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Ameliorative Role of *Jania Rubens* Alga Against Toxicity of Heavy Metal Polluted Water in Male Rats

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Abstract: Elimination of heavy metals from contaminated water is a significant issue. Algae can be used as the detoxifying agent of these metals. This study was achieved to assess the efficacy of red marine alga (*Jania Rubens*) opposed to the initial modifications that may be concerning the toxicity of heavy metal-polluted water on male rats. 45 male Wistar rats were separated into 3 groups (15 rat/ group): Group I (control); Group II (Heavy metal group) and Group III (Treated group). There were significant increases in the levels of SGPT, SGOT, ALP, creatinine, urea, UA, malondialdehyde (MDA), glutathione peroxidase (GPx), protein carbonyl (PC) and significant decrease of body weights& levels of blood hemoglobin, total protein, albumin, catalase, SOD, and GSH in the heavy metal group in comparison with control. Meanwhile, the administration of treated water to rats restores all parameters to normal level in the treated group. Highly significant increase in Metallothionein (MT) levels in hepatic and renal tissue of heavy metal group compared to control, that restored to normal in the treated group. Also, Ni, Cd and Pb metals accumulated in liver, kidney and brain tissues were measured in all studied groups. Our results suggested the ameliorative effect of red marine algae treated water opposed to the toxicity of heavy metals in liver, kidney and brain tissues.

Keywords: Heavy Metals, Water Treatment, Red Marine Algae, Metallothionein, Oxidative Stress

1. Introduction

Aquatic pollution with heavy metals represents most environmental and health problem worldwide [1]. Because of rapid industrialization, civilization and population growth, heavy metals excessive release into the environment [2]. Metals can be accumulated in living organisms over a period of time and causing health problems [3]. They can exert their toxicity to living organizations by generating reactive oxygen species which lead to oxidative stress [4]. Heavy metal induced oxidative stress cause several diseases including hepatic injury, renal damage, cardiovascular diseases and inflammatory diseases [5& 6]. Antioxidants can inhibit oxidation or neutralize the harmful effects of ROS leading to the limited risk of oxidative stress [7]. Alteration in the antioxidant defense systems is considered important mechanism for heavy metal-induced toxicity [8].

Removal of heavy metals from wastewater has become the subject of considerable interest owing to strict legislation [9]. Numerous novel approaches have been studied to develop an alternative frugal and more efficient metal removal technique [10]. Bio-sorption has recently considered as one of the most applicable technologies for wastewater treatment [11]. Algae considered as a favorable bio-sorbents [12], due to their high

sorption uptake, high selectivity, abundance in seawater and fresh water, low cost, reusability and no toxic waste generation [13]. The purpose of our research is to examine the effect of heavy metal-polluted water treated by the red marine alga (*J. Rubens*) on biochemical parameters in male wistar rats.

2. Materials and Methods

2.1. Rats

Forty-five adult male Wistar rats (135±15) g, average 8 weeks old, obtained by the Animal House of the Research Institute of Ophthalmology, Egypt was used. Rats were settled in standard circumstances of light, moisture, and warmth with nourishment in balls and tap water available. This study was achieved as the guiding principles of the Animal Care and Use, confirmed by the Ethics Committee for Animal Research of the Research Institute of Ophthalmology, Giza, Egypt.

2.2. Study Design

After one week of acclimatization, the rats were separated into three groups (15 rats/ group). Control group (I); Heavy metal group (II): animals took a mixture of (3.5 mg/L $Pb(NO_3)_2$, 3 mg/L $Cd(NO_3)_2$ and 2.5 mg/L $NiSO_4$) dissolved in distilled water for 90 days; Group III (Treated group): Rats received the previous water which was first treated by *J. Rubens* alga for 90 days. All rats were daily noted for clinical markers and symptoms of toxicity and mortality, while body weights and water consumption were weekly recorded.

