotometry at 755 nm.

0.5ml of RFD and 1ml of sodium carbonate (0.5% Na2CO3) were introduced into volumetric flasks. 100 µl of the dilution of the methanolic extract was Added There to, the volume of the solution was adjusted to 5mL with distilled water. After a 30 min incubation, the absorbance was measured by a SHIMADZUUV-1202spectrophotometer. The total tannin content was determined by extrapolation on a calibration curve obtained from a series of dilutions of tannic acid (MS 10 mg / ml). The results were expressed in milligrams of tannic acid equivalent per gram of dry plant material (mg EAT / g DM).

Determination of flavonoids:

Principle: The quantification of the flavonoids was carried out by a method adapted by Zhishen et al. (1999), based on the formation of complexes between flavonoids and aluminum trichloride. The complexes produced, yellow in color, absorb in the visible range at 510nm.

500 µl of the crude extract is mixed with 2 ml of distilled water, and added with 150 µl of 5% sodium nitrite (NaNO2). After 5 min of incubation, 100 µl of 10% aluminum trichloride is added to the mixture. After a further 6min incubation, add 1ml of 1M sodium carbonate. The mixture is completely stirred in order to homogenize the content and the absorbance of the solution is determined at 510 nm against a blank. A calibration curve is carried out in parallel under the same operating conditions using quercetin as positive control. The total flavonoid content is expressed in milligram (mg) equivalent of quercetin per gram of dry plant material (mg EQ / g DM).

Evaluation of the anti-free radical activity by the DPPH test:

The anti-free radical activity of the extract of Inula viscosa L. was evaluated in vitro, by the DPPH test. This spectrophotometric method uses the violet-colored DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical as a reagent, which turns yellow, in the presence of free radical scavengers, and is reduced to 2,2'-diphenyl-1 -picrylhydrazine (Cuendet et al., 1997; Burits and Bucar, 2000). This makes it possible to follow the discoloration kinetics at 517 nm.

Evaluation of anti-inflammatory activity:

The anti-inflammatory activity of methanolic extract from the aerial part of Inula viscosa L. is evaluated by the protein denaturation method described by Alhakmani et al. (2013), with some modifications. By this method, acetylsalicylic acid (aspirin) is an anti-inflammatory drug used as a benchmark anti-inflammatory.

T The test consists of two experiments. In the first experiment, the reaction mixture consists of 2 ml of extract of opuntia Ficus Flower. (methanolic) at different concentrations (2-1.6-1.2 mg / ml) and 2.8 ml of distilled water adjusted to pH = 6.4 with HCl, then mix with 2 ml of egg albumin. Incubate at 37 ° C for 10 minutes. In the second experiment, the extract of opuntia ficus Flower. is replaced by acetylsalicylic acid for each of the experiments, a control is prepared. It consists of 2ml of distilled water in place of the inule extract and acetylsalicylic acid, 2.8ml of distilled water and 2ml of egg albumin. Denaturation is induced by placing the reaction mixture in a water bath heated to 72 ° C for 5 minutes. After cooling, the absorbance is measured at 660n in using distilled water as a white.

Protocol HPLC

HPLC (high performance liquid chromatography) is an analytical separation technique. first, we prepared the methanolic extract as we saw in the study of the components of opuntia ficus indica flowers, then we put 5 mg of opuntia ficus indica flower extract in100 ml of distilled/directed water and 900 µl of methanol (MeOH). Chromatographic analysis was performed using Shimadzu Prominence-I LC-2030C (Japan) HPLC liquid chromatography with UV detector, C18 (4.6 x 25 mm, 5 µm) chromatography column (USA). column is maintained at 45°C.

Chemical Product

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

Animals

Fifty-five (55) rats from the Pasteur Institute in Algeria. each weighing 196±8 g. were raised in the pet store of the Badji Mokhtar-Annaba University. They were subjected 15 days period of adaptation to the conditions of the pet store (T°22C°+2C°, 40% humidity and a clear and dark photoperiod of 12 hours/12 hours). Fifty-five rats were divided into 06 equal groups.

Group 1 (C), which is the control group, each rat receives 1 ml of tap water by gavage, Group 2 (F1), which is the positive control group, each rat receives 1.5 ml of the extract aqueous extract of opuntia ficus indica flowers by gavage at 250 mg/kg body weight, group 3 (F2), which is the positive control group, each rat received 1.5 ml of the aqueous extract of opuntia flowers ficus indica by gavage at a dose of 500 mg/kg of body weight, group 4 (Pb) which is the group treated with lead, each rat received 1 ml of Pb acetate solution (50 mg/Kg of body weight) by gavage. , Group 5 (F1+Pb), each rat receives the combination of Pb acetate (50mg/kg/body weight) and the aqueous extract of Opuntia ficus indica flowers (250 mg/kg bw by gavage. Group 6 (F2+Pb), each rat receives the combination of Pb acetate (50 mg/kg/body weight) and the aqueous extract of Opuntia ficus indica flowers (5 mg/kg bw) by gavage. were treated for four weeks. The animals were weighed once a week throughout the experimental period. At the end of the treatment period, the rats were sacrificed by decapitation. The organs (testis and epididymis) were removed, weighed and a part was frozen at -20°C for the assay of markers linked to oxidative stress and the other part was fixed in formalin in order to make the histological sections.

Studied Parameters

At the time of the sacrifice, the blood was collected in heparini zed tubes and then centrifuged at 3000 rpm for 10min. The plasma obtained was stored at -20 °C along with the which had already been weighed, till further analysis. Animals' treatments were authorized by the Ethical Committee of Animal Sciences at the University of Badji Mokhtar-Annaba, before starting the experimental work Semen analysis.

The sperm analysis is carried out immediately after the sacrifice, by taking a sample of the epididymis, estimated at 1 µl, which is diluted with a physiological solution of NaCl at 0.09%, then 5 ml of the mixture is deposited in an empty chamber slide (Gold Cyto model) The results are read and analyzed under an electron microscope on a Nikon Eclipse microscope (Nikon E200-LED) with phase objective (x4), Connected to a computer (CASA) at the using a group analysis of spermatozoa (SCA®, Microptic, Barcelona, Spain), automatic sperm analysis allowed the calculation of concentration, velocities, vitality, velocity parameters (VCL, VSL, VAP), and cross-beat frequency (BCF), were automatically calculated. For the DNA fragmentation test, a diluent semen sample at a concentration of 5 to 10million per millilitre was used (n = 4).the test was performed using the Gold Cyto DNA kit (Gold Cyto Biotech crop. Room 1602, 6F, NO, 16) as illustrated in the instruction manual. The spermatozoa were recognized and automatically classified into fragmented spermatozoa. According to established analysis criteria.

Hypo - osmotic swelling (HOS) test

The study of sperm vitality is carried out by carrying out the Bright Vit Protocol. This test was developed to measure two functional aspects of sperm, namely living sperm in a simple way and secondarily the quality of cell membrane integrity and the ability of the cell membrane to resist osmotic swelling without bursting. Bright vit, a neosine negrosine solution stains dead cells pink, living cells will remain white. This analysis is done by exposing a drop of sperm, extracted from the tail of the epididymis, to a hypo osmotic solution consisting of a negrosine neosine .

