JJBS

Volume 13, Number 2, June 2020
ISSN 1995-6673
Pages 213 - 218

Jordan Journal of Biological Sciences

Effect of Edible Bird's Nest Supplement on Hepato-renal Histomorphology of Rats Exposed to Lead Acetate Toxicity

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Received May 20, 2019; Revised July 29, 2019; Accepted August 8, 2019

Abstract

Lead acetate (LA) has been shown to cause hepato-renal damages through induction of oxidative stress. Edible bird's nest (EBN) has reportedly been shown to alleviate these damages, but no studies have been conducted on this area. The present study was aimed to evaluate the protective effects of EBN supplement on the liver and kidney of rats exposed to LA toxicity. Five groups of rats were used and grouped as follows: group 1 (positive control, C), was given distilled water; group 2 (positive control, T0), was administered with LA (10 mg/kg BW); and groups 3 (T1), 4 (T2), and 5 (T3), were given LA (10 mg/kg BW) plus graded concentrations of 30, 60, and 120 mg/kg BW of EBN, respectively. At day 35, blood was collected via cardiac puncture, serum was used for biochemical analysis, and rats were euthanized to collect liver and kidney for histo-morphological study. Laboratory analysis revealed significantly elevated liver enzymes, urea and creatinine levels in the T0 and T1 compared to C and T3 ($p<0.05$). The level of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was significantly lower in the T3 and C compared to T0 and T1 ($p<0.05$). Histo-morphological studies showed that exposed rats to LA without EBN supplement with portal and central vein dilatation and congestion evidenced by hepatocyte necrosis and degeneration as well as increased number of kupffer cells, while degree of damage was decreased in EBN treated groups. The animals in T3 showed ameliorating effects against LA toxicity, as well as decreased number of kupffer cells. In T0 and T1 rats, histopathological lesions of the kidneys were characterized by the degenerations of the tubular system, while T2 and T3 groups showed no such lesions. In conclusion, the findings showed that EBN can protect the hepatic and renal tissues from the damaging effects of LA toxicity and modulate biochemical alterations.

Keywords: EBN, lead acetate, liver, kidney, Kupffer cell

1. Introduction

Adverse effects of industrialization and urbanization increase the risk of daily exposure to a variety of chemical contaminants. Lead (Pb) is considered one of most important hazardous and cumulative environmental pollutants. Environmental levels of lead have increased more than 1000-fold over the past three centuries as a result of human activity and due to increasing worldwide use of leaded gasoline (ASDR, 2007). Lead has a negative impact on animal and human physiological, behavioral and biochemical functions, including liver, kidneys, reproductive system, hematopoietic system, central and peripheral nervous system, cardiovascular system, and endocrine glands (ASDR, 2007). Lead acetate can accumulate in human and animal tissues and can be induced by various factors such as age or hormone production to re-release Pb into the general circulation. Consequently, it also has long half-life (Horiguchi et al., 2013; Rzymski et al., 2014). Several studies have shown that Pb toxicity has a negative effect on vital organs associated with hepatic and renal damage (Farrag, 2007; Assi et al., 2017 a). One of the important mechanisms underlying Pb toxicity is the induction of oxidative stress because of reactive oxygen species and the depletion of the antioxidant defense system (Gupta, 2011). Edible bird's nest (EBN) is a natural product from swiftlet saliva and is traditionally considered a powerful medicine used by the Chinese community for centuries to alleviate several ailments (Koon, 2002). Research on various aspects of EBN has recently gained momentum, including its nutritional and health benefits. The traditional beliefs are largely supported by scientific explanations. Results from emerging researches have shown that EBN has a number of biological properties. These biological properties of EBN may have an impact on hepatic and renal diseases (Yida et al., 2015). Studies carried out in areas other than toxicity on the influence of EBN have reported important biological properties of EBN, which may reflect its ameliorating effect on the influence of heavy metal toxicity. EBN has been shown to be a potent antioxidant, but its role in mitigating the effect of Pb toxicity on liver and kidney is unknown. Therefore, the general objective of the present study was to evaluate the ameliorating effects

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of EBN supplement on hepato- renal histomorphology of rats exposed to lead acetate (LA) toxicity.