By the finish of the search, the rats were fasted overnight and anesthetized using ether, and the blood was gathered through retro-orbital venous plexus. Blood samples collected (with and without EDTA) for plasma and serum then centrifuged for 15 min. The red blood cells were mixed four times its volume with a cold saline solution (0.9% NaCl), centrifuged at 3000 rpm for 15 min at 4°C, then the erythrocyte lysates were collected.

Whole blood used for determination of Hb level. Serum was used for the determination of SGPT, SGOT, ALP, TP and albumin as hepatic markers; urea, creatinine, and UA as renal markers; PC as oxidative markers and TNF-α as a pro-inflammatory cytokine. Plasma was used for the determination of MDA and CAT levels as oxidative markers; Erythrocyte lysate used for the determination of GPx, SOD, and GSH as oxidative markers. The liver, kidneys, and brain were immediately removed, rinsed with an ice-cold physiological saline solution (0.9% NaCl) and stored at –80°C for metal testing. Portions of the isolated liver and kidneys were weighed, homogenized, centrifuged and the resulting supernatants were saved at –20°C for hepatic and renal metallothionein assay. Moreover, sections of the liver, kidneys, and brain of each group were immediately excised and fixed in 10% formalin for histopathological examination.

2.3. Biochemical Analysis

2.3.1. Hepatic Markers Assays

SGPT and SGOT enzymes were estimated according to assays of Gella *et al* [14]; ALP, TP and serum albumin were evaluated according to assays of Tietz et al [15] Gornall *et al* [16] and Doumas *et al* [17] respectively

2.3.2. Blood Hemoglobin (Hb)

Blood Hb concentration was determined according to Betke *&* Savelsberg [18].

2.3.3. Renal Markers Assays

Creatinine, Urea, and U.A were evaluated according to the assay of Fabiny & Ertingshausen [19], Tabacco *et al* [20] and Fossati *et al* [21] respectively.

2.3.4. Oxidative Stress Marker Tests

MDA level was measured by the technique of Ohkawa *et al* [22]. Protein carbonyl (PC) content was measured by the technique of Reznick & Packer [23]. The activity plasma CAT was determined following the method of Aebi [24]. The levels of SOD, GPx, and GSH in erythrocyte lysate were determined following Nishikimi *et al* [25], Paglia *&* Valentine [26] and Beutler *et al* [27] respectively.

2.3.5. Tumorigenicity Biomarker Assay

ELISA technique was used for determination of TNF-α in serum according to Aggarwal [28].

2.3.6. Metallothionein Determination Assay

Metallothioneins represent a specific biomarker of metal exposure; it was determined in both liver and kidney tissues by the method of Viarengo *et al* [29].

2.3.7. Metal Analysis Assay

The concentrations of lead, cadmium and nickel in liver, kidneys and brain tissues were determined by graphite furnace atomic absorption spectrophotometry (AAS) according to the method of IAEA [30].

2.4. Histological Examination

The liver, kidney and brain tissues were removed and set in 10% buffered formaldehyde, and put in ascending gradients of ethanol, cleared in xylene and embedded in paraffin blocks. Sections of 5 µm thickness of the tissues were prepared using a rotary microtome, stained with hematoxylin and eosin (H&E) stain and observed under microscope [31].

2.5. Statistical Analysis

SPSS program was used for statistical analysis. Data variables from more than two groups were evaluated with one-way analysis of variance (ANOVA) and Post hoc-LSD (least significant difference). The results were explicited as mean ± standard error (S.E). Differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Clinical Markers, Body Weight and Water Consumption

During the experimental period neither mortality nor obvious clinical signs, including rashes, allergy, hair loss, decreased defecation, or vocalization upon handling were observed.

The mean weekly body weights in the heavy metal group exhibited a significant decrease in comparison to control. While a significant increase in the body weight in the treated group in comparison to heavy metal group. However, non-significant changes were detected among the treated and control groups (Figure 1a). Also the mean weekly water consumption within both the heavy metal and treated groups showed no significant alternations as compared to control (Figure 1b).

