



Protective effect of *Yucca Schidigera* extract against lead induced-toxicity in New Zealand male rabbits

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ABSTRACT

The present research was aimed to detect the deleterious effects of lead on the biochemical, histopathological, and reproductive performances of Newzealand rabbit and *Yucca schidigera* extract role in protecting the tox impacts. sixty mature male Newzealand rabbits were divided into four groups, each one subdivided into 3 replicates. The treatments as follows: group 1 control basal diet, group 2 basal diet + 150 mg Pb/kg bwt, group3 basal diet +yucca (100 mg/kg diet), and group 4 basal diet + Pb (150 mg Pb /kg diet) + yucca (100 mg/kg diet). lead exposure group showed decreasing sperm concentration, and motility in comparison with control, and adding yucca extract can improve reproductive performance concerning control. Aspartate aminotransferase, alanine aminotransferase, creatinine, and uric acid were significantly elevated in Pb while, yucca treated groups and co-exposure to yucca with Pb were significantly decreased than the lead alone group. Pb-exposed group significantly decreased total antioxidant capacity, superoxide dismutase activities and reduced glutathione content, and significant elevation in malondialdehyde and nitric oxide. Co-exposure to yucca with lead elevate the antioxidant parameter and reduced MDA and NO than lead alone. Co-exposure to yucca+ Pb reduced the level of Pb residues in hepatorenal tissue than the lead-exposed group. The results of adding yucca exhibited have a protective effect against the lead-induced inhibitory effects on biochemical and reproductive performances of rabbit and yucca have improved the toxic impacts induced by lead in rabbit

Keywords: Rabbit, *Yucca*, residue, HPLC, histopathology.

1. Introduction

Lead is among the most ubiquitous pollutants especially in the industrials area (Klaminder et al., 2006). Most heavy metals were found around the factories in the air or in its wastes which include the batteries, painting substances, printing inks, or the wastes which were generated from petrol in most of the countries. Lead can also be one of the components of corrosive gas or liquid tank linings, piping, and equipment, as well as in magnetic resonance in imagining and nuclear medicine(Farag et al., 2018). Lead intoxication has been known to generate ROS and oxidative damage induction as the primary mechanisms of its toxic effect resulted from damage of the cell structure, peroxidation of lipid, and DNA and protein damage (Kasperczyk et al., 2012). Due to the obvious general toxic impacts induced by oxidative damage due to Pb exposure, they still explore the beneficial effect of some of medicinal plants and herbs with antioxidant properties and its ability in chelation of heavy metals which may be useful in amelioration the toxic impact that induced (Patrick, 2006). Multiple heavy metal ions include (Pb, Hg, As, Cd, Cu, Lithium, Nicle, Chromium)) have a variety of negative effects on reproductive performance in males and females that may lead to subfertility or infertility, abortions, congenital performance (Apostoli and Catalani, 2011).

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Through using different animals models it was shown that the lead has a primary effect on the hypothalamus-pituitary gland toxic effect which mainly affect the on testicles and acts on the reproductive organs (Sokol et al., 2002). Lead-induced reproductive dysfunction as distinct morphological and biochemical changes, including low sperm quality and abnormal shape and also low testosterone and androgen level in serum in male rabbits (El-Nattat et al., 2000) and also another studies have been explained how was lead exposure reduced the semen quality after exposure to it (Telişman et al., 2007).

Yucca schidigera is one of the members of "Agavaceae,". It seems to be widely a medicinal plant with100% natural content(Balázi et al., 2013). *Yucca* has a lot of beneficial properties as anti-inflammatory, anticarcinogenic, antioxidant, immunostimulant, hypoglycemic growth promoter, and hypocholesterolemia. (Alagawany et al., 2016). It is acts as a source for many active principles as antioxidants resveratrol, saponins, and different enzymes (Alagawany et al., 2016; Farag et al., 2017). The steroid saponins of yucca extract were approved by GRAS (Generally Recognized as Safe) given by FDA (Food and drug administration) for dietary human consumption (Tenon et al., 2017). The adding of yucca extract to feed may lead to enhancement in the activity of antioxidants through reducing the induction of oxidative stress generation and lipid peroxidation in mice (Ince et al., 2013). Also, (Alagawany et al., 2016) recorded that, yucca may lead to improve SOD activity and GSH level, and MDA in serum in laying hens was reduced. Glutathione peroxidase and catalase activities were elevated in case of consumption in rabbit rations (Ashour et al., 2014). Therefore, the primary object of this research study was to explore the toxic effects of lead on growth and reproductive performance, serum biochemical parameters, and oxidative stress parameters, and also the residual concentrations of lead in the liver and kidney were measured. So that, the role of *Yucca schidigera* extract was become very clear in ameliorating the toxic impacts induced by lead.

2. Materials and Methods

2.1. Animals, experiment design, and treatments

A total number of 60 mature Newzealand male rabbits at 3.5month of age with an initial body weight of 1700 ± 200 g were examined clinically to be in good health and free of external and internal parasites. They were put in the same management and hygienic conditions in galvanized wire cages good ventilated houses automatically. All rabbits were completely divided into the randomized design for 4 treatments. Rabbits were fed a pelleted diet and freshwater adlibitum during the whole experimental period. Each group was subdivided into 3replicates with 15 rabbits. Rabbits were fed the basal diet with or without supplemental Pb or yucca that was formulated to meet rabbit requirements according to NRC (1994). The treatments were as follows: group 1 control was consumed the basal diet, group 2 was consumed the basal diet + 150 mg Pb/kg bwt. 1/10 LD50 (Ahur et al., 2018), group3 was consumed basal diet +yucca (100 mg/kg diet)(Chrenková et al., 2012), and group 4 basal diet + Pb (150 mg/kg diet) + yucca (100 mg/kg diet) 3times per week/per os for 65d. Rabbits also were maintained on a 17 h light: 7 h dark cycle throughout the trial. The protocol of the research and procedures were approved by Animal Health Research Institute (AHRI), Tanta Branch,