Analysis of Oxidative Stress Markers

100 mg of testis samples and epididymis were taken and placed in test tubes for measurement. For the determination of glutathione (GSH) using the method of Cory and Weckbecker. The principle of this assay is based on the measurement of the optical absorbance of 2-nitro-5-mercapturic acid. 200 mg of each tissue was placed in the presence of 8 ml of a solution of ethylene diamine tetra acetic acid (EDTA) at 0.02 M, then cold ground using an ultra-Sound mill (4 °C) to obtain a homogenate. Read optical densities at 412 nm against white. The testicular and epididyme total proteins were quantified according to the colorimetric method of Bradford. by using CoomassieBlueG-250 (Sigma, St. Louis, USA) Malondialdehyde (MDA) was estimated by using the method of Ohkawa et al., 1979. The determination is based on the formation in an acidic and hot medium (100 ° C) between MDA and thiobarbituric acid (TBA) of an absorbent-colored pigment at 530 nm, extractable by organic solvents such as butanol. the enzymatic activity of glutathione peroxidase (Gpx) was measured by the method of Flohé and Günzler (1984). This method is based on the reduction of hydrogen peroxide (H2O2) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the influence of GPX. read optical densities at 412 nm against white

Histological examination of samples

Tissue samples are immediately fixed in buffered formaldehyde (10%), the samples are then dehydrated with a series of ethanol, cleaned with xylene, embedded in paraffin, cut using a 3 µm microtome. The tissue sections are mounted on gelatinized slides, then stained with eosin hematoxylin, then examined under a light microscope (Optika B-350). A second toluidine blue stain is used (Appendix I). The microscopic photos are taken with a digital camera (Optikam B5) according to the recommendations (Aeffner et al., 2018). Tissue samples are immediately fixed in buffered formaldehyde (10%), the samples are then dehydrated with a series of ethanol, cleaned with xylene, included in paraffin, cut using a microtome at 3 µm. The fabric sections are mounted on gelatinized blades and then stained with hematoxylin eosin then examined under an optical microscope (). A second color of toluidine blue is used (Appendix I). Microscopic photos are taken with a digital camera (Optikam B5) according to the recommendations (Aeffner et al., 2018).

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 software prism7.

Resultats

Quantitative evaluation of the phenolic compounds of Opuntia ficus indica flowers (OFI)

The quantitative analysis of the phenolic compounds of the methanolic extract of the flowers of Opuntia ficus indica affirm the richness of this species in Total polyphenols, the Tannins as well as the flavonoids.

Table 1: the quantitative analysis of the phenolic compounds of the methanolic extract of the flowers of Opuntia ficus indica affirms the richness of this species in Total polyphenols, the Tannins as well as the flavonoids.

Phenolic compounds	Total polyphenols (mg EAG/g DM)	Flavonoïdes (mg EAG/g DM)	Tannins (mg EAG/g DM)
Teneur (OMFE)	970,4503	16,85013	883,835

EAG/g DM): Milligram equivalent of gallic acid per gram of dry matte

Evaluation of anti-free radical activity by the DPPH test (Diphenyl-picryl-hydrazy)

The results obtained show that the methanolic extract of OFI flowers has an exceptional antioxidant effect against the DPPH radical with an IC50 of the order of 0.843mg/ml compared to: Inhibitory concentration of 50% of the ascorbic acid IC50 = 0.290mg/ml.

Table 2: the quantitative analysis of the phenolic compounds of the methanolic extract of the flowers of Opuntia ficus indica affirms the richness of this species in Total polyphenols, the Tannins as well as the flavonoids

50% inhibitory concentration	IC50 (mg/ml)
Ascorbic Acid	0,290 mg / ml
Flower extract concentration	0,843 mg /ml

Evaluation of anti-inflammatory activity

The results obtained show that the methanolic extract of *OFI* Flowers has an anti-inflammatory activity in vitro. The addition of increasing concentration of the methanolic extract of the *OFI* flowers of the plant to the albumin solution prevents the precipitation of this protein.

Table 3: the quantitative analysis of the phenolic compounds of the methanolic extract of the flowers of *Opuntia ficus indica* affirms the richness of this species in Total polyphenols, the Tannins as well as the flavonoids

Concentration of flower extract mg/ml	% inhibition methanolic extract
100	13,39
200	16.12
300	32.79
500	55.74
750	84.43

Qualitative analysis of flavonoids

The analytical separation technique allows the identification of the phenolic compounds of the aqueous extract of the flowers of Opuntia ficus indica. Chromatographic profiles of aqueous extracts of prickly fig flowers analysed by HPLC show several spikes which appear to contain several types of phenolic compounds. The comparison between the reference molecules (standards) and the phenolic compounds of the aqueous extract of the flowers of Opuntia ficus indica is based on retention time (TR), that is, the height of the spike. So, we identified phenolic compounds such as flavonoids, and Iso quercetin, the other phenolic compounds are not identified because of a lack of standards. According to chromatographic profiles the aqueous extracts of Opuntia ficus indica flowers are rich in phenolic compound.

Chromatogram

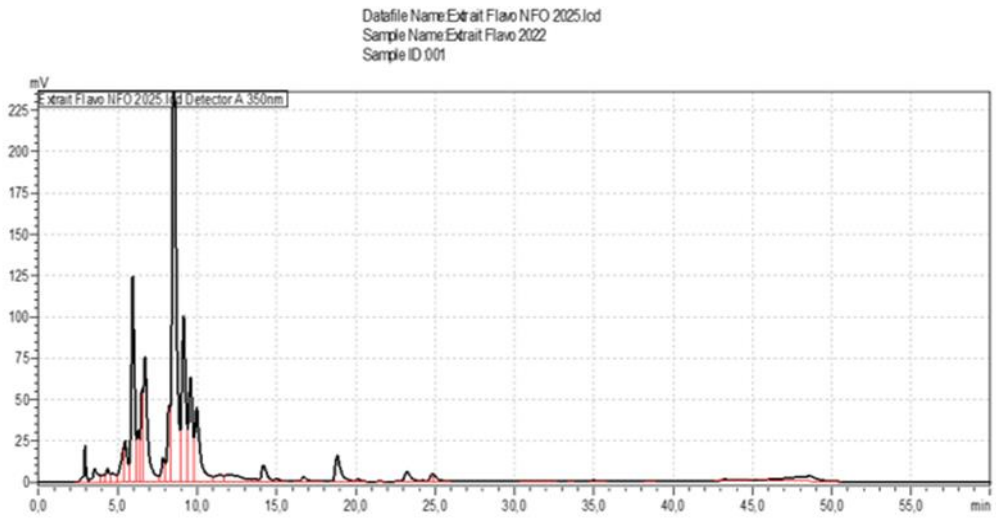

Figure 1: Chromatogram of the aqueous extract of Opuntia ficus indica flower.

The analytical separation technique allows the identification of the phenolic compounds of the aqueous extract of the flowers of Opuntia ficus indica. Chromatographic profiles of aqueous extracts of prickly fig flowers analysed by HPLC show several spikes which appear to contain several types of phenolic compounds. The comparison between the reference molecules (standards) and the phenolic compounds of the aqueous extract of the flowers of Opuntia ficus indica is based on retention time (TR), that is, the height of the spike. So, we identified phenolic compounds such as flavonoids, and Iso quercetin, the other phenolic compounds are not identified because of a lack of standards. According to chromatographic profiles the aqueous extracts of Opuntia ficus indica flowers are rich in phenolic compound.

General Assessment

General assessment During the experimental period, the rats of the control group (C) and the positive groups (F1, F2) were in very good condition unlike the group treated with lead (Pb) which presented a set of changes in the behavior of the animals such as rigor, aggressiveness, and exhaustion, as well as a decrease in body gain and the appearance of lymph nodes in some rats.

Organs and total body Weights

The treatment of rats with lead at 50 mg/kg of body weight caused a non-significant decrease in the body weight of the rats compared to the control group (C) and the positive control groups (F1, F2). On the other hand, a non-significant increase in the weight of the rats in the groups treated with the Pb association and the aqueous extract of OFI was observed compared to the Pb group, observed and administration of the aqueous extract of OFI flowers at 250 mg/kg and 500 mg/kg by gavage did not cause any significance in body mass in the different groups of rats. The treatment of rats with lead (Pb) also induces a significant reduction in the absolute weight of the testes and epididymis compared to the control group (C) and the positive control groups (F1, F2).Co-administration of the aqueous extract of OFI flowers with lead acetate caused a significant increase in the absolute weight of the testicles and epididymis in the combination groups, especially (F2+Pb) compared to the (Pb) group.

Table 4: The total body weight (g) and the absolute weight (g) of reproductive organs (mean±SEM) of Wistar rats exposed to lead acetate and co-administrated with the aqueous extract of OFI for 4 consecutive weeks.

