INTRODUCTION

Lead (Pb) compounds due to human activities is a high-consumed metal mineral (Ilychova et al., 2012). Although lead toxicity has been considered since ancient times, it is still an important environmental and occupational health problem (Flora et al., 2012). Prolonged exposure to low doses of lead can cause renal and hepatic problems because of its slow excretion and cumulative property (Ercal et al., 2013). Among the soft tissues, liver tissue followed by the kidney cortex and medulla is the largest depot of lead (Haouas et al., 2014). According to (Bharali, 2013), Pb toxicity-induced liver damage may be the result of pro-oxidant and antioxidant imbalance, which can lead to oxidative damage to essential biomolecules such as proteins, lipids, and DNA by producing reactive oxygen species (ROS). Many cultures use the pomegranate (Punica granatum) as a traditional remedy. Pomegranate fruit, juice, and peel have a high antioxidant capacity that can inactivate the byproducts of oxidative catabolism (Ibrahim et al., 2016). These compounds include polyphenols, particularly those that are polyphenolic, ellagitannins, condensed tannins, and anthocyanins.

Super Para magnetic Iron Oxide Nanoparticles SPIONs are essential and fascinating metallic nanomaterials that reveal useful biomedical applications, such as in vivo cell tracking, targeted delivery of molecules or genes, magnetic resonance imaging (MRI), hyperthermia, transfection, tissue repair, and magnetic separation technologies (e.g. rapid DNA sequencing). According to Wang et al., (2014), SPIONs are precipitated magnetite (Fe3O4) or maghemite (g-Fe2O3) cores that range in size from 5 to 20 nm. The requirement for a novel, potent and non-toxic anti-oxidant derived from natural sources has increased. The above-mentioned anti-oxidants are employed to reduce various xenobiotics-induced toxicities in experimental animals and are regarded as safe therapeutic agents (Awad et al., 2020). Therefore the present study aimed to evaluate the ameliorative effect of an aqueous extract of pomegranate peel and iron oxide nanoparticles on the oxidative damage induced by lead intoxication in rats.

MATERIAL AND METHODS

2.1. Chemicals:

Lead (II) acetate 3-hydrate (CH3coo)2 Pb.3H2o was produced from ADWIC. Iron (III) chloride hexahydrate and Iron (II) chloride tetrahydrate were obtained from Alpha Chemika-INDIA, Sodium hydroxide (99.0%) was purchased from SRL Chemicals, and Citric acid mono hydrate from (East Chem). P. granatum fruit peel from (local Egyptian market).

Keywords
lead acetate, Nanoparticles, Green mediated synthesis, lead residue

ABSTRACT

Forty-Two albino rats were allocated into six groups, each group of seven rats. Group 1 of control rats administrated saline, Group 2 was given citric acid, Group 3 received 500mg/kg b.wt of green synthesized iron oxide nanoparticles, Group 4 rats were given 15mg/kg of lead acetate daily. Group 5 were given 250 mg/kg of green synthesized mediated iron oxide nanoparticles and 15mg/kg b.wt of lead acetate day after day and Group 6 which received 500mg/kg of green mediated synthesized iron oxide nanoparticles and 15mg/kg b.wt of lead acetate day after day for 45 days. The result showed significant adverse effects of lead acetate include significant increases in serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, and uric acid. Also, it showed significant decreases in total protein and albumin. Moreover it showed increasing in Malondialdehyde (MDA) levels; whereas superoxide dismutase (SOD) and glutathione reduced (GSH) levels are declines in both hepatic and renal tissue. These results confirmed by alteration of histological architecture of both liver and kidneys. The majority of the changes in the studied parameters were reversed in groups taken green mediated synthesized iron oxide nanoparticles using Punica granatum. In conclusion, green mediated synthesized IONPs using Punica granatum were effective in ameliorating lead induced hepato-renal toxicity in rats.
Green-mediated synthesized iron oxide nanoparticles prepared by extract the pomegranate peel extract (PPE) then green synthesis of IONPs in PPE by Precipitation method according to (Yusefietal.,2020) then dissolve by citric acid mono hydrate from (East Chem).