Agriculture Research Centre (ARC), Giza, Egypt with approved date: (AHRI;12/2020)

2.2. Collection of blood and hepatic tissue sampling

At the end of the experiment, samples of blood, Liver, Kidney, and reproductive organs were collected from the rabbits. Blood samples were left 30 min in a test tube for coagulation then centrifuged (3000 rpm for 15 min) and put at -20°C until using for measuring the testosterone, T3, and T4 hormones, and liver and kidney enzymes. Rapidly, hepato-renal tissue was removed and washed with 0.9%NaCl saline. Then the samples were cut into two sections; one section was used for the detection of lead residue. Another section was used for histopathological examination

2.3. Tested chemicals

Lead acetate (99.6%) was taken from El-Gomhoria Chemical Co., Egypt. Yucca schidigera extract was taken from Free Trade Egypt Company (El-Behera, Egypt). And all rest of chemicals and reagent which used in this study was purchased from Sigma (St. Louis, MO) in analytical grade.

2.4. Semen collection and analysis

Semen samples from males of 15 New Zealand were collected by using the artificial vagina. The animals were killed, the epididymal content of each control and treated rabbits were collected through the cutting of the tail of the epididymis and gently squeezed it on a glass slide to estimate the sperm motility, sperm cell count, and sperm abnormalities according to the method described by (Bearden and Fuquay, 1980). Physical semen characteristics were evaluated in the freshly collected semen. Ejaculate volume (ml) and Initial mass motility of raw semen were estimated as described by (Melrose, 1970). Detection of live/dead spermatozoa percentage was performed according to (Blom, 1950). The percentage of abnormal spermatozoa was determined in a smear prepared for live/dead sperm test. Low eosin solution was used to detect sperm cell concentration (Smith and Mayer, 1955).

2.5. Determination of serum hepato-renal function

Using the commercial kits in the laboratory, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity, and total protein were detected as described by (Reitman and Frankel, 1957), and, (Dumas et al., 1981) respectively. Furthermore, creatinine, urea, and uric acid were detected by spectrophotometer as recorded by (Perakis and Wolff, 1984), (Searcy, 1967), and (McGowan et al., 1983) respectively

2.6. Oxidant/antioxidant detection in serum

Malondialdehyde (MDA) the indicator of oxidative stress and the reduced glutathione (GSH) which is considered a biomarker of antioxidant parameters, total antioxidant capacity (TAC), Superoxide dismutase (SOD), nitric oxide (No) were determined spectrophotometrically as recorded by (Ohkawa et al., 1979), (Beutler, 1963), (Kiuchi et al., 1982), (Sun et al., 1988) and (Gabor and Allon, 1994) respectively.

2.7. Determination of testosterone, T3, and T4 hormones

Blood samples for thyroid hormones (T3 & T4) and testosterone hormone determination were taken and left to clot at room temperature followed by centrifuging. The serum samples were separated and frozen until the analysis.

Determination of thyroid hormones (T3 & T4) and testosterone hormone in serum:

Testosterone concentrations in the collected serum were measured by an accurate and precise developed high-performance liquid chromatography (HPLC) method.

Chemicals and reagents: The 3,3',5'-triiodo-L-thyronine (T3) ($\geq 95\%$), L-thyroxine (T4) ($\geq 98\%$), and testosterone ($\geq 99\%$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid and ethyl acetate were purchased from Sigma-Aldrich (Madrid, Spain) and methanol from MERCK (Madrid, Spain). All solvents and reagents were used in the present study were HPLC grade. HPLC water was obtained by ultrafiltration (Millipore Milli Q system, Bedford, MA, USA).

Chromatographic conditions: The parameters were summarized in table (1) as described by Häkkinen et al. (2018)

Calibration standards and quality control (QC) samples:

Stock standard solutions (100.0 ng/mL) were prepared in methanol. This solution when kept to be stable at -180°C for 2 months (Samanidou et al., 2007). Working standards were prepared in blank serum by the appropriate dilution for each hormone at 0.02, 0.05, 0.5, 1, 2, 5, 10 ng/mL

and analyzed as described in sample analysis. Quality control samples (QS) used in method verification were 3 levels: low (0.2 ng/mL), medium (1 ng/mL), and high (10 ng/mL) according to (USP, 2019)

2.8. Determination of Lead Residues

Lead residue in hepato-renal tissues was detected from treated rabbits and control by using Atomic absorption spectrophotometer model Senss AA, Australia according to spectrophotometer according to (AOAC, 1990)

2.9. Histopathological examination

Afterward, the fresh samples from liver, kidneys, and testicles were obtained from control and lead intoxicated rabbits and the group treated with yucca extract, and the fourth treated group was immersed+ in neutral formalin solution 10% for at least one day. After the preservation process, the traditional paraffin embedding technique was used. 5 μm thick sections were taken from the prepared paraffin blocks and the sections were stained by hematoxylin and eosin according to the method recorded by (Suvarna et al., 2013)

2.10. Statistical Analysis

Data were analyzed statistically using the SaSS program with one-way ANOVA. The mean differences between treatments were detected using (Sas, 2001). Means were separated using least-square means of the same program ($P < 0.05$).

3. Result

3.1. Bodyweight and semen analysis

The influence of dietary yucca on growth performance in rabbits in the lead intoxicated group suffered a reduction in final body weight versus the control and no significant changes were detected in other treated groups. The testicles weight has no significant changes in all groups, while there is a reduction in the epididymis weight in the lead-induced toxicity group versus the control. Also, the yucca-treated group suffered from some lowering in weight concerning control, and no significant changes were detected in the yucca +Pd treated group versus to control and yucca group. There was a significant reduction was observed in sperm concentration and sperm motility in the Pb-intoxicated group, while there were no changes were detected in yucca +Pb treated group. The sperm abnormality was detected as a very significant high in the Pb-intoxicated group, while in a treated group no detect abnormalities in sperm as shown table (2).