Groups	Body weight	Testis	Epididymis
C	246,6 ± 9,495	1,514 ± 0,035	0,459 ± 0,013
F1	250,9 ± 10,73	1,524 ± 0,033	0,504 ± 0,023
F2	253,4 ± 11,24	1,564 ± 0,025	0,565 ± 0,020$^{a:**}$
Pb	229,8 ± 3,693	1,32 ± 0,030$^{a:**, bc:***}$	0,329 ± 0,009$^{abc:***}$
F1+Pb	235,1 ± 5,238	1,501 ± 0,029$^{d:**}$	0,499 ± 0,014$^{d:***}$
F2+Pb	241,7 ± 7,655	1,557 ± 0,030$^{d:***}$	0,526 ± 0,020$^{d:***}$

Spermatozoa Concentration and Motility

The results indicate a significant decrease ($p<0.05$) in the concentration and motility of spermatozoa in the group (Pb) compared to the control group (C) and the positive control groups (F1, F2).However, administration of water extract from Opuntia ficus indica flowers in all groups combined was significantly higher than in lead-poisoned rats.

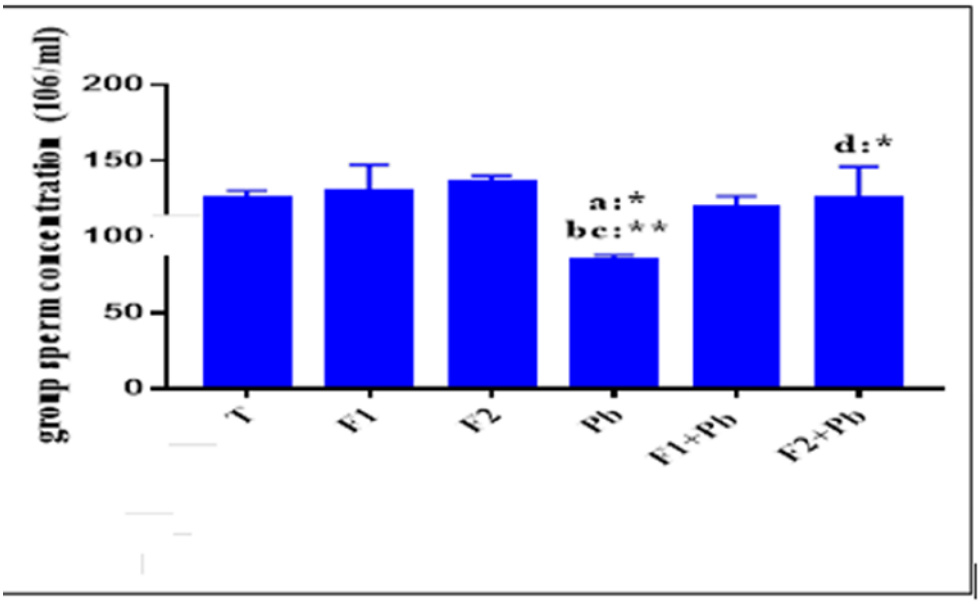

Figure 2: the concentration of spermatozoa in rats exposed to lead and combined with the aqueous extract of Opuntia ficus indica flowers for 4 weeks of treatment.

Sperm Velocity

He results show that the fast, medium and slow sperm and velocity parameters. The sperm velocity and velocity parameters (VCL, VSL, VAP and BCF) of the Pb group sperm were significantly lower than those of the control groups. The velocities and VCL, VSL, VAP and BCF of the sperm of the combined groups were significantly higher than those of the (Pb) group especially of the combined (F2 + Pb) group and were not statistically different from the control group (Figure).

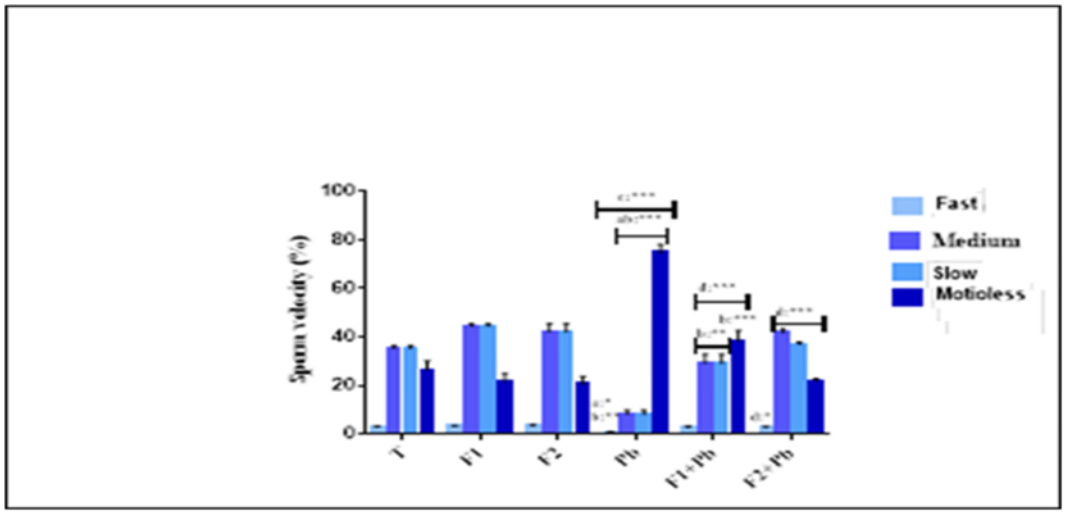

Figure 3: Sperm velocity (fast, medium, slow and motionless) of rats exposed to lead and combined with the aqueous extract of Opuntia ficus indica flowers for 4 weeks of treatment.

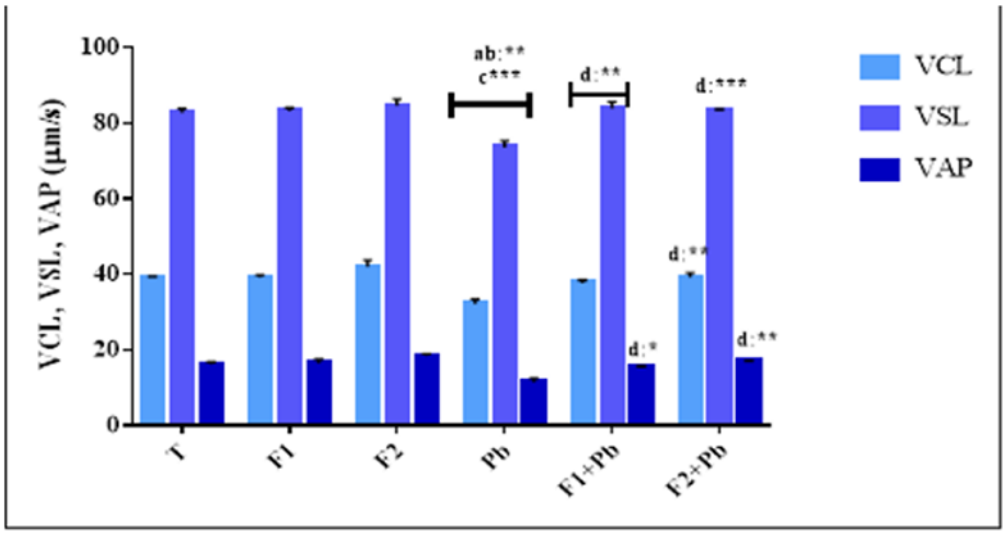

Figure 4: VCL (curvilinear velocity), VSL (straight-line velocity), VAP (velocity average path), levels of rats exposed to lead and combined with the aqueous extract of Opuntia ficus indica flowers for 4 weeks of treatment.