2.2. Experimental design:
Forty-Two adult albino rats purchased from Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt. The animals were acclimated for one week at the hygienic laboratory criteria. Rats were housed in plastic cages kept under constant environmental conditions and fed on fresh standard pellet and given tap water throughout the study. 42 albino rats were divided in to seven groups each one contain6 rats were established by random selection. First group (Control group) administrated (1ml of saline) daily. The second group received (1ml of citric acid) daily as vehicle. The third group administrated (500mg/kg b.wt of IONPs in P. granatum peel extract) daily. The fourth group received (15mg/kg of lead acetate day after day) according to (Javorac et al. 2021). The fifth group received (250 mg /kg of IONPs in P. granatum) daily then lead acetate (15mg/kg body weight) day after day; and while the sixth group administrated (500mg/kg of IONPs in P. granatum) daily then lead acetate (15mg/kg body weight) day after day in this study rats were dealing via oral gavage throughout the 45 day experiment.

The Ethical Committee of the Faculty of Veterinary Medicine, Benha University, Egypt, approved all protocols used in this experiment (Ethical No:BUVF0TMB-06-23)

2.3. Sampling:
Rats were anesthetized with Isoflurane one day after the final treatment. Blood samples from the retro-orbital plexus were collected to separate the serum, which was then kept at -20C for bio-chemical examination. The liver and kidneys were divided to three sections. One piece was immersed in 10% neutral buffered formalin for histopathological examination; piece was immersed in ice-cold phosphate-buffered saline (PBS) for anti-oxidant testing. Final piece was kept-at-20C for lead residue analysis.

2.4. Biochemical assays:
2.4.1. Evaluation serum biochemical markers:
Serum ALT, AST, ALP (Babson et al., 1966), Total protein and albumin levels (Doumas et al., 1971; Koller and Kaplan, 1984), urea level (Fawcett and Scott, 1960), and creatinine (Blass et al., 1974) values were measured by using commercial kits (Spectrum Diagnostics company, Egypt) and strictly following to the instructions provided by the manufacturers.

2.4.2. Assessment of anti-oxidants markers:
One gram of liver and kidney tissue specimen were homogenized by using electrical homogenizer with 5ml of phosphate buffer pH7.4. Malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) were evaluated using the manufacturer's approved techniques (Bio-diagnostics, Egypt).

2.4.3. Assessment of lead residue in liver and kidney tissue:
A wet digestion for tissues according to (Mason, 1991). Atomic absorption spectrophotometric procedure was used for the evaluation of lead as qualified in Perkin Elmer catalog of atomic absorption model 2380, U.S.A (1982). Atomic absorption with a single slot burner head was used at a wavelength of 248.3 nm for lead.

2.5. Histopathological examinations:
At the ending of the experiment, rats in all groups were euthanized and small tissue specimens were collected from liver and renal tissue immediately fixed in 10% neutral buffered formalin. After proper fixation, tissue paraffin sections of 4 µm thickness were routinely prepared and stained with haematoxylin and eosin according to (Bancroft and Gamble 2008). A Nikon Eclipse E800 microscope with an Olympus camera was used for histopathological evaluation and microscopic images photographing.

2.5. Statistical analysis:
One-way ANOVA and Duncan's multiple range tests were used in the analysis. Using SPSS software version 25 (Chicago, USA), data were assessed. The distinctions were statistically significant at P<0.05. All information was presented as means ± SD.

3. RESULTS
3.1. Effect of green-mediated synthesized iron oxide Nanoparticles using Punica granatum extract and/or lead acetate on serum biochemical parameters:
Oral administration of lead acetate revealed significant increase in serum activities of AST, ALT, ALP, urea, creatinine, and uric acid, while showed significant decrease in serum total proteins, albumin, compared to the 1st group (control), 2nd and 3rd group given saline, citric acid and 500mg/kg b.wt of IONPs in PPE. While co-administration with green-mediated synthesized iron oxide Nanoparticles using Punica granatum extract showed significant decrease on the serum levels of AST, ALT, ALP, urea, creatinine, uric acid and Moreover significant elevation on serum total proteins and albumin (Table 1) (P<0.05)