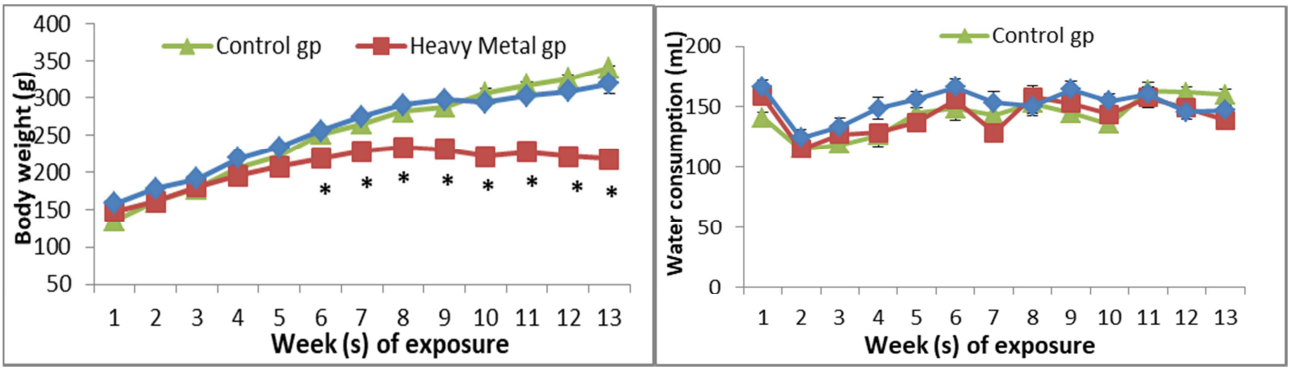

Figure 1. *Mean ± S.E. of a: body weights (g) and b: water consumption (mL) of rats in the studied groups.*

3.2. Biochemical Results

There was a significant increase in the levels of SGPT, SGOT and ALP in the heavy metal group in comparison to control, while by comparing the treated group with the heavy metal group; a significant decrease in SGPT and SGOT activities and non-significant alterations in ALP activity were demonstrated. However, no significant changes in SGPT and ALP activities were detected between the treated and control groups, while a significant elevation was detected in SGOT activity in treated groups comparable with control group (Table 1).

Serum levels of both total protein (TP) and albumin were significantly reduced in the heavy metal group comparable with control. Furthermore, between the treated and heavy metal groups the serum TP level was elevated in the treated group and non-significant changes were detected for serum albumin. However, no significant alterations were observed for serum TP level in the treated group in comparison with control, also a significant decrease in serum albumin level in treated group comparable with control was detected. A significant increase was detected in serum TNF-α level in both the heavy metal and treated groups in comparison with control. A highly significant decline in TNF-α level was detected in the treated group in comparison to heavy metal group (Table 1).

There was high significant decline in blood hemoglobin concentration in the heavy metal group in comparison with control. With respect to the treated group, there was a highly significant increase in blood hemoglobin level compared to heavy metal group. While, there were non-significant changes between the treated and control groups (Table 1).

There was a high significant elevation in levels of creatinine, urea and UA in the heavy metal group in comparison with control. Also a significant elevation of creatinine and urea levels in treated groups in comparison with control, conversely, non-significant alteration in UA level was detected between both control and treated groups. Furthermore, there was a highly significant decrease in both urea and UA concentrations in the treated group in comparison with heavy metal group. While non-significant variations in creatinine levels detected in the treated group in comparison to heavy metal group (Table 1).

Table 1. *Mean±S.E. for the activities of rat serum SGPT, SGOT and ALP and levels of TP, albumin, TNF- α, blood hemoglobin, creatinine, urea and UA in the different groups.*