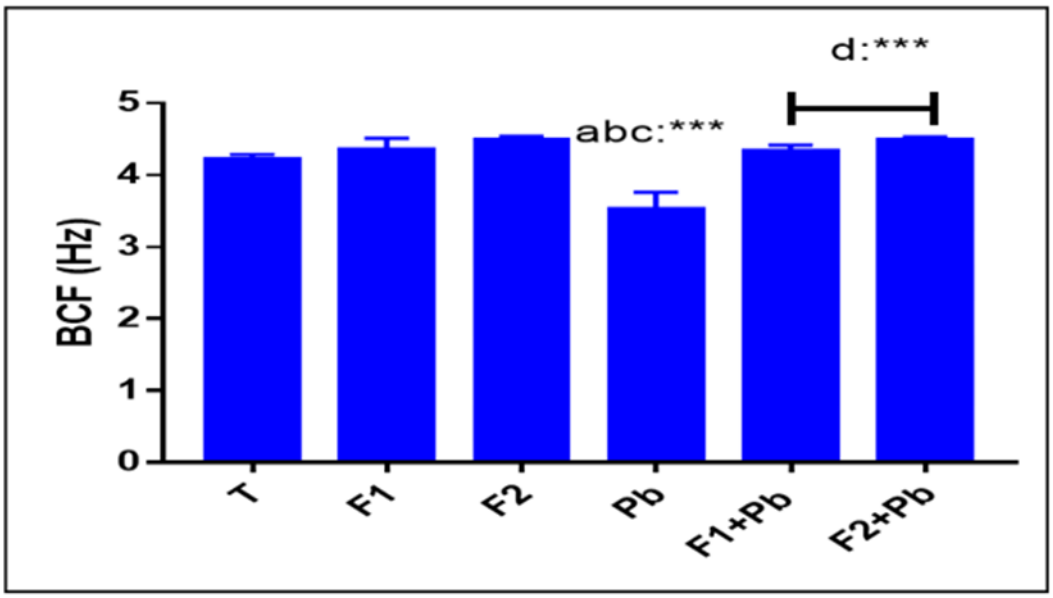

Figure 5: Trajectory crossover frequency (BCF) of rats exposed to lead and combined with the aqueous extract of Opuntia ficus indica flowers for 4 weeks of treatment.

Sperm vitality

the results indicate that the percentage of dead sperm in the (Pb) group was significantly higher compared to living sperm in the control group (C) and the positive control groups (F1,F2). However, the results reveal that the treatment of rats with the aqueous extract of the flowers of Opuntia ficus indica in the combined groups (F1+Pb), (F2+Pb) succeeded in preventing lead-induced alterations, which we observed a significant increase in the percentage of living spermatozoa and the p dead spermatozoa was remarkably low, especially in the group (F2+Pb).

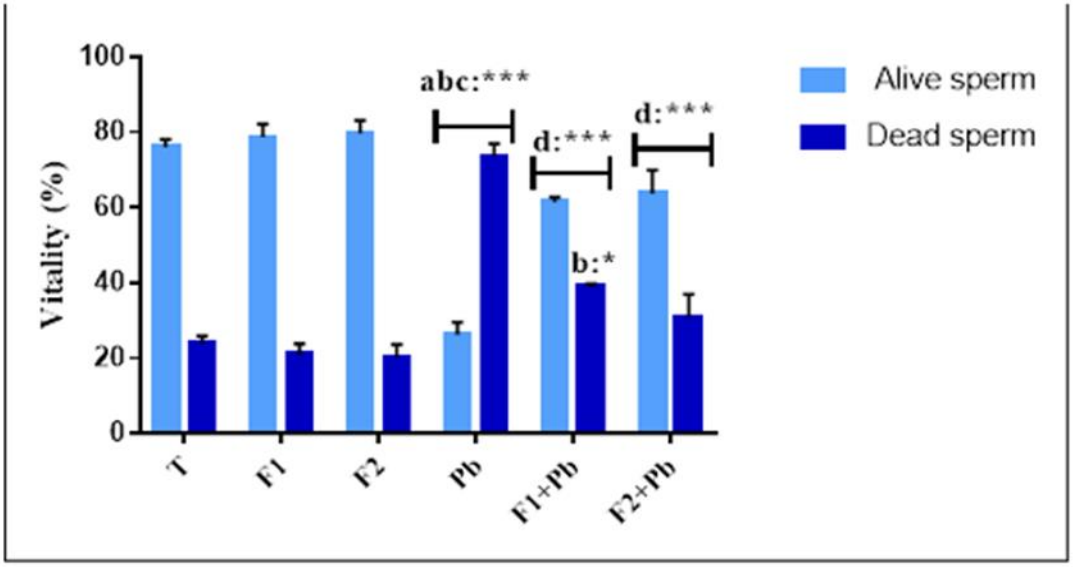

Figure 6: Percentage of live and dead spermatozoa (%) of rats exposed to lead and combined with the aqueous extract of Opuntia ficus indica flowers for 4 weeks of treatment

Testosterone levels

The results are shown in the figure ().indicate a significant decrease in the level of testosterone in the group (Pb) compared to the control group (T) and the positive control groups (F1, F2). On the other hand the supplementation of the aqueous extract of the flowers (OFI) in the groups of the combination (F1+Pb), (F2+Pb) improved the results, especially in the group (F2+Pb).

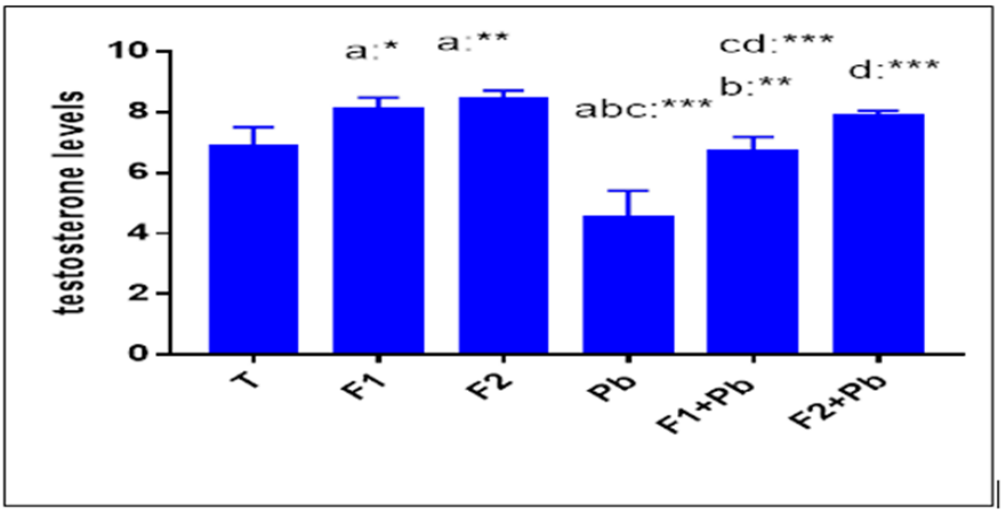

Figure 7: Testosterone levels of rats exposed to lead and combined with the aqueous extract of Opuntia ficus indica flowers for 4 weeks of treatment.

DNA Fragmentation

The results show a significant increase in DNA fragmentation in group (Pb) by contribution to control group (C) and groups (F1, F2).at the same time, a significant decrease in DNA fragmentation in the combined groups (F1+Pb) and especially in the group (F2+Pb).while F1 had levels close to those of the control group. while F2 was better than group (C) and positive control groups

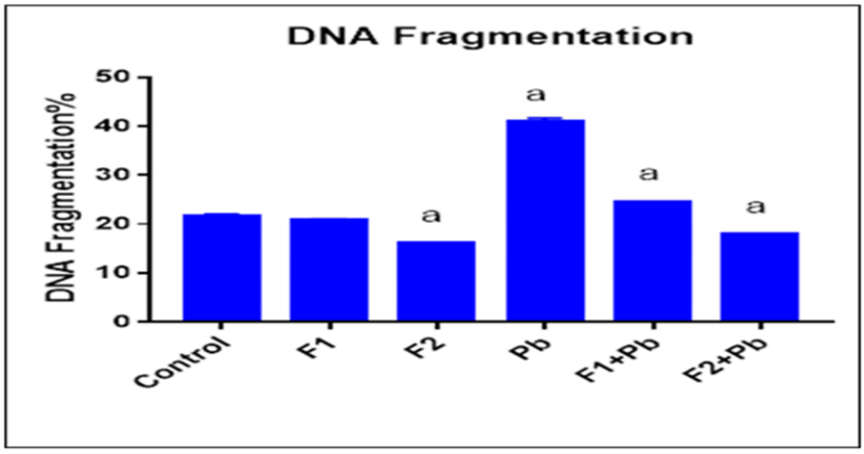

Figure 8: Percentage of DNA fragmentation (%) of rats exposed to lead and combined with the aqueous extract of Opuntia ficus indica flowers for 4 weeks of treatment.