3.2. Liver and kidney function

In the lead exposure- group, Liver ALT and AST have recorded a significant elevation concerning control. In contrast, decreasing in levels of AST and ALT were recorded in yucca treated group. ALT and AST levels in the Pb +yucca group were significantly reduced than Pb exposure alone. However, the level of ALT did not return to control value while ALT level reach control levels. In the same, kidney parameters the value of urea, creatinine and uric acid were recorded in an elevation with control. Alternatively, lower levels of urea, creatinine and uric acid were observed in yucca treated group. Urea, creatinine, and uric acid levels in Pb +yucca group were decreased significantly than Pb alone and better reduction. As the level of urea, creatinine and uric acid were return to control value versus to control as shown in table (3).

3.3. Antioxidant assay detection

The lipid peroxidation acts as evidence for MDA, and No formation was significantly elevated in the Pb exposed group compared to the control group. The level of MDA in yucca and Pb + yucca groups was significantly reduced than the lead group alone, and a better effect was observed in the Pb + yucca group. Meanwhile, the MDA level in the yucca+ Pb group did not reach to control while No level reach to control level. The Pb-intoxicated group showed significantly decreased in TAC, GSH level, and SOD activity in the serum of rabbits compared to the control. Yucca treated group has enhanced antioxidant parameters TAC, GSH, and SOD and were better enhancement for these parameters in yucca +Pb treated group. TAC and GSH levels returned to control and in SOD activity were increased than control level as a shown table (4).

3.4. Determination of lead residue

The tissue analysis for measuring the lead residue indicated that the highest level of accumulated lead was found in Pb intoxicated group in the liver and kidney tissue in comparison with control. Yucca treated group showed a significant reduction in lead residue level. Reduction of Pb residue level in Pb+ yucca treated group is better as in kidney tissue was reach to control level as a shown table (5).

3.5. Testosterone thyroid hormones using HPLC

Results of intra-lab validation of analytical methods: the analytical methods were verified and validated according to USP, (2019) and the obtained results were summarized in table (6) and figures (a,b,c,d.) showing that the used method was accurate, precise specific, and robust.

Results of testosterone and thyroid hormones:

The levels of analyzed hormones in analyzed serum were shown in figure (e), which indicates the effect of yucca and lead on testosterone and thyroid hormones levels (ng/ml) in healthy rabbits. The results indicated that there were no significant changes were detected in testosterone, T4, T3 hormones in all treated groups with each other's

3.6. Histopathological Examination

The group intoxicated with lead exhibited pathological lesions in kidneys, liver, and testis,

(Fig.1), the kidneys exhibited severely dilated blood capillaries engorged with coagulated blood (A) the interstitial blood capillaries between the renal tubules engorged with coagulated blood (B) thrombus in the blood capillaries in the renal pelvis was recorded (C) necrosis of the endothelium lining the glomerular tuft capillaries, and splitting of the glomerular tuft capillaries (D), severe necrosis of the epithelium of the renal tubules with loss of nuclei and cellular casts in the lumen (E) accumulation of a huge number of inflammatory cells interstitial nephritis was reported (F)

(Fig.2) The group treated with yucca and lead exhibited minimizing of the interstitial nephritis and inflammatory cells with a normal lumen of blood capillaries (A) the medium-sized artery appeared normal (B), the glomerular tuft capillaries were normal glomerular tuft capillaries with normal glomerular wide space (long arrow) normal wide capillary free from coagulated blood (C) and renal tubular epithelium were healthy intact epithelium. The small-sized arterioles engorged with blood (D) The group treated with yucca only exhibited medium-sized artery dilated filling with blood (E), the glomerular tuft capillaries appeared healthy, and the renal tubule normal intact epithelial lining (F). The control group yucca group exhibited renal tissues normal with normal small size arterioles (G)

(Fig.3) The liver intoxicated with lead exhibited necrotic dissociated hepatic tissues with sloughing of the abundant area of hepatic tissues (A), the portal area showing portal vein thrombosis, inflammation and necrosis of the wall of the hepatic ducts (B) with an accumulation of a huge number of inflammatory cells (C), partially thrombosed portal vein with inflammation of the wall and accumulation of inflammatory cell per the wall of the vein (periportal) and proliferation of fibroblasts (D), newly formed bile duct along the portal tract (E) thrombosis of the central vein (F).

(Fig.4) The group treated with yucca and lead exhibited regeneration of the wall of the hepatic duct and the portal vein normal filled with few hemolyzed bloods (A) normal central veins with healthy hepatic tissues (B) the group treated with yucca exhibited normal portal area with normal hepatic duct (C) The control group exhibited normal hepatocytes portal area and central veins.

(Fig.5) The testis exhibited necrosis of the spermatogenic cells, loss of spermatids, and interstitial accumulation of inflammatory cells (A) edema between testis tubule (B), and proliferation of peritubular fibroblasts of the basement membrane with severe loss of spermatocytes and Sertoli cells (C).

(Fig.6) The group treated with yucca and lead exhibited elongation of pachytene phase (stage of spermatocytes meiosis and increase chromosome chiasmata (the stage of chromosomal exchange) with a high yield of spermatids with intact spermatogenic cells and Sertoli cells (A) the yucca group exhibited the same increase in chromosomes chiasmata and high spermatids number (B), the control group showing normal spermatogenic series normal chromosomal content (C)

4. Discussion

The exploring the environmental pollutions and discover its toxic impacts on living organisms, the researchers decided to use these different levels of pollutants on various animal models under known media, specific doses, and periods with definitive biochemical parameters to detect the biological changes in the specific target organs and tissues and there are many toxic and pathological changes were induced by heavy metal (Ates et al., 2008).