	Group I	**Group II**	**Group III**
	(Control group)	**(Heavy metal group)**	**(Treated group)**
SGPT (U/L)	52.93 ± 1.86	65.47 ± 4.92[a]	50.27 ± 1.67[c]
(percentage change)		(↑23.6%)	(↓5.02%)
SGOT (U/L)	76.60 ± 3.10	138.0 ± 4.77[b]	92.73 ± 5.25[a,d]
(percentage change)		(↑80.1%)	(↑21.0%)
ALP (U/L)	133.13 ± 4.51	150.13 ± 4.65[a]	137.60 ± 5.09
(percentage change)		(↑12.7%)	(↑3.35%)
TP (g/dL)	6.99 ± 0.11	6.60 ± 0.07[a]	7.09 ± 0.10[c]
(percentage change)		(↓5.57%)	(↑1.43%)
Albumin (g/dL)	4.11 ± 0.08	2.51 ± 0.39[b]	3.09 ± 0.09[a]
(percentage change)		(↓38.9%)	(↓24.8%)

	Group I (Control group)	Group II (Heavy metal group)	Group III (Treated group)
TNF-α (pg/mL)	29.51 ± 0.61	63.18 ± 0.46[b]	41.66 ± 3.56[a,d]
(percentage change)		(↑114.09)	(↑41.1%)
Blood hemoglobin (g/dL)	14.53 ± 0.30	11.70 ± 0.19[b]	13.84 ± 0.32[d]
(percentage change)		(↓19.4%)	(↓4.74)
Creatinine (mg/dL)	0.43 ± 0.02	0.81 ± 0.09[a]	0.69 ± 0.06[a]
(percentage change)		(↑88.3%)	(↑ 60.4%)
Urea (mg/dL)	39.40 ± 0.86	60.13 ± 2.09[b]	45.47 ± 1.52[a,d]
(percentage change)		(↑52.6%)	(↑15.4%)
UA (mg/dL)	1.44 ± 0.05	2.21 ± 0.12[b]	1.66 ± 0.04[d]
(percentage change)		(↑53.4%)	(↑15.2%)

a: $P< 0.05$, b: $P< 0.001$ vs. Control group
c: $P< 0.05$, d: $P< 0.001$ vs. Heavy metal group
$P> 0.05$ is non-significant.

Table 2 has shown a high significant increase in both MDA and PC levels in the heavy metal group in comparison with control. When comparing the treated group with heavy metal group, a significant depletion in both MDA and PC levels was detected. Conversely, non-significant changes were indicated in the treated group in comparison with control.

There was a high significant decline in activities of CAT, SOD and GSH in the heavy metal group in comparison with control. Significant elevations in activities of CAT, SOD and GSH were detected in the treated group compared with heavy metal group. By comparing the treated group to control CAT activity was significantly decreased, while non-significant differences were determined with SOD and GSH activities. Also a significant elevation was detected in avtivity of GPx in both the heavy metal and treated groups in comparison with control. Furthermore, a high significant decline was detected in the treated group compared to heavy metal group (Table 2).

Table 2 exhibited a high significant elevation in hepatic MT level in the heavy metal group in comparison with control group. Also a high significant decline in hepatic MT level in the treated group in comparison to heavy metal group was detected. However, non-significant alterations were determined among treated and control groups. Table 2 also showed a significant elevation in renal MT concentration in both the heavy metal and treated groups in comparison with control group. Though, a significant decline was determined in renal MT level in the treated group in comparison to heavy metal group.

Table 2. *Mean±S.E. for the levels of plasma MDA, serum PC and erythrocyte GSH, activities of (SOD, CAT and GPx), levels of rat hepatic and renal MT in the different groups.*

	Group I (Control group)	Group II (Heavy metal group)	Group III (Treated group)
MDA (nmol/mL)	14.43 ± 0.44	20.21 ± 0.86[b]	16.35 ± 0.51[d]
(percentage change)		(↑40.06%)	(↑13.3%)
PC (nmol/mL)	2.82 ± 0.08	6.56 ± 0.08[b]	3.12 ± 0.11[d]
(percentage change)		(↑132.6%)	(↑10.6%)
CAT (U/L)	647.76 ± 24.11	423.46 ± 19.64[b]	568.44 ± 20.30[a,d]
(percentage change)		(↓34.6%)	(↓12.2%)
SOD (U/g. Hb)	1718.26 ± 50.36	1373.33 ± 40.91[b]	1586.89 ± 66.12[c]
(percentage change)		(↓20.07%)	(↓7.64%)
GPx (mU/mL)	173.78 ± 9.20	328.11 ± 12.72[b]	211.39 ± 9.47[a,d]
(percentage change)		(↑ 88.8%)	(↑21.6%)
GSH (mg/dL)	63.82 ± 2.08	56.20 ± 1.98[a]	64.79 ± 1.76[c]
(percentage change)		(↓11.9%)	(↑1.51%)
Hepatic MT (µmole) x 10^{-5}	37.71 ± 4.64	82.53 ± 7.51[b]	59.76 ± 5.66[d]
(percentage change)		(↑118.8%)	(↑58.4%)
Renal MT (µmole) x 10^{-5}	41.58 ± 4.42	98.73 ± 5.26[b]	56.34 ± 7.47[a,c]
(percentage change)		(↑137.4%)	(↑35.4%)