Malondialdehyde Level

The results obtained in our experiment show that gavage of lead acetate for 4 weeks caused a significant increase in the concentration of MDA, in the testes and epididymis compared to the control group (C) and the positive control groups (F1, F2).. However, treatment of rats with OFI flower suspension in the combined (F1+Pb) and (F2+Pb) groups successfully prevented the deleterious effect of lead acetate. Exhibited a significant decrease in MDA concentration compared with (pb)-treated group.

Glutathione Concentration

The enzymatic activity of GSH in the testes and epididymis of rats was significantly reduced in the lead-treated group compared to the control group (C) and the positive control groups (F1, F2).However, the activity of GSH in the combined treatments (F1+Pb , F2+Pb) was significantly higher than in the (Pb) group .

Glutathione Peroxidase Activity

We observed that testicular and epididymal glutathione peroxidase activity was significantly reduced in rats exposed to lead acetate compared to control group (C) and positive control groups (F1, F2).However, the treatment of rats with Opuntia ficus indica flower suspension in the combined groups (F1+Pb) and (F2+Pb) successfully prevented the toxic effect of lead acetate. Showed a significant increase, especially in the (F2 + Pb) groups.

Groups	umol GSH/mg proteins GSH	Umol Gpx/mg proteins GPX	nmol/100 mg tissues MDA
C Testis	49,06 ± 1,592	41,43 ± 0,7	5,386 ± 0,223
Epididymis	53,06 ± 0,951	7435,2 ± 0,451	4,977 ± 0,015
F1 Testis	53,2 ± 0,730^{a} : *	43,88 ± 0,731	5,177 ± 0,181
Epididymis	56,35 ± 0,275^{a} : *	35,9 ± 0,511	4,898 ± 0,023
F2 Testis	59,93±0,456^{a} :*** ;^{b} :*	41,43 ± 0,774^{a} :**	4,926 ± 0,041
Epididymis	57,95 ± 0,463^{a} : ***	42,28 ± 0,746^{ab} :**	4,833 ± 0,019
Pb Testis	33,2 ± 1,691^{abc} :***	43,88 ± 0,731^{abc} :***	10,78 ± 0,063^{abc} :***
Epididymis	34,32 ± 0,554abc :***	17,53 ± 0,807^{abc} :***	

			10,28 ± 0,032^{abc} :***
F1+Pb Testis Epididymis	48,31±2,329^{b}:**;^{cd}:*** 52,17 ± 1,038^{b}:**;^{cd}***:	38,89±0,874^{b} :** ;^{cd} :*** 29,9 ± 0,809^{abd} :***	6,162 ± 0,069^{a}:* ^{b}:** ;^{cd} :*** 5,634 ± 0,23^{ab} :** ;cd :***
F2+Pb Testis Epididymis	55,32 ± 1,503^{a} :*** ;^{c} :* ;^{d} :*** 56,01 ± 0,762d :*** ;^{c} :*	41,55 ± 0,493^{c} :* ;^{d} :*** 29,9 ± 0,809^{abd} :***	5,442 ± 0,209^{d}:***;^{e*} 5,136±0,145^{d} :*** ;^{e}:*

Table 5: Testicular and epididymal GSH concentration, GPx activity, and MDA level (mean±SEM) of Wistar rats exposed to lead acetate and co administrated with the aqueous extract of Opuntia ficus indica flowers for 4 weeks of treatment.

Histological study

The estimated histological examination of the testis of different groups of rats after four (4) weeks of treatment is mentioned in the figures. The histological study of the testicle of the control group, showed several seminiferous tubes, each tube consists of a central lumen bordered by a seminiferous epithelium containing germ cells, the testicles of the positive control (F1, F2) present preserved histological aspects like healthy tissue (C). Rats exposed to lead acetate (50 mg/kg) by gavage, showed some changes which are noted according to their severity, we noticed dystrophic parenchyma, widening of interstitial spaces. The lumens of the seminiferous tubules are empty or few spermatozoa, the cell layers in the seminiferous tubules are scarce thus vascular congestion. On the other hand, the rats treated with the (F1+Pb) and (F2+Pb) combinations demonstrated a normal structural organization of the testicles in the two combination groups (F1+Pb, F2+Pb), a well-defined epithelium. The protective effect of aqueous extract of OFI flowers revealed recovery of spermatogenesis, seminiferous tubules become more normal, and seminiferous lumen is enriched with spermatozoa. The epididymis is a very coiled canal where the maturation of the spermatozoa takes place (acquisition of their forward mobility essential to their fertilization functions).The epididymis of the control group shows a well-structured tissue architecture, the central lumen presents a density of spermatozoa, same observation in the histological sections of the positive control groups (F1 and F2).Exposure to lead acetate caused alterations, we observed architectural disorganization (photo No. 5), fibrosis of the wall of the epididymal tubes, with narrowing of light and absence of germ cells compared to the group control and positive control

groups (F1, F2). However, co-administration of aqueous extracts of OFI flowers in groups (F1+Pb and F2+Pb) at different doses improved the results, which was revealed by well-defined tissue architectures, normal cell distribution, increased density.sperm in the epididymal lumen.

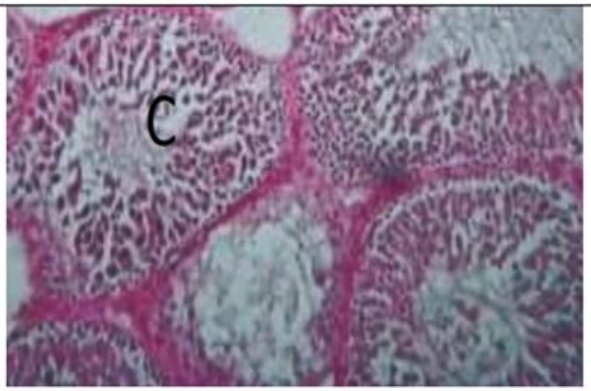

Photo 1: control (C)

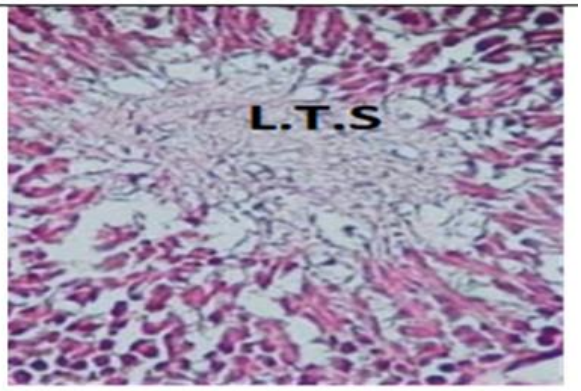

Photo 2 : Control (séminifères tube)

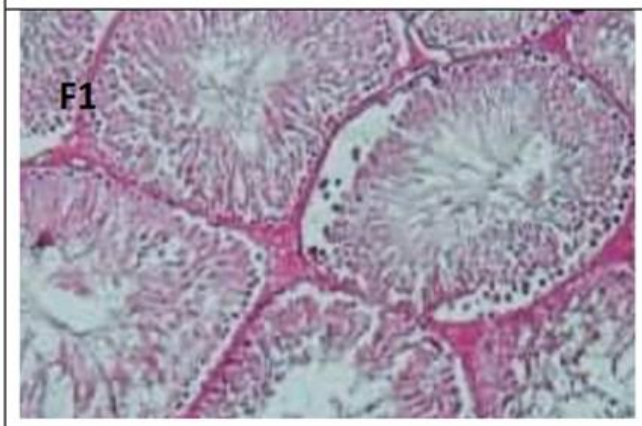

Photo 3 : Positive control (F1)