The data which reported in these findings indicated that the elevating of Pb concentrations and increasing the duration of exposure may result in increasing percent of abnormality in sperm morphology and reduced sperm motility resulted from increased incidence of sperm with abnormal

morphology (Krockova et al., 2016). In addition, lead caused a significant reducing weight of epididymis, the number of spermatozoa, the motility of sperm, the level of testosterone, and a significantly elevated number of abnormal sperm (Alhassan et al., 2010). Another study which made on the paint factory workers which reported that have low sperm velocity which may be due to reducing the activity of the cell after being exposed to Pb, which was supported by high seminal plasma fructose levels. The exposure to Pb will perturb the cellular nutritional support system that is important for cellular motility as it reduces the seminal plasma total protein with a concomitant rise in free amino acid level (Naha and Manna, 2007)

The improvements in the sperm quality include (%live sperms, abnormalities, concentrations, and motility) may be attributed to the antioxidant effects of medicinal plants. Antioxidants break or prevent the lytic activity within semen fluid (Yang et al., 2006), thereby reducing lipid oxidation which leads to the deterioration of semen quality (Sekiwa et al., 2000).

Yucca is a very important source of saponins, resveratrol, different enzymes, and antioxidants molecules (Alagawany et al., 2016; Farag et al., 2017) which play a critical role in improving the semen quality in this study in (Pb+yucca) and yucca treated group.

It was known that lead poisoning causes hepatotoxicity and nephropathy (Abdel-Moneim et al., 2015). The biochemical results of this study indicated that AST, ALT, urea, creatinine, and uric acid levels were elevated in the serum of the lead-intoxication group compared to the control. The elevation of ALT and AST activities creatinine, urea and uric acid concentration may due to the toxic effect of lead (El-Tantawy, 2016). In this research, the elevation of hepatic enzyme activities is due to the tissue injury induced by lead that destructs liver cells and leads to the release of these enzymes in serum (Halliwell, 1994) or may be due to the generation of free radicals which elevate the destructive alteration of liver under the exposure to lead (Ibrahim et al., 2012).

Chronic lead intoxication resulted in renal damage with hyperuremia and hypercreatinemia (Cledes and Allain, 1992). The elevation of serum urea, uric acid, and creatinine level indicated renal damage induced by lead. Kidneys are sensitive organs was affected by the substance which has a toxic injury as they go directly to the blood plasma and reach to the kidney. In this study, there are cyto-histological alterations after being exposed to lead. Pb affects the structure and molecular architecture of GBM and plays an important role in the changes of glomerular capillary wall permeability, which lead to microalbuminuria and may be very essential neuropathology pathogenicity resulting in renal dysfunction (Sánchez et al., 2001). While the improvement which was recorded in liver and kidney parameters in the treated group (lead +yucca) and in yucca treated group due to that the yucca extract has an important role as its extract affects on the nitrogen metabolism in the body and lead to decreasing urea concentration in the blood (Piacente et al., 2005).

The oxidative status of the animals must be affected when it exposed to lead. Lead has been recorded that elevates the level of ROS generation resulted in disruption of the antioxidant system and cell damage through lipid peroxidation and nucleic acids and amino acids oxidation (Pandya et al., 2012; Winiarska-Mieczan, 2013). The inhibitory effect of Pb has led to disturbance in the antioxidant system. The cell becomes attack by ROS and ended by to oxidative damage (Liu et al., 2010). This indicates the causes of lowering in SOD and CAT activities in Pb -exposed group as recorded in this study. Also, Lead may be attached with SH -group which directly interfere with GSH and reducing its level (Pandya et al., 2012). This may be explaining the observed decrease in the level of GSH in kidney and pancreatic tissues of Pb -administered groups. Similarly, previous studies reported the altered antioxidant status in Pb -exposed animals and workers (Baranowska-Bosiacka et al., 2012; Dai et al., 2013). Additionally, Pb binds to -SH groups of various proteins, as enzymes, that lead to reducing in the activity some of them as SOD and CAT (Flora et al., 2012; Matović et al., 2015).

The elevation in the level MDA which used for evaluating the antioxidant defense system. The same results were reported by (Alagawany et al., 2015; Ashour et al., 2014) who reported that consuming some of the herbs or their extract elevate the level and the activity of antioxidant systems like SOD and GSH-Px and reduce MDA in response to decreasing ROS generation. Yucca extract is very rich in different biological substances, steroidal saponins, and polyphenolic compounds such as RES and

yuccaols A, B, C, D, and E (Alagawany et al., 2015). Flavonoids, Saponins, and polyphenolic substances have good anti-inflammatory and antioxidant effects and the pivotal role of extracts of yucca is used in diabetes, cardiovascular diseases, and cancers treatment (Amin et al., 2018; Selamoglu and Ozgen, 2016). And also, the phenolic substance and RES from extract of yucca may reduce free radical formation and LPO in the blood platelets (Olas et al., 2003). RES has a powerful antioxidant activity in overcome free radicals which generated by heavy metal intoxication as CAT, GSH-Px, SOD, GST and NADPH quinone reductase (Frag et al., 2018; Rubiolo and Vega, 2008).

Statistically, there were significant changes was detected in Lead level in hepatorenal tissue, lead is competitive with irons for binding, and the liver is a rich source of iron, so it is considered a very important reason in which why the level of Lead was elevated in the liver than the kidney. Liver act as good iron source so its consumption by human has increased over the years in many parts of the world (Siddiqui et al., 2006), consequently, may lead to bad effects on the health of the public. Using the yucca extract may decrease Pb residues due to its high antioxidant effect which acts as a chelator and this dual benefit makes (Frag et al., 2018).

The obtained results refer to there were no significant changes were detected in serum levels of testosterone, T4, and T3 hormones in all treated groups in relation to each other after its determination using highly sensitive, accurate, and precise HPLC method. These results agree with that reported by Marchlewicz (1994); (McGregor and Mason, 1990) who found that testosterone serum levels were unaffected by lead intoxication. But some studies have noted functional disorders in synthesis of testosterone as reduced testosterone levels in lead-exposed animals accompanied by decreased libido (Martynowicz et al., 2005) or even higher in a low-level lead-exposed in acutely intoxicated animals (Kempinas et al., 1990). The extract of *Yucca schidigera* plant was not affect testosterone and thyroid hormones serum levels, this agree with Kucukkurt et al. (2016) who found that there was not any difference between the total T4 and T3 in the *Y. schidigera* extract groups compared to control group.