a: $P< 0.05$, b: $P< 0.001$ vs. Control group
c: $P< 0.05$, d: $P< 0.001$ vs. Heavy metal group
$P> 0.05$ is non-significant.

3.3. Tissue Metal Accumulation

There was a significant increase in accumulation of heavy metals (Cd, Pb and Ni) in hepatic, renal and brain tissues in the heavy metal group compared to control. When the treated group in comparison to the group of heavy metal, a significant decreases in metal levels in hepatic and renal tissues, while decrease only in lead level in the brain tissue were observed. In contrast, no significant variations in these metal levels (in all tissues) were detected in the treated group in comparison with control (Table 3).

Table 3. *Mean±S.E. for the levels of Pb, Cd and Ni in liver, kidney and brain tissues in the different groups.*

	Group I (Control)	Group II (Heavy metal)	Group III (Treated)
Liver tissue			
Pb (µg/g tissue)	0.016 ± 0.0049	0.088 ± 0.0239[a]	0.031 ± 0.0083[c]
Cd (µg/g tissue)	0.0011 ± 0.0003	0.1185 ± 0.0268[a]	0.0518 ± 0.0137[c]
Ni (µg/g tissue)	0.022 ± 0.0038	0.0541 ± 0.0048[a]	0.035 ± 0.0052[c]
Kidney tissue			
Pb (µg/g tissue)	0.047 ± 0.0127	1.0461± 0.2631[a]	0.3794 ± 0.0834[c]
Cd (µg/g tissue)	0.0021 ± 0.0005	0.0322 ± 0.0124[a]	0.0043 ± 0.0012[c]
Ni (µg/g tissue)	0.016 ± 0.0035	0.07 ± 0.0083[b]	0.042 ± 0.0080[c]
Brain tissue			
Pb (µg/g tissue)	0.0031 ± 0.001	0.0248 ± 0.0073[a]	0.0055 ± 0.0012[c]
Cd (µg/g tissue)	0.0014 ± 0.0004	0.0064 ± 0.0017[a]	0.0039 ± 0.0009
Ni (µg/g tissue)	0.09 ± 0.0179	0.1317 ± 0.0074	0.11 ± 0.0206

3.4. Histological Results of Rat Liver Tissues

The hepatic tissues of control rats displayed the normal structure of hepatic lobule (Figure 2 (a, b)). In the heavy metal group showed vacuoles with degeneration of hepatocytes, stimulation of Kupffer cells and focal necrosis of hepatocytes correlated with inflammatory cells infiltration (Figure 2 (c, d)). However, in the treated group, congestion of central vein and hepatic sinusoids as well as stimulation of Kupffer cells was found (Figure 2 (e, f)).