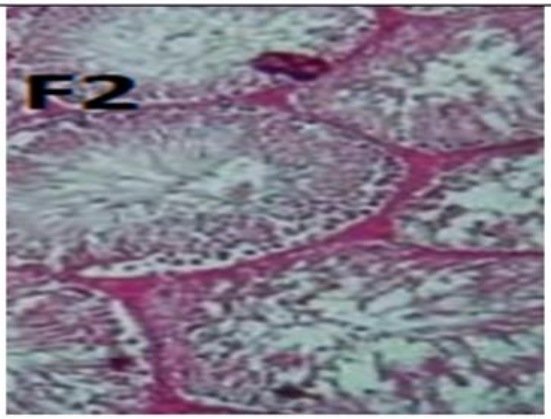

Photo 4:Positive control (F2)

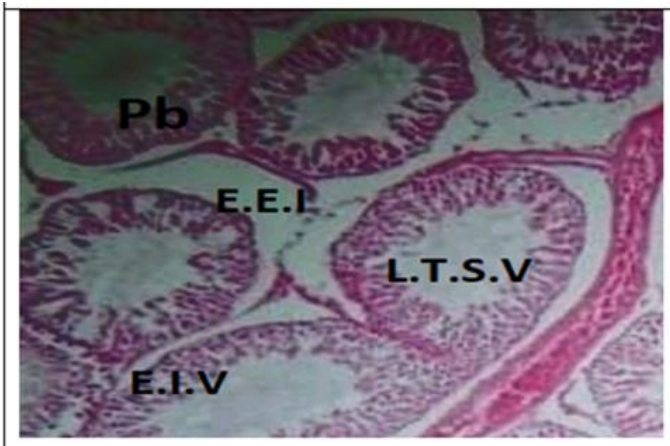

Photo 5: the toxic effect of lead acetate

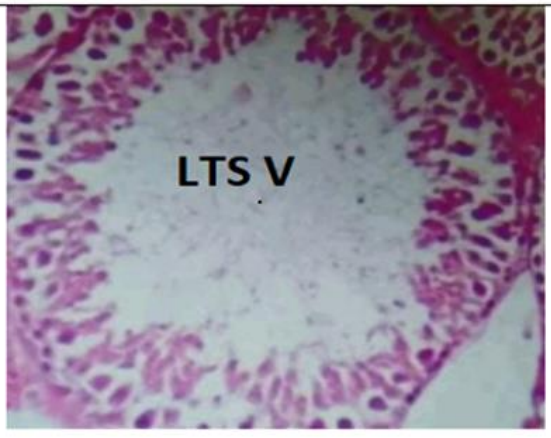

Photo 6: the toxic effect of lead acetate)

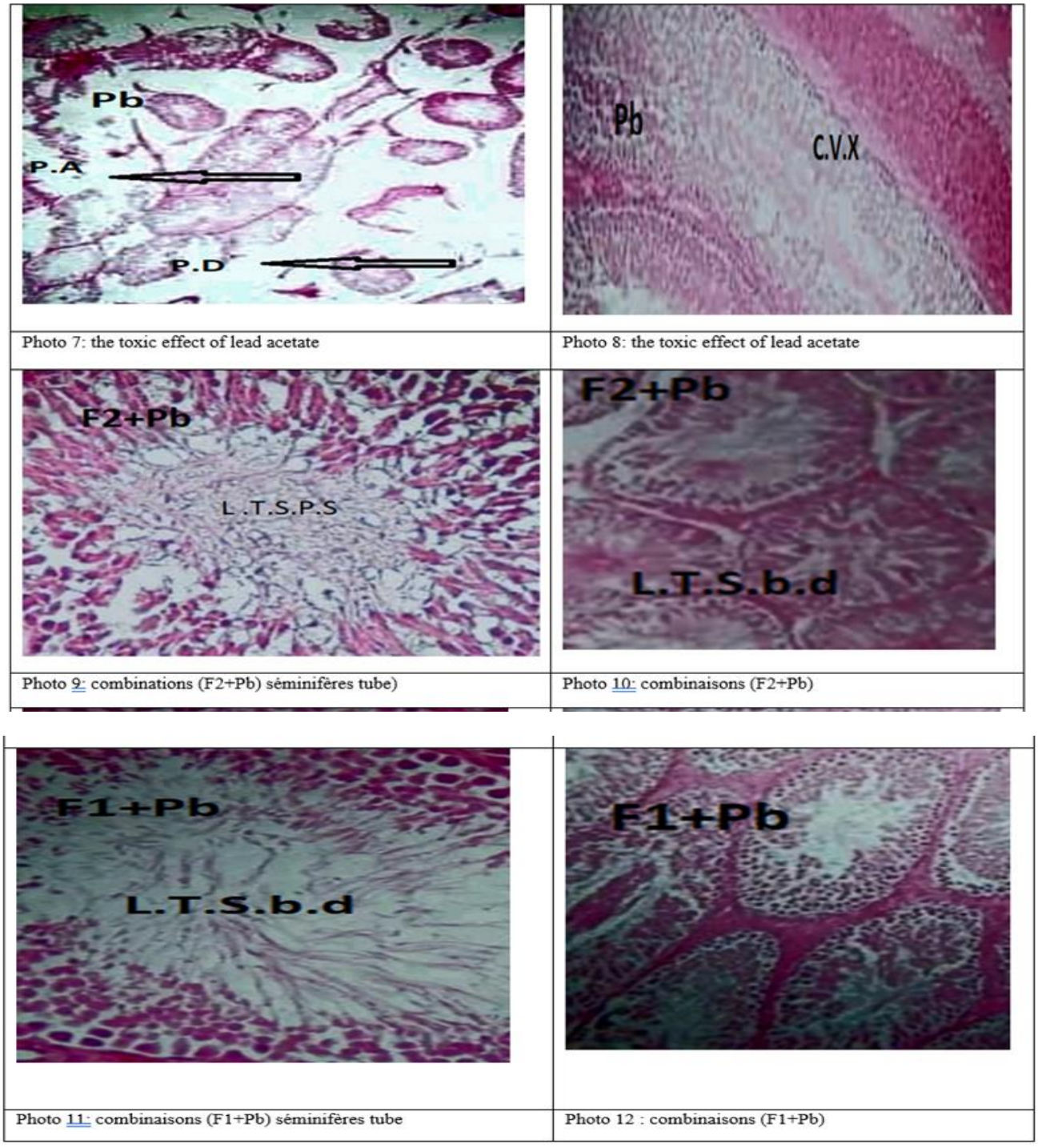

Photo 7: the toxic effect of lead acetate

Photo 8: the toxic effect of lead acetate

Photo 9: combinations (F2+Pb) séminifères tube)

Photo 10: combinaisons (F2+Pb)

Photo 11: combinaisons (F1+Pb) séminifères tube

Photo 12 : combinaisons (F1+Pb)

Figure 9: Characteristic of the testicles of rats exposed to lead acetate and co-administered with aqueous extract of OFI flowers for 4 weeks of treatment.(G×40).

The control testicle (C) composed of well-defined seminiferous tubules (Photo N°01). The seminiferous tubule consisting of a central lumen bordered by a seminiferous epithelium (Photo N°2).Those of the positive controls (F1, F2) have a histological appearance like healthy tissue(Photos N°03 and N°04).The testicles treated with 50mg/kg of lead acetate present disorganized seminiferous tubules, empty interstitial space (L.I.V), lumen of the seminiferous tubules are empty (LTS.V) and enlargement of the interstitial spaces (EEI) (photo N°5, 6, 7,8).The

testicle reveals an atrophic parenchyma (AP) and dystrophic parenchyma (PD) (PhotoN°9). Presence of vessel congestion (CVx).