The histopathological examination indicated coagulated blood and thrombus formation in kidneys and liver caused by lead intoxication as reported by Köklü et al. (2003) which suggested that the thrombocytes, that may activate the factors of thrombocyte aggregations, resulting in elevating the adhesive and aggregation activity in rabbits during lead exposure. The electron microscopy showed blood platelets revealing an abundant vacuolization with disordered for the distribution of the serotonin and glycogen granules. and founded that the chronic lead intoxication increased photochemically which lead to platelet aggregation in cerebral micro-vessels of mice in vivo. Barman et al. (2014) indicated that the chronic Pb intoxication of animals has elevate the levels of PLT, platelet distribution width and these changes like which reported by (Rastogi, 2008) reported that acute lead intoxication leads to renal dysfunction and it characterized by general dysfunction in tubular transportation mechanisms (Fanconi syndrome) and in morphology that showed a degenerative changes in the tubular epithelium and the nuclear inclusion bodies containing Pb protein complexes.

Chronic exposure to lead to an increase the incidence of renal destruction, which is characterized by tubulointerstitial and glomerular dysfunction, leading to hypertension, chronic renal failure, and hyperuricemia. Also, the glomerular filtration rate (GFR) for (creatinine clearance, blood urea nitrogen, serum creatinine) were affected.

The changes in liver reported were agreed by (Sipos, 2003) who cleared that, Lead leads to the generation of free radicals that lead to hepatic damage which appeared in the picture of necrotized tissue that was observed in a histopathological study. Lead intoxication the main cause of hepatic damage in higher level, while the low level induces cholecystitis, may be due to the changing of normal biliary processes. Histopathological sections confirmed that the lead caused lymphocyte infiltration and severe periportal inflammatory reaction, which may lead to cirrhosis.

The changes recorded in testis supported by Ekeh et al. (2015) who reported that that histological changes that appeared in the testicles of the intoxicated group by lead were architecture degeneration of tissue, vacuolization, necrosis, maturing spermatogenic cells in seminiferous tubules, thick basement membrane, myoepithelial cell and scanty Leydig cells which decreased the testicular sperm count. Also, lead has a toxic

effect on sperm quality and oxidative status. These findings indicate that Pb is found in testicles that inducing severe morphological and biochemical destruction that affect the reproductive performance of male birds and although scenario exposure (Leidens et al., 2018).

The antithrombotic effect of yucca was reported by (Olas et al., 2008) who showed that yucca is enriched with polyphenols that has the ability to reduce aggregation and generation of oxygen and free radicals platelet adhesion in platelets. The improvement effect of yucca on hepatorenal tissue was supported by (Cigerci et al., 2009) who approved that yucca extract has multiple antioxidant molecules like phenolics which maybe prevent the extent of lipid peroxidation. Also, these have an anti-inflammatory effect that may reduce the nuclear transcription factor NF- κ B. NF κ B which responsible for inducible nitric oxide synthase (iNOS) synthesis that is responsible for nitric oxide formation. Resveratrol substance is one of the contents of yucca that have the ability to reduce lipid peroxidation resulted in elevating its ability to scavenge the secondary reactive radicals and this mechanism, may play a good role against the formation of superoxide and hydrogen peroxide during the normal metabolic activity. Also, Reham et al. (2019) recorded the protective effect of yucca on liver and kidneys histology intoxicated with ammonia in fish. The regenerative effect of yucca on testis supported by (Balázi et al., 2013) who suggested that the addition of *Yucca schidigera* in the normal feed had good health effects on sperm parameters and quality as it was shown in the yucca group which registered sperm with high motility in comparison with the control group.

Conclusion

From the reported results of this study, we concluded that the rabbit which exposed to lead in their diets resulted in adverse impacts on the body performance parameters (biochemical and reproductive) and registered abnormal semen quality and biochemical parameters changes of liver and kidney function and the inhibitory effect on the antioxidant system. These adverse effects were accompanied by oxidative destruction which was concluded by lowering antioxidant molecules and enhanced lipid peroxidation. On the other hand, using yucca extract as a feed additive naturally in rabbit diets may lead to alleviate the deleterious effects of Pb and via improving the semen quality and enhanced liver and kidney function and antioxidant parameters with its ability to eliminate the lead from liver and kidney.

5. Disclosure statement

The authors have no conflicts of interest.

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Table 1): The optimized chromatographic parameters

Parameter													
Mobile phase	0.2 mM NH4F in water (eluent A) and 0.2 mM NH4F in methanol: water 95: 5 (v/v) (eluent B). <table border="1" style="margin-left: 20px;"> <tr> <th>Time (min.)</th> <th>Eluent B (%)</th> </tr> <tr> <td>0-3</td> <td>57</td> </tr> <tr> <td>3-7</td> <td>63</td> </tr> <tr> <td>7-12</td> <td>95</td> </tr> <tr> <td>12-14.1</td> <td>100</td> </tr> <tr> <td>14.1-16</td> <td>57</td> </tr> </table>	Time (min.)	Eluent B (%)	0-3	57	3-7	63	7-12	95	12-14.1	100	14.1-16	57
Time (min.)	Eluent B (%)												
0-3	57												
3-7	63												
7-12	95												
12-14.1	100												
14.1-16	57												
Needle wash solution	50% acetonitrile												
Flow rate	0.8 ml/min												
injection volume	50 μ l												
The column temperature	40°C												
UV-Detector	236 nm												

Table 2. Showed that, the effect of *Yucca schidigera* extract against Lead induced-toxicity in newzealand male rabbit on semen quality, testis weight, epididymis weight and final body weight for 65 days pb, yucca; Pb +lead, (treated group).