Table 1: Effect of green mediated synthesized iron oxide Nano particles using Punica granatum extract and/or lead acetate on serum biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Urea</th>
<th>Uric acid</th>
<th>Creatinine</th>
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<tbody>
<tr>
<td>G1</td>
<td>21.6±2.54</td>
<td>44.3±2.54</td>
<td>90.3±1.40</td>
<td>6.9±0.35</td>
<td>5.3±0.22</td>
<td>20.6±3.02</td>
<td>1.7±0.03</td>
<td>0.4±0.09</td>
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<tr>
<td>G2</td>
<td>26.3±0.95</td>
<td>48.9±0.82</td>
<td>110.3±2.71</td>
<td>9.4±0.12</td>
<td>6.0±0.12</td>
<td>22.8±0.78</td>
<td>1.8±0.12</td>
<td>0.4±0.01</td>
</tr>
<tr>
<td>G3</td>
<td>28.1±0.64</td>
<td>46.9±0.61</td>
<td>109.9±2.94</td>
<td>9.1±0.14</td>
<td>5.9±0.07</td>
<td>23.5±0.40</td>
<td>1.8±0.02</td>
<td>0.4±0.00</td>
</tr>
<tr>
<td>G4</td>
<td>40.9±1.4</td>
<td>61.3±1.4</td>
<td>109.9±2.94</td>
<td>9.1±0.14</td>
<td>5.9±0.07</td>
<td>23.5±0.40</td>
<td>1.8±0.02</td>
<td>0.4±0.00</td>
</tr>
<tr>
<td>G5</td>
<td>69.2±1.4</td>
<td>124.8±1.4</td>
<td>269.3±2.4</td>
<td>4.5±0.13</td>
<td>3.2±0.14</td>
<td>52.5±0.14</td>
<td>5.0±0.28</td>
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<tr>
<td>G6</td>
<td>42.6±0.8</td>
<td>76.5±0.8</td>
<td>188.7±4.8</td>
<td>7.6±0.06</td>
<td>5.0±0.05</td>
<td>36.4±0.57</td>
<td>2.6±0.05</td>
<td>0.5±0.01</td>
</tr>
<tr>
<td>G7</td>
<td>5.1±0.8</td>
<td>93.2±0.4</td>
<td>226.9±3.59</td>
<td>6.1±0.09</td>
<td>4.5±0.05</td>
<td>41.5±0.81</td>
<td>3.5±0.10</td>
<td>0.7±0.01</td>
</tr>
</tbody>
</table>

Mean values with different superscript letters in the same column are significantly different at (P<0.05)
3.2. Effect of green-mediated synthesized iron oxide Nanoparticles using Punica granatum extract and/or lead acetate on hepatic and renal anti-oxidants parameters:
Oral administration of lead acetate revealed significant increase in level of both hepatic and renal MDA, while there were significant decrease in levels of both hepatic and renal GSH and SOD compared to the 1st group (control), 2nd and 3rd group saline, citric acid and 500mg/kg b wt of green-mediated synthesized iron oxide nanoparticles using Punica granatum extract While co-administration of green mediated synthesized iron oxide nanoparticles using Punica granatum extract showed significant decrease in the levels of MDA and Moreover significant elevation in of both hepatic and renal, GSH and SOD (Table2). (P<0.05)

| Table (2): Effect of green mediated synthesized iron oxide Nano particles using Punica granatum extract and/or lead acetate on antioxidants parameters: |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                               | Hepatic GSH     | Hepatic MDA     | Hepatic SOD     | Renal GSH       | Renal MDA       | Renal SOD       |
| G1                             | 0.10±0.003a     | 16.5 ± 2.46     | 130.4±45.60     | 0.94±0.028b     | 37.6±1.23a      | 1120.6±101.86b  |
| G2                             | 0.10±0.016a     | 24.1±3.34     | 124.8±55.65     | 0.87±0.036a      | 42.4±4.20a      | 986.7±45.27b    |
| G3                             | 0.16±0.017b     | 28.3±4.03      | 122.0±111.26    | 0.96±0.026b      | 33.7±4.74b      | 928.1±21.26b    |
| G4                             | 0.03±0.015b     | 94.3±7.41      | 260.8±49.44     | 0.06±0.016b      | 107.5±7.32      | 69.8±12.33      |
| G5                             | 0.13±0.03a      | 47.1±6.27      | 757.6±29.64     | 0.84±0.110a      | 73.9±4.37a      | 323.9±36.20a    |
| G6                             | 0.11±0.033b     | 63.8±3.05      | 505.6±47.89     | 0.64±0.271a      | 97.9±26.74      | 168.1±14.76b    |

Mean values with different superscript letters in the same column are significantly different at (P<0.05).