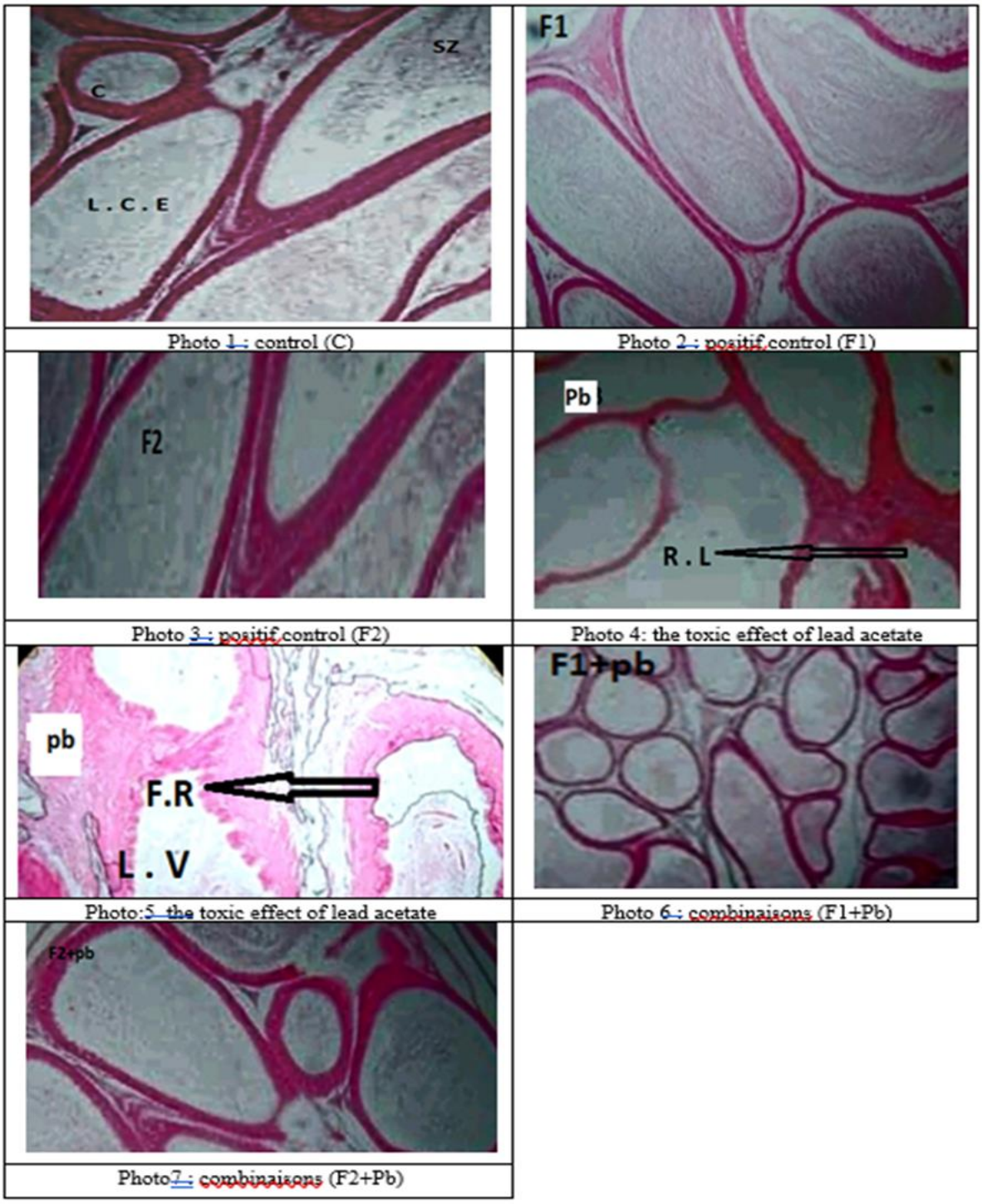

Figure 10:Characteristic of the epididymis of rats exposed to lead acetate and co-administered with aqueous extract of OFI flowers for 4 weeks of treatment.(G×40).

(C, control; PB, lead acetates; F1, positive control (aqueous extract of Opuntia ficus indica flowers at 250 mg/kg dose); F2; positive control (aqueous extract of Opuntia ficus indica flowers at

500mg/kg dose); F1+b combination of lead and (aqueous extract of the flowers of Opuntia ficus indica at 250 mg/kg); F2+Pb; combination between lead and (aqueous extract of the flowers of Opuntia ficus indica at 500 mg/kg dose). L.C. E; light from efferent ducts; R. L; Narrowing of light; F. R; wall fibrosis; L. V; empty light; S Z; sperm.

Discussion

Fertility physiology in mammals is very sensitive to disturbances in the body by exogenous agents. Numerous studies indicate a decrease in the number and quality of male sex cells in recent years induced by several pollutants (Jegou, 1996, debouche et al., 2018). Heavy metals that pose serious ecological problems, because of their presence in the biosphere, and have the ability to accumulate in the tissues of living organisms (Drif et al., 2019). Lead is a cumulative toxic element that causes changes in rat sperm quality and is an inducer of oxidative stress (WHO, 2017). Disrupts spermato genesis (Anomaa et al., 2017; Wani et al., 2015). Lead acetate induces in male rats functional and morphological abnormalities on spermatozoa, disrupts spermatogenesis, steroidogenesis, acrosome function, neuroendocrine system, this causes male infertility (Anomaa et al., 2017; Wani et al., 2015; INRS, 2018). As well as the number, motility of sperm and increased fragmentation of DNA (Akcha et al., 2020; Li et al., 2018; Wani et al., 2015). Perhaps explained by tissue atrophy induced by inhibition of spermatogenesis, so could be related to the harmful effects of lead on the hypothalamo-pituitary-testicular axis (Abdel-Emam, 2021). Other studies also reported that ingestion of lead acetate reduced the diameter or thickness of seminiferous tubes, a neuroendocrine disruption that leads to testicular atrophy (Didolkar et al., 1988; Chanel et al., 2017). On the other hand, a mitigating effect is observed on body weight and sexual organs, in groups treated with the combination of aqueous extract of OFI and lead especially in the lot (F2+Pb). Our results are consistent with studies by Ncibi et al., 2008; Leo et al., 2010; Gouthamchandra et al., 2010. Explained this protection by the antioxidant capacity of the OFI flower extract, these bioactive components act as traps and lipid peroxidation inhibitors of ROS. According to phytochemical screening, the aqueous extracts of OFI flowers present its richness in phenolic compound. In the present study we observed a positive correlation between testicular weight, epididymis and testosterone concentration in the experimental groups. Our results are consistent with studies by Mokhtari et al., 2011 confirmed that the experimental groups that received 50 and 100 mg/kg of lead acetate showed a significant decrease in testosterone levels. Other recent studies have shown that the toxicity of lead in rats treated with 50 mg/kg lead acetate results in reductions in serum FSH, LH, testosterone, body weight and testis, as well as 17β-Steroid hydroxy dehydrogenase was significantly reduced (Abdel-Emam, 2021). Other studies have shown that the decrease in testosterone concentration may be mainly due to the toxic effect of lead on Leydig cells (Anomaa et al., 2017). So, the alteration of spermatogenesis during lead exposure can be attributed to hormonal imbalance at the hypothalamic-pituitary axis, this translates into the reduction of follicular-stimulating hormone -luteinizing (FSH), the gonadotropin-liberating hormone (Gn, and testosterone concentration (Doumouchtsis et al., 2009, Rezaei et al., 2018; Ahmed and Abdel-Emam, 2019). In contrast, the combination of flower extract (OFI) with lead significantly improved serum testosterone levels. According to (Sakly et al., 2014). Showed that the administration of OFI flower extract (250 and 500 mg/ kg) in rats against nickel-induced toxicity in the testes of rats resulted in a significant recovery of testosterone, follicle-stimulating hormone