	CTR	Pb	Yucca	Pb+ Yucca
Testis weight	4.81±0.12a	4.40±0.12a b	4.23±0.09 b	4.60±0.25ab
Epiphysis weight	01.6±0.06a	1.10±0.12b c	1.30±0.12 ab	1.43±0.03b
Sperm Conc	31.87±0.85a	20.17±0.93 c	24.83±1.4 1b	31.50±1.27a
Sperm Motility	75.00±2.89a	53.33±8.82 b	65.00±2.8 9b	77.33±2.33a
Sperm Abnormality	10.67±0.67c	17.00±0.58 a	14.67±0.8 8b	12.00±0.58c
Final body weight	1216.67±16.6 7a	916.67±44. 1b	1100±100 ab	1066.67±66.67 ab

Il values were expressed as mean \pm standard errors (n=15)

All values indicated by different letters are significantly different between groups within the same rows ($p \leq 0.005$)

Table 3. Showed that, the effect of *Yucca schidigera* extract against Lead induced-toxicity in newzealand male rabbit on serum liver and kidney function parameters for 65 successive days. pb, yucca; Pb +lead, (treated group).

	CTR	Pb	Yucca	Pb+Yucca
ALT Iu/ml	34.8±0.58cb	66.8±2.08a	32.20±0.66c	37.20±1.2b
AST Iu/ml	41.6±0.93c	104.2±1.85a	41.00±0.89c	50.60±0.68b
urea mg/dl	25.8±0.80b	56.60±3.22a	26.60±0.51b	30.20±0.49b
Creatinine mg/dl	0.89±0.02bc	02.14±0.09a	00.84±0.03c	01.02±0.02b
Uric acid mg/dl	2.90±0.17b	06.42±0.36a	02.82±0.26b	03.22±0.05b

All values indicated by different letters are significantly different between groups within the same rows ($p \leq 0.005$)

Table 4. Showed that, the effect of *Yucca schidigera* extract against Lead induced-toxicity in newzealand male rabbit on serum oxidative stress/antioxidant parameters for 65 successive days. pb, yucca; Pb +lead, (treated group).

	CTR	Pb	Yucca	Pb+Yucca
TAC m.mol/ml	1091.4±41.18 b	777.2±22.2 c	1313.4±54.58 a	1065.8±19.37 b
MDA u/ml	03.38±0.20c	10.04±0.33 a	02.86±0.07c	04.28±0.19b
No u/ml1.8	01.88±0.10b	06.68±0.36 a	01.24±0.05c	02.10±0.07b
GSH mg/dl	45.40±1.75a	20.20±1.36 b	20.20±1.36b	46.20±2.58a
SOD u/ml	19.20±0.86b	06.46±0.82 d	23.20±0.97a	16.18±0.36c

All values indicated by different letters are significantly different between groups within the same rows (p<0.005)

Table 5. Showed that, the effect of *Yucca schidigera* extract against Lead induced-toxicity in newzealand male rabbit on liver and kidney lead residues for 65 successive days. pb, yucca; Pb +lead, (treated group).

	CTR	Pb	Yucca	Yucca+Pb
Pb residue/liver	0.064±0.02c	0.516±0.04a	0.054±0.01c	0.160±0.02b
Pb residue/Kidney	0.106±0.01b	1.818±0.14a	0.100±0.01b	0.232±0.03b

All values indicated by different letters are significantly different between groups within the same rows (p<0.005)

Table (6): Intra-lab validation results

Parameters	Testosterone	T3	T4	Accepted levels	
Range	0.2-10 ng/ml				
Slope	1032	636.06	364.13		
Retention time (min.)	7.88	5.3	6.7		
Intercept	- 29.748	18.627	6.9649		
Correlation coefficient (R)	0.9999	0.9995	0.9998	> 0.99	
LOD (ng/ml)	0.0013	0.003	0.27		
LOQ (ng/ml)	0.0038	0.009	0.84		
Recovery (%)	87- 93	91- 95	90- 94	85- 120	
Intra-day precision (RSD %)	0.03	0.07	0.11	≤ 1	
Inter-day precision (RSD %)	0.7	0.34	0.82	≤ 2	
Robustness (Pooled RSD %)	1.2	1.03	1.08	≤ 6	
System suitability testing (SST)	Peak area (RSD%)	0.21	0.1	0.17	< 1%
	Theoretical plate	10500± 112	7150± 84	8333± 72	> 2000
	Tailing factor	0.06± 0.005	0.08± 0.006	0.06± 0.002	≤ 2