3.3. Effect of green-mediated synthesized iron oxide Nanoparticles using Punica granatum extract and/or lead acetate on lead residues in liver and kidneys tissues:
Co-administration of green-mediated synthesized iron oxide nano particles using Punica granatum extract showed significantly decrease lead accumulation in liver and kidneys tissues compared to lead acetate group. While in comparing between high and low dose of green-mediated synthesized iron oxide nanoparticles using Punica granatum extract the result showed that in liver tissue low dose more effective than high dose, in contrast to kidneys residues showed high dose more effective than low dose (Table3) (P<0.05)

| Table (3): Effect of green mediated synthesized iron oxide Nano particles using Punica granatum extract and/or lead acetate on lead residues in liver and kidney tissues: |
|---------------------------------|-----------------|-----------------|
|                               | Hepatic lead residue | Renal lead residue |
| G1                             | 0.08±0.03b      | 0.27±0.10a      |
| G2                             | 0.09±0.06b      | 0.28±0.14a      |
| G3                             | 0.29±0.21b      | 0.10±0.10a      |
| G4                             | 1.17±0.34b      | 0.98±0.29b      |
| G5                             | 0.23±0.14b      | 0.22±0.13a      |
| G6                             | 0.42±0.26b      | 0.09±0.05b      |

Mean values with different superscript letters in the same column are significantly different at (P<0.05).

3.4. Effect of green mediated synthesized iron oxide Nanoparticles using Punica granatum extract and/or lead acetate on hepatorenal tissues:
The examined liver and kidneys of rats in group1 (control) and group 2 (citric acid) revealed normal histological structure of the hepatic and renal parenchyma (Fig. 1 A & B). While the examined liver in group 3 showed aggregation of a few mononuclear cells and mild congestion of portal blood vessels with mild dilatation of hepatic sinusoids, the examined kidneys revealed slight congestion of some renal blood vessels and glomerular tufts with eosinophilic casts in some of the lumens of some renal tubules (Fig. 1 D). In contrast, in group 4 (lead acetate toxicity) the liver showed severe congestion of the portal vessels with focal areas of necrosis infiltrated with inflammatory cells (Fig. 1 C). Multifocal vacuolar and hydropic degeneration of the hepatocytes were prevalent. Focal mononuclear leukocytic infiltration was observed in some examined liver sections. The examined portal areas revealed severely congested portal blood vessels with mild perivascular edema. Bile ductal hyperplasia along with formation of newly bile ductules was seen (Fig. 1 D). In addition, some portal blood vessels were thickened and hyalinized and showed vesiculation in their tunica muscularis. Furthermore, the examined kidneys in this group showed congestion of renal blood vessels with focal coagulative necrosis at the corticomedullary junction. Desquamated necrotic epithelia were also seen in the lumens of renal tubules (Fig. 1 E). These necrotic areas sometimes infiltrated with mononuclear inflammatory cells (Fig. 1 F). Occasionally, peritubular interstitial mononuclear cellular infiltration was seen. There was glomerular tuft proliferation as well as significant thickening and hyalinization of their Bowman's capsules. Severe glomerular tuft congestion with periglomerular mononuclear leukocytic infiltration was also seen. In group 5, administration of 250mg/kg b wt of green-mediated synthesized iron oxide nanoparticles attenuate the hepatorenal damage induced by lead acetate. The examined liver revealed only congestion with vacuolar degeneration of hepatic cells (Fig. 2 A). Mild bile ductal hyperplasia with eosinophilic debris in the lumen of some bile ducts were also seen (Fig. 2 B). Similarly, nearly all of the kidneys tested in this group had normal histological structure with mild congestion in the glomerular tuft. However, necrosis of few tubular epithelia was seen in some examined kidneys (Fig. 2 C). Desquamation of lining epithelial cells of some renal tubules with eosinophilic debris in their luminae were found in few examined kidneys (Fig. 2 D). In Group 6 Green-mediated synthesized iron oxide nanoparticles at a
concentration of 500mg/kg b.wt alleviated lead acetate-induced hepatorenal damage. However this protective effect was weak than that of green-mediated synthesized iron oxide nanoparticles (250mg/kg b.wt) where the examined liver in group 6 revealed small focal areas of coagulative necrosis with mononuclear leukocytic infiltration. Centrolobular hepatic degeneration in the form of vacuolar and hydropic degeneration of hepatic cells were detected (Fig. 2 E). There were severe congestion of the portal arteries, moderate perivascular edema, and activation of Von Kupffer cells. In addition, mild hyperplasia of the bile duct with newly formed bile ductules and mononuclear infiltration were seen in some portal areas (Fig. 2 F). Furthermore, the kidneys of rats in the same group showed mild congestion of the renal blood vessels and intertubular blood capillaries as well as mild segmentation of the glomerular tuft. In addition, degeneration and even necrosis of the renal tubular epithelium with the presence of eosinophilic casts in the lumens of renal tubules were occasionally found (Fig. 2 G & H).