(FSH) levels and luteinizing hormone (LH). The therapeutic power of OFI flower extract is validated and could be explained by several cumulative factors, including phenolic compounds, flavonoids and polysaccharides of plant origin, that act as traps and inhibitors of lipid peroxidation of ROS via hydrogen (Leo et al., 2010; Gouthamchandra et al., 2010). In the present study the administration of lead acetate to rats showed a clear toxic alteration in the parameters of sperm biology, the results of which indicate that the concentration, vitality and different types of sperm velocity significantly reduced after oral gavage of lead acetate and velocity parameters (VCL, VSL, VAP, BFC) were decreased. Analysis of sperm mobility informs us about their essential kinetic qualities: speed, vitality and progressivity in a straight line. These factors are essential for fertilization of the oocyte. Our results are in perfect synchronization with those of Pizent et al., 2012; Abdel-Emam, 2021. Numerous studies have reported that lead exposure disrupts the parameters of sperm biology leading to male infertility (Kumar, 2018; Martin et al., 2017; Wahab et al., 2019). According to Naha et al., 2005, Moreover, Li et al., 2018; Wani et al., 2015 demonstrated that high concentrations of lead in drinking water cause decreased sperm motility, density, and viability. Also, they lead to significant morphological abnormalities, and alteration of endocrine function of reproductive organs (Chowdhury, 2009). Lead-induced spermato genesis (Abdel-Emam, 2021). Metal ions induce Sperm concentration is reduced in our study can be attributed to dysfunction apoptosis of Leydig cells so the decrease of all sperm cell populations, in particular, the sperm and sperm forms (Shubina et al., 2016;Corpas et al., 2002). The deleterious effects of lead on different types of sperm velocity can be attributed to changes in the intermediate part, particularly in mitochondria, axoneme and flagella function (Castellini et al., 2009). By also inducing phosphorylation of axonem proteins or by reducing the production of ATP in sperm cells that are necessary for the wavy movement of sperm (Aitken and Baker, 2006). In addition, the decrease in sperm concentration and viability in our experiment could be reasonably assumed also on lead-induced oxidative stress. Which, it was noted that lead causes excessive production of ROS could also be related to mitochondrial dysfunction leading to lipid peroxidation whose consequences, decreased sperm motility, concentrations, viability, increased DNA fragmentation and apoptosis (Sainath et al., 2011;Chowdhury, 2009). Lead also affects sperm membrane morphology (Shubina et al., 2016). The exploration of male infertility in first line is based on the analysis of the spermogram and spermocytogram, these two examinations give an overall idea of the fertilizing potential of the spermatozoon, but does not inform us on the quality of sperm DNA. Lead acetate in our research caused increased fragmentation of DNA. DNA damage causes disruption of cell functions including somatic and germ cells (Akcha et al., 2020). These results are certainly explained as reported by several studies, by the deleterious effect of ROS when present in high concentrations, which induces peroxidation of the lipids of the spermatic membrane and leads to the deterioration of the axonal structures of spermatozoa, thereby reducing their mobility (asthenospermia (Saleh et al., 2002). On the other hand; by the Caspases, mediators of apoptosis that are found in high concentration in the sperms of individuals characterized by asthenospermia (Hamamah et al., 2000). On the other hand, oral administration of the aqueous extract of Opuntia ficus indica with lead in the groups (F1+Pb, F2+Pb) attenuated the biological parameters of sperm against lead toxicity whose results, leading to an improvement in fertility parameters (concentration, viability, velocity, kinetic parameters (VCL, VSL, VAP, BFC) were significantly increased compared to the toxic group (Pb) with no significant change in comparison with the control group, plus decreased DNA fragmentation. Indeed, the antioxidant property of the

aqueous extract of OFI improved the biological parameters of sperm in combination groups (F1+Pb, F2+Pb). Its results are supported by (Sakly et al., 2014). Also showed that the addition of OFI flowers improved sperm parameters, testosterone levels, sperm concentration and mobility in sheep. Studies by (Allai et al., 2016) have reported that prickly pear flowers have beneficial and mitigating effects against DNA fragmentation in men's sperm. They observed a significant reduction in sperm DNA fragmentation as well as an improvement in sperm quality, mobility and viability. Indeed, our phytochemical results confirm that the methanolic extract of OFI flowers exerts an exceptional antioxidant and anti-inflammatory function. Lead is an exogenous poison that can induce ROS production, disrupt and inhibit the activity of free radical scavenging enzymes, improve lipid peroxidation and cause tissue damage (WHO, 2017). In our experiment sub chronic exposure of rats to lead acetate for 4 weeks resulted in a significant increase in MDA thus significantly reducing the level of GSH and Gpx enzyme activity in the testes and epididymis. Several literatures shows that lead induces a significant decrease in the activities of antioxidant enzymes GSH, GPX and CAT in animals, and a significant increase in the level of expression of Malondialdehyde (MDA) (Chen et al., 2019, Han et al., 2017). Our results are consistent with (Piasecka et al., 1997; Gavrić et al., 2017). Explained that lead has a high affinity for sulfhydryl groups and direct interaction with biological membranes inducing lipid peroxidation, and decreased free radical trapping enzymes and glutathione. Lead ions also lead to lipid peroxidation of the sperm membrane due to its richness in polyunsaturated fatty acids that could cause serious damage to the membrane, this can change the structure of the sperm and the integrity of the membrane, on the other hand changes in the DNA (Aitken et al., 1987; Chen et al., 2004; Hou et al., 2018). A recent study has shown that oxidative stress plays an important role in Pb-induced cell damage that not only influences the antioxidant defense system, but also leads to tissue damage (Ademuyiwa et al., 2009). Our histological study confirms tissue damage induced by lead. Nrf2 is an essential regulator between the cellular antioxidant response and the xenobiotic metabolism of heavy metals that are generally multifactorial, with ROS being the main contributor to heavy metal toxicity (Rehman et al., 2018). However the aqueous extract of OFI managed to protect the organs to study against oxidative stress induced by lead in groups (F1+Pb, F2+Pb) and bring them back to normal levels. We noted the decrease in the concentration of MDA as well as the increase in the concentration of GSH, and the activity of Gpx in the testicles and epididymis. These results are consistent with other studies that confirm that the administration of the aqueous extract of Opuntia ficus indica flowers to protect organs from oxidative stress induced by several xenobiotics (Hamed et al., 2014; Chougui et al., 2013; Ammar et al., 2015). Showed that OFI flower extract had antioxidant effect because of these bioactive compounds. According to Sakly et al., 2014; Allai et al., 2016 in turn demonstrated that they managed to overcome oxidative damage induced by nickel at the testicular level and It was sported that OFI flowers managed to prevent lead-induced stress (BENKAHOUL, 2018). lead leads to depletion of glutathione and proteins, leading to increased production of reactive oxygen species such as peroxide ion, hydroxyl radical and H O ROS increase lipid peroxidation by oxidation of polyunsaturated fatty acids of membrane phospholipids. These alterations modify the fluidity of membranes (Liu et al., 2012; Laamech et al., 2017). In testicles and epididymis our results agree with (Piasecka et al., 1997; Gavrić et al., 2017). Nrf2 is an essential regulator between the cellular antioxidant response and the xenobiotic metabolism of heavy metals that are generally multifactorial, with ROS being the main contributor to heavy metal toxicity (Rehman et al., 2018). Our histological study confirms tissue damage

induced by lead. However, the aqueous extract of OFI flowers managed to protect the organs from oxidative stress induced by lead in the groups (F1+Pb, F2+Pb) and bring them back to normal levels. We noticed the decrease in the concentration of MDA as well as the increase in the concentration of GSH, and the activity of Gpx in the testicles and epididymis. These results are consistent with other studies that confirm that the administration of aqueous OFI flower extract to protect organs from oxidative stress induced by several xenobiotics (Hamed et al., 2014; Chougui et al., 2013; Ammar et al., 2015) showed that OFI flower extract had antioxidant effect because of these bioactive compounds. According to Sakly et al., 2014; Allai et al., 2016 in turn demonstrated that they managed to overcome oxidative nickel-induced damage to the testicle. It has been planted that OFI flowers have successfully prevented oxidative stress induced by lead (Chahdoura et al., 2014; Rabhi et al., 2015; BENKAHOUL, 2018).

In conclusion, lead is a toxic element disrupts sperm quality parameters, induces oxidative stress, affecting testicular MDA, GSH and Gpx. Co-administration of aqueous extracts of Opuntia ficus indica with lead successfully maintained the levels of oxidative stress markers near their normal physiological values and improved sperm quality. OFI flower extract has an exceptional antioxidant effect by reducing lead toxicity.

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