Liquid-liquid extraction of T3, T4, and testosterone

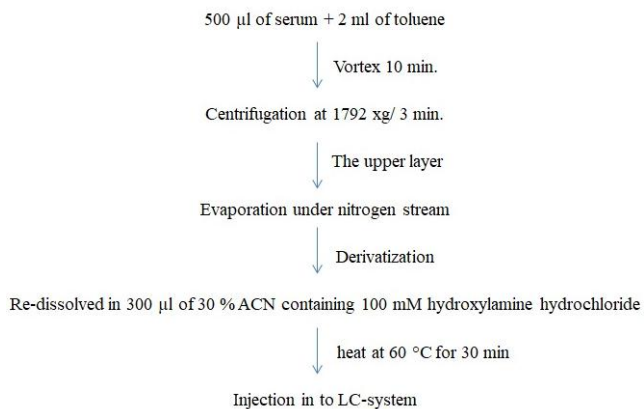


Figure (a): Standard curve of testosterone conc. versus peak area

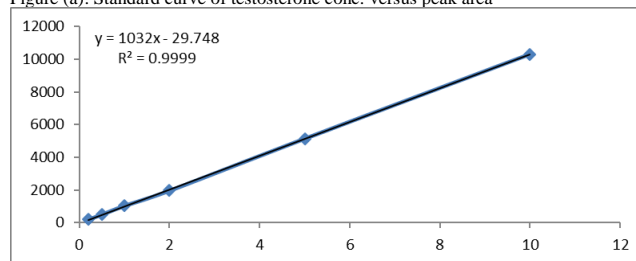


Figure (b): Standard curve of T3 conc. versus peak area

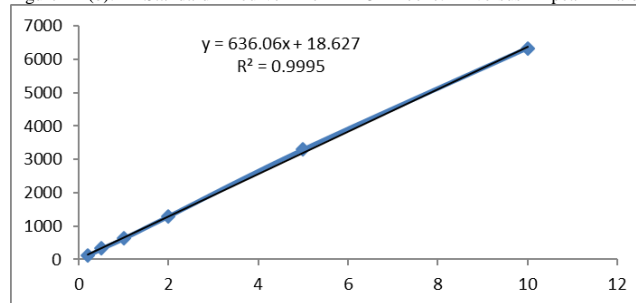


Figure (c): Standard curve of T4 conc. versus peak area

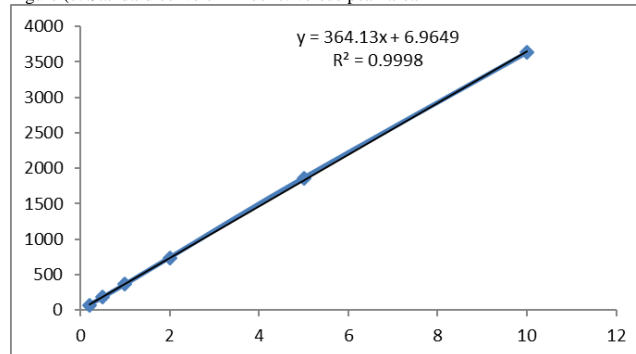


Figure (d): Chromatogram of T3, T4, and testosterone in serum at a conc. of 0.5 ppb

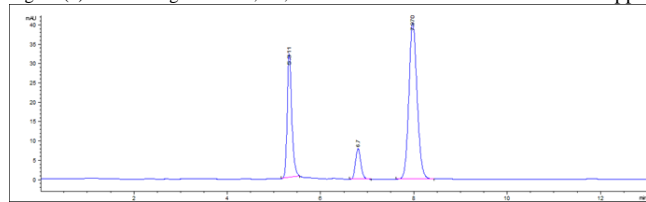


Figure (E): showed no effect of yucca extract on lead (Pb) intoxication on T3, T4, and testosterone in serum in newzealand rabbit

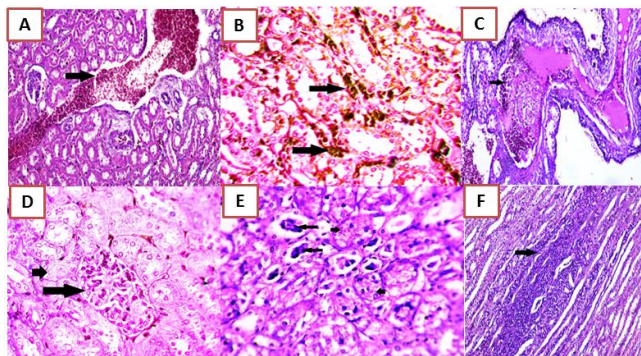
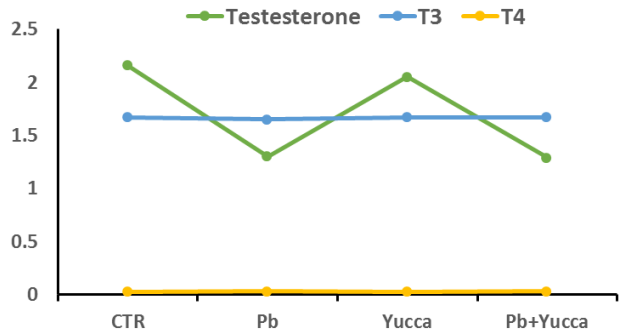


Fig.(1): lead group kidneys showing severely dilated blood capillary highly engorged with coagulated blood StainH&EX200 (A), kidneys showing severe coagulated blood in the interstitial blood capillaries between renal tubuli in kidneys parenchyma (StainH&EX400) (B), Thrombus formation in kidneys in blood vessel of renal pelvis StainH&EX200 (C), kidneys showing necrosis of the endothelium lining the glomerular tuft capillaries (long arrow) , and splitting of the glomerular tuft capillaries necrosis of renal tubuli epithelium StainH&EX600 (D), severe necrosis of the renal tubular epithelium (small arrows) and cellular casts in the lumen of renal tubules (longa Arrows) StainH&EX200 (E), kidneys showing accumulation of huge number of inflammatory cell StainH&EX200 (F).

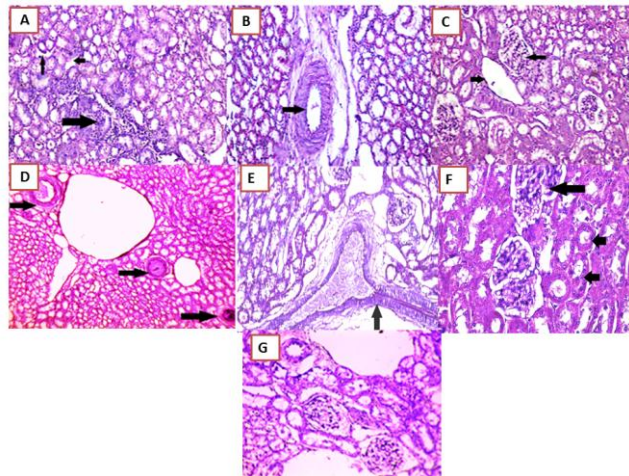


Fig.(2)Group treated with yucca and lead showing minimizing of the accumulated inflammatory cells thick long arrows, free of the interstitial blood capillaries from congested coagulated blood (StainH&EX100) (A), normal medium sized artery (StainH&EX100) (B), glomerular tuft capillaries with normal glomerular wide space (long arrow) normal wide capillary free from coagulated blood (short arrow) (StainH&EX100) (C), Yucca and lead group increase arteriolar blood supply dilated arterioles filled with blood (D), Dilated medium sized artery filled with blood supply StainH&EX200 (E), Normal renal glomerular tuft (long arrow) with normal renal tubular epithelium (short arrows) (StainH&EX400) (F), Control kidney showing normal glomerular and small sized arteriole normal StainH&EX200 (G)