Figure1: Photomicrographs of (A) liver (control) showing normal histotarchitecture of liver (B) kidney (control) showing normal renal corpuscles and convoluted tubules (C) liver (Group 4) showing focal necrosis mixed with mononuclear cells (D) liver (Group 4) showing bile ductal hyperplasia with portal edema. (E) Kidney (Group 4)
showing desquamated necrotic epithelia in lumen of renal tubules. (F) Kidney (Group 4) showing necrosis of renal tubules infiltrated with inflammatory cells. H&E stain X200

Figure 2: (A-C) Photomicrographs of (A) Liver (Group 5) showing congestion of portal blood vessel and vacuolar degeneration of hepatocytes. (B) Liver (Group 5) showing mild bile ductal hyperplasia with eosinophilic debris in the lumen. (C) Kidney (Group 5) showing congestion of glomerular tuft and intertubular capillaries with necrosis of few tubular epithelia (D) Kidney (Group 5) showing pyknosis of nuclei of some renal tubules with eosinophilic debris in their lumens (E) Liver (Group 6) showing centrolobular hepatic degeneration (F) Liver (Group 6) showing extensive degeneration of hepatocytes, bile ductal hyperplasia and mononuclear infiltration
of the portal area (G) Kidney (Group 6) showing necrosis of the renal tubular epithelium (H) Kidney (Group 6) showing esinophilic casts in the lumens of renal tubules. H&E stain X200

4. DISCUSSION

In the current study, administration of lead acetate led to a significantly higher level of serum AST and ALT activity than in the control groups, which may be related to increased cell membrane permeability or hepatic cell damage caused by lead poisoning. Our findings match with those of (Shalan et al., 2005). According to (Suradkar et al., 2009) and (Kaplan et al., 1970), the dangerous effects of lead on the liver and kidney might cause liberation of ALP. According to Gaskill et al., (2005) and (Ibrahim et al., 2012), in addition to the generation and alteration of free radicals in the liver tissue, cellular necrosis can cause the release of AST and ALT from the cell cytoplasm According to Farida et al., (2012), elevated levels of ALP were indicative of liver injury or biliary obstruction, which would impair blood flow to the liver. the green synthesized iron oxide nanoparticles using *Punica granatum* extract regenerate the cell of bile duct resulted in normal level of ALP as clear in pathological examination (FIG 2F). Lead acetate also resulted in a reduction in albumin and total proteins in the serum in the present research. These parameters were raised by the co-administration of green-mediated produced iron oxide nanoparticles using *Punica granatum* extract in this investigation. According to (Ahmed and Shalaby., 1999) and (Goering, 1993) lead exposure can cause damage to the liver and kidneys. Lead can also attach to plasma proteins, which can interfere with hepatocytes' ability to synthesize protein which confirmed by histopathological examination in liver tissue.