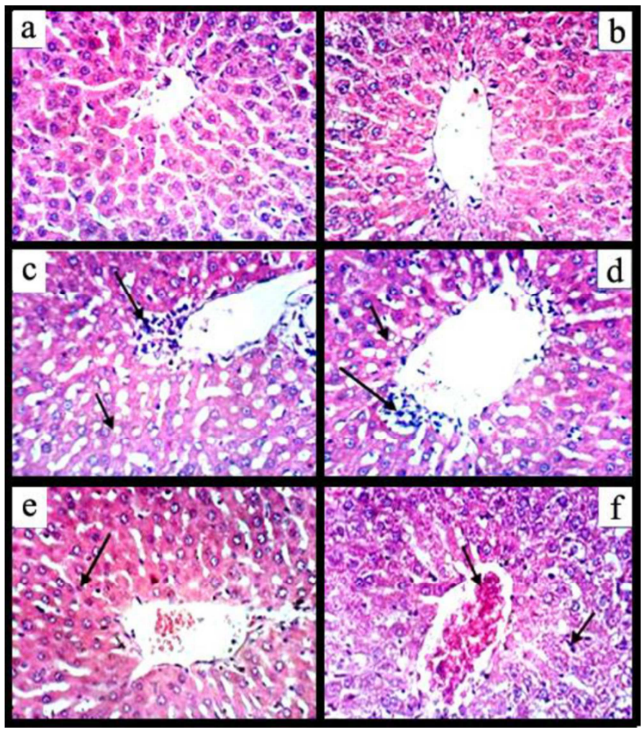

Figure 2. *A photomicrograph of the liver of control rats (a,b) showing normal hepatocyte histological structure (H&E X400). (c,d): Showing liver sections of heavy metal group with vacuolar degeneration of hepatocytes, Kupffer cells activation and focal necrosis of hepatocytes associated with inflammatory cells infiltration (H&E X400). (e,f) showing congestion of central vein and slight activation of Kupffer cells in the treated group. (H&E X400).*

3.5. Histological Results of Rat Kidney Tissues

Renal tissues of control rats showed the normal structure of renal parenchyma (Figure 3a). The heavy metal group exhibited vacuolation of epithelial cells of renal tubules, thickening and obstruction of glomerular tuft as well as congestion of intertubular blood vessels (Figure 3 (b,c)), necrosis of renal tubular epithelium and focal renal hemorrhage (Figure 3d). The treated group revealed thickening of glomerular basement membrane (Figure 3e), other sections revealed apparent normal renal parenchyma (Figure 3f).

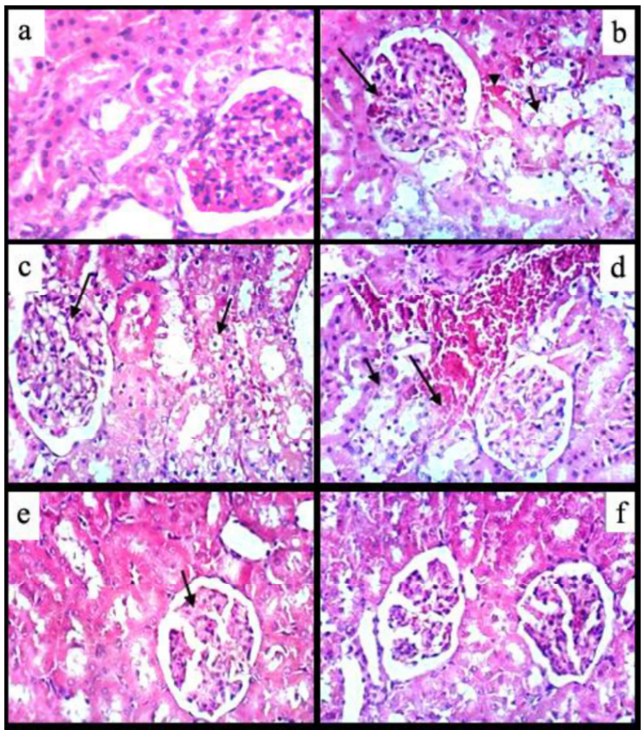

Figure 3. *Photomicrograph of kidney of control group showing normal histological structure of renal parenchyma (a) (H&E X400). (b-d): Photomicrograph in kidney of heavy metal group. Showing vacuolation of epithelial lining renal tubules, thickening of glomerular tuft and congestion of intertubular blood vessels (b,c), necrosis of renal tubular epithelium and focal renal hemorrhage (d) (H&E X400). (e,f): The treated group revealed thickening of glomerular basement membrane (e), apparent normal renal parenchyma (f) (H&E X400).*

3.6. Histological Results of Rat Brain Tissues

The brain tissue of control rats showed the normal structure of brain tissue (Figure 4a). The heavy metal group exhibited necrosis of neurons, focal gliosis and cellular edema (Figure 4(b,c)). Improved histopathological picture was noticed in rats from treated group (Figure 4d).