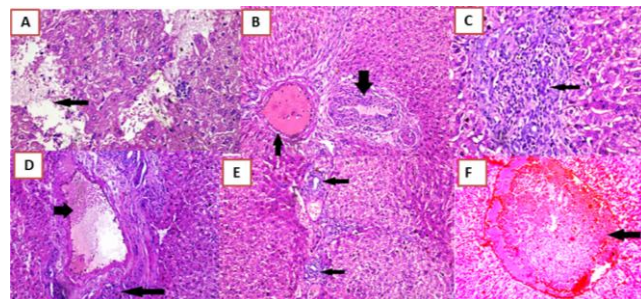


Fig.(3): Necrotic dissociated hepatic tissues with sloughing of abundant area of hepatic tissues (arrow) (StainH&EX400)(A), thrombus formation in the portal vein (thin arrow) ,inflammation of the wall of hepatic duct of gall bladder and necrotizing wall(thick arrow) StainH&EX200 (B), ; lead group accumulation of large number of inflammatory cells lymphocytes (StainH&EX400)(C), Liver showing partially occlude thrombus of portal vein severe proliferation of peri portal vein fibroblasts and inflammatory cells (long arrow) (StainH&EX400) (D), newly formed bile ductulus along the portal area (StainH&EX400) (E), Completely occluded thrombus central vein StainH&EX200 (F)

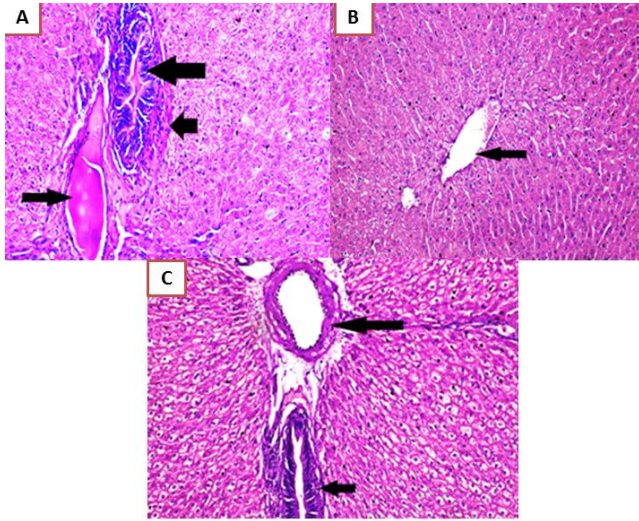


Fig.(4)The group treated with yucca and lead exhibited regeneration of the hepatic ducts (thick arrows)wall and the portal vein normal filled with few hemolyzed blood (thin arrow) (StainH&EX400) (A), yucca and lead normal central vein with healthy hepatic tissues(StainH&EX100) (B), Yucca group only showing normal portal artery and normal epithelium hepatic duct (StainH&EX200) (C).

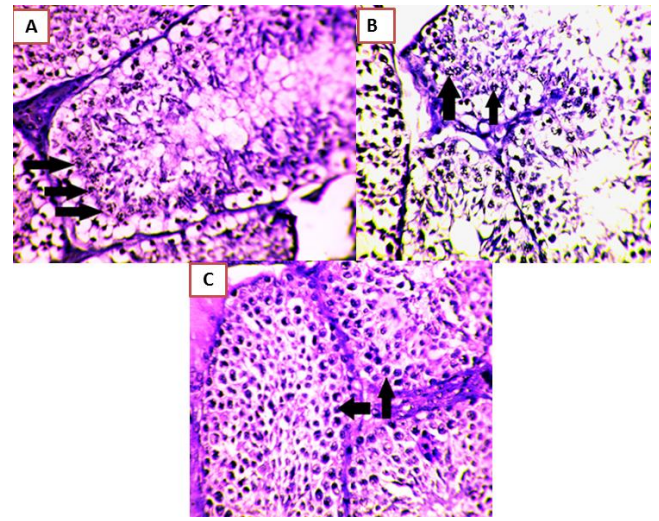


Fig. (6)yucca and lead elongation of pachytene phase (stage of spermatocytes meiosis and increase chromosome chiasmata (the stage of chromosomal exchange) (arrows)with high yield of spermatids (StainH&EX400)(A), yucca only elongation of pachytene phase (stage of spermatocytes meiosis and increase chromosome chiasmata (the stage of chromosomal exchange) with high yield of spermatids (StainH&EX400)(B) , Control testis with low chromosomes in pachytene phase (arrows)(StainH&EX400) (C)

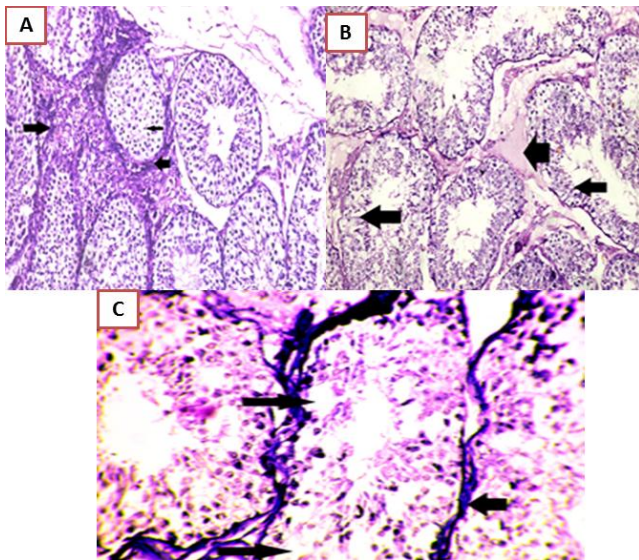


Fig (5):lead intoxicated group testis with severe accumulation of interstitial inflammatory cells (thick arrows)(StainH&EX200) (A), Severe necrosis and degeneration of the spermatogenic cells with intratubular edema (thick arrow) necrosis of spermatocytes long arrow)(StainH&EX200) (B), Severe loss of spermatocytes and sertoli cells (long arrows)with proliferation of fibroblasts basement membrane (short arrows)(StainH&EX400) (C)