To assess renal function, study showed elevated serum urea and creatinine levels. Urea and creatinine elevations of lead treated group might be due to impairment of kidney function and considered as functional evidence of nephrotoxicity (Qu et al., 2002). While by using green mediated produced iron oxide nanoparticles using *Punica granatum* extract, serum urea and creatinine levels were significantly reduced. Our result is agreed with Cheong and Roh's (2006).This may be due to iron oxide nanoparticles and pomegranate peel extract possess strong antioxidant properties (Ibrahim et al., 2016, and Awad et al., 2020). Oxidative stress plays a crucial role in the development and progression of renal diseases. By reducing oxidative stress, these substances may help protect the kidneys from damage and improve renal function.

Regarding to antioxidiant parameters, it was found that lead acetate-intoxicated rats had lower levels of GSH and SOD and higher level of MDA as lipid peroxidation indicator that agree with (Azoz and Raafat, 2012). While by using green mediated synthetic iron oxide nanoparticles using *Punica granatum* extract the levels of these biomarkers were balanced. These findings were in order for the biological system to detoxify the reactive intermediates or repair the damage, there must be a balance between the generation of free radicals and its capacity (Flora, 2002).

Regarding to lead residues in tissues (Table3). The liver and the kidneys are also known to play a major role in the elimination of lead (Goyer and Chirian, 1979) and hence, account for the toxic actions (Lockitch, 1993). According to (Suleman et al., 2011), lead exhibited a substantial capacity for bioaccumulation in tissues.

While co-administration by green mediated synthetic iron oxide nanoparticles using *Punica granatum* extract the levels of lead residues in liver and kidneys were decreases it may be due to iron oxide nanoparticles has a role for removal of lead from the body. The results nearly agreed with (Nahed et al., 2017) that used pomegranate peel extract. While in comparing between high (group 6) and low dose (group 5) of green-mediated synthesized iron oxide nanoparticles using *Punica granatum* extract the result showed that in liver tissue low dose more effective than high dose, in contrast to kidneys residues showed high dose more effective than low dose. That may due to the absorbed Pb is excreted primarily in urine as routes of excretion (Chamberlain et al., 1978), so residue of lead in kidney in group 6 showed improvements than group 5.

Regarding to histopathological result our study showed focal areas of necrosis in the hepatic parenchyma. According to (El-Neweshy and El-Sayed., 2011), lead acetate-intoxicated rats revealed severe vacuolar degeneration in hepatocytes, congestion of hepatic sinusoids, and the bile ducts showed varying degrees of hyperplasia of their lining epithelial cells with the presence of newly formed bile ductules. According to (Dewanjee et al., 2013; El-Sayed et al., 2015) and Nahed et al. (2017) the liver of lead-treated rat had similar alterations.

There were significant degenerative alterations in the renal cortex’s lining epithelium of the convoluted tubules agreed with (El-Bahrserum et al., 2021). According to (Shafie et al., 2016), the livers of rats given 10 and 30 ppm of nano iron displayed normal histological alternation (Sarbaksh and Vafa., 2016) demonstrated that the kidneys of rats treated with10 and 30 ppm nano-iron revealed that normal histological structures of glomeruli and renal tubules while showed mild inflammatory cell infiltration in between renal tubules in the nano-iron-treated group. The nano-iron treated group with 60 ppm showed mild inflammatory cell infiltration.

From our biochemical and histopathological findings in group 5 (low dose) administrated 0.5 ml was the best outcome achieved compared with group 6 (high dose) which received 1 ml of daily green-mediated produced iron oxide nanoparticles using *Punica granatum* extract co administrated with lead acetate due to higher doses of IONPS can lead to increased accumulation in healthy tissues or organs, which may not be desirable. Additionally, the clearance of IONPS from the body can be dependent on dose. If the dose is too high, it may overwhelm the body's clearance mechanisms, leading to prolonged exposure or potential toxicity. A lower dose can allow for more efficient clearance, reducing potential side effects. It's important to note that the ideal dose of iron oxide nanoparticles for lead removal may vary depending on the specific application, lead concentration, and other factors. Furthermore, the use of high doses should be balanced with factors such as cost, potential environmental impact, and safety considerations.

5. CONCLUSION

Iron oxide nanoparticles that were produced through green technology using *Punica granatum* extract may have a
positive effect because their constituent parts have free radical scavenging abilities. So, it could protect the liver and kidneys from damage caused by lead toxicity.

6. REFERENCES