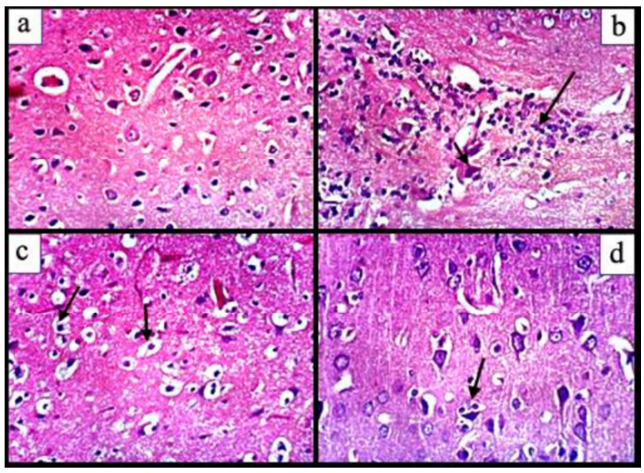

Figure 4. *Photomicrograph of brain of control group showing histological structure (a) (H&E X400). (b,c): The heavy metal group showed necrosis of neurons, focal gliosis and cellular edema (H&E X400). (d) Improved histopathological picture of treated group (H&E X400).*

4. Discussion

Heavy metal pollution has become one of the most environmental health problems [32], they can accumulate in the vital organs as liver, kidneys, heart, brain and bone, causing several adverse effects. Also displace the vital nutritional minerals, so banning their biological roles [33]. Microorganisms as algae or bacteria have been successfully used as biosorbents for the detoxification of heavy metals [34].

The current study showed a significant reduction in the weight of rat body in the heavy metal group in comparison with control. Our result agrees with Jadhav *et al*. [35]. However, treated group showed an effective restoration of the body weight nearly to that of the control group, which probably due to the improvement of biochemical parameters. With respect to water consumption, no significant alternations were detected between the studied groups. This finding agrees with that obtained by Markiewicz-Górka *et al*. [4].

Hepatic and renal parameters could serve as biomarkers for early detection of heavy metal pollution [36]. This research recorded significant increase in SGPT, SGOT and ALP activities in the heavy metal group in comparison with control, which may suggest degenerative effects of heavy metals on liver cells [37]. In our research, significant reductions in TP and albumin levels were detected in the heavy metal group in comparison with control, these results confirmed by Bhattacharjee *et al*. [38].

Our results exhibited that administration of heavy metals led to a highly significant elevation in plasma creatinine, urea and uric acid levels in comparison with control. This significant elevation is closely related to renal function impairment and indicated the nephrotoxic effects of metals [39]. On the other hand, treated group showed obvious reversion of the hepatic and renal markers almost close to that of the control, showing the ameliorative influence of alga against heavy metal-polluted water. In our results, a highly significant decline in the blood hemoglobin level was detected in the heavy metal group in comparison with control. Our finding was matched with that reported by Yuan *et al*. [40] who stated that accumulation of toxic metals in kidney, spleen, and liver might suppress the activity of these hematopoietic tissues, leading to anemia. According to the present results, there was a significant elevation in blood hemoglobin concentration in the treated group relative to heavy metal group, which could be due to reduction of the toxic effects of metals.

The results of the current study revealed a high significant elevation in TNF- α level in the heavy metal group in comparison with control. This matched with Hussein *et al*. [41]. This elevation may be due to the immune-modulatory actions of these xenobiotics on the immune cells [42]. However, the treated group showed suppression of inflammatory response as indicated by decreased level of TNF- α, which could be due to the reduction of oxidative insult.

Oxidative stress was one of the well-known mechanisms of heavy metal-induced toxicity [43]. The current study showed highly significant increase in plasma MDA and serum PC levels in the heavy metal group in comparison with control group. Our findings were confirmed by Reckziegel *et al*. [44] and Padma *et al*. [45] who attributed such significant elevation in lipid peroxidation and protein carbonyl levels in Cd-intoxicated rats to extreme generation of free radicals. However, this elevated level of lipid peroxidation products and protein carbonyl content were found to be significantly decreased reaching almost control level in the treated group. The present study showed highly significant decrease in erythrocyte SOD and plasma CAT activities in heavy metal group relative to control. These findings were in accordance with those achieved with Hussein *et al*. [46] and Apaydın *et al*. [39]. The decline in activities of SOD and CAT could be refered to oxidative stress and excessive production of ROS-induced by metals [47]. In our results, a highly significant elevation in erythrocyte GPx activity was detected in heavy metal group relative to control. This result matched with that reported by Reddy *et al*. [48]. The increase in GPx activity could be a compensatory adaptive mechanism of the antioxidant system to combat the ROS generation-induced by toxic metals [49].

The current study exhibited a significant decline in erythrocyte GSH level in heavy metal group in comparison with control, this agree with that reported by Reckziegel *et al*. [44] who attributed this depletion in GSH level to the high affinity of metal for sulfhydryl groups of GSH, interfering with the antioxidant activity and leading to its inhibition. In

contrast, all these alternations in antioxidant activities and levels induced by heavy metal intoxication were significantly restored to normal level in the treated group.

Another important mechanism related to heavy metals toxicity is induction of metallothionein synthesis [50]. The present study showed highly significant elevations in both hepatic and renal MT levels in the heavy metal group compared to control. Alternatively, treated group exhibited marked decrease in MT levels in relative to control. Our findings matched with that described by Abdelmigid *et al*. [51] and Šveikauskaitė *et al*. [52]. Therefore, MT level is proposed as a potential biomarker for monitoring heavy metal exposure [53].

Heavy metals are non-biodegradable and can be bio accumulated in different organs producing probable health risks [54]. The present study showed significant increase in Pb, Cd and Ni levels in both hepatic and renal tissues of heavy metal group when compared with control. This finding was confirmed by Reckziegel *et al*. [44] who stated that increased Ni level in mice kidneys after Ni treatment could be explained by the high affinity of metallothionein for Ni. Moreover, the obtained results revealed significant elevations in Pb and Cd levels in the brain tissues of the heavy metal group in comparison with control. This significant increase may be referred to the high degree of toxicity of lead and cadmium and their detrimental effects on various organs [55]. Also the brain can be considered as important organ for metal accumulation due to slow excretion [56]. The treated group showed significantly decreased accumulation of Pb, Cd and Ni in the different tissues compared to the heavy metal group.

Heavy metals can cause various histopathological alterations in many tissues [40]. In the present study, there were vacuolar devolution of hepatocytes, activation of Kupffer cells and focal necrosis of hepatocytes correlated with inflammatory cells infiltration in liver of heavy metal group. This agrees with Samir & Zine., [57] and Bhattacharjee *et al*. [38].

Also in our study, heavy metals administration resulted in kidney histopathological changes as thickening and congestion of glomerular tuft as well as obstruction of tubular blood vessels, necrosis of renal tubular epithelium and focal renal hemorrhage. This matched with Apaydın *et al*. [39].

The present study showed marked alternations in the brain tissues of heavy metal group includes necrosis of neurons, focal gliosis and cellular edema. This result matched with Cobbina *et al*. [58] and Reddy *et al*. [48].

5. Conclusion

In conclusion, this study is considered to be the first that investigated the ameliorative action of *Jania rubens* alga against toxic effects-elicited by a mixture of Pb^{+2}, Cd^{+2} and Ni^{+2} employed as drinking water to male Wistar rats. As a result, *J. rubens* may be taken into consideration as a promising, efficient and safe biosorbent for decontamination of heavy metals and purification of wastewater.

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