2. Materials and Methods

2.1. EBN preparation

EBN was purchased from Nest Excel Resources Sdn Bhd, maintained at 25 ℃ to 30 ℃. EBN extract was prepared in accordance with Chinese tradition as indicated by the local suppliers. The samples were cleaned, dried at room temperature, and ground into powder using a mixer (BUCHI-400, Switzerland). The ground EBN extract was maintained at 4 ℃. EBN solution was prepared by dissolving 1 g of EBN powder in 100 mL of distilled water, followed by heating in a water bath at 60 ℃ for 45 min. Lastly, the EBN solution was cooled down to room temperature and administered to the rats at doses based on their body weights (BW).

2.2. Preparation of lead acetate solution

Lead acetate with molecular formula Pb (C2H3O2)2, was purchased commercially from Oxford Lab. Co., India (CAS: 6080-56-4). A 1% (w/v) solution of lead acetate in distilled water was initially prepared and individual rats of the treated groups were given orally at a dose of 10 mg/kg of body weight using a gavage tube (straight 18 gauge, China).

2.3. Animals and experimental design

The study used 30 female Sprague–Dawley rats (12 weeks of age) from the Animal Resource Center, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). The rats were housed in groups for acclimatization period of 7 days with free access to water and standard rat diet (Gold Coin Brand Animal Feed). Approval to the use of rats for the experiment was obtained from the Institute for Animal Care and Use Committee, UPM (UPM/IACUC/AUP-R009/2016) and animals were handled in accordance with the guidelines. After 7 days of acclimatization, the rats were randomly grouped into 5 with 6 animals per group and administered LA at 10 mg/kg BW with oral supplement of EBN according to Albishtue et al. (2018a). Rats were divided into five groups, namely: Control (C), given distilled water orally; Positive control (T0), given LA (10 mg/kg); Treatment 1 (T1), given EBN (30 mg/kg) and LA (10 mg/kg), orally, daily; Treatment 2 (T2), given EBN (60 mg/kg) and LA (10 mg/kg); and Treatment 3 (T3), was given EBN (120 mg/kg) and LA (10 mg/kg). Both LA and EBN were administered orally once daily for a period of 35 days. The rats were euthanized after 35 days by CO_2 asphyxiation method following a general anaesthesia procedure, which included injection of ketamine (30 mg/kg BW) and xylazine (10 mg/kg BW) and blood collected by cardiac puncture according to the method of Albishtue et al. (2018 b).

2.4. Evaluation of biochemical parameters

An automated chemistry analyzer (Siemens, USA) was used to assess biochemical parameters from the sera obtained from the whole blood of the rats according to the methods of Assi *et al*. (2017 a).

2.5. Macroscopic and microscopic examinations of the liver and kidney

After sacrificing the rats, the livers and kidneys were excised and physically examined for any evidence of gross tissue abnormality. The tissue samples were fixed in 10% formalin for 24 h, processed, sectioned, and stained using hematoxylin and eosin (H&E) for histomorphological examinations. Stained tissues were examined under light microscopy and viewed with image analyser microscope. Three microscopic areas of similar region for each tissue sections were studied at various magnifications (40x and 100x magnifications). Sections were randomly selected for analysis using the Medical Image Analyser (Motic Image plus 2.0, China). The samples were observed under the microscope for histomorphological changes. Numbers of hepatocyte vacuolations and pyknotic hepatocytes in an area were counted at a magnification of 40x. Lesion severity was scored from 0 to +3 (0, no lesion; 1, mild lesion (<30%); 2, moderate (<60%); 3, severe (>60%) (Assi *et al*., 2017a). Number of Kupffer cells of the liver in an area was counted at a magnification of 40x.

Similarly, degeneration score of the tubular systems of the kidney in an area was also counted at a magnification of 40x using the same image analyser. Three microscopic areas of similar region for each tissue sections were studied. Lesion severity was scored according to Assi *et al*. (2017a) from 0 to +3 (0; no lesion; 1, mild lesion (<30%); 2, moderate (<60%); 3, severe (>60%)).

2.6. Statistical analysis

All data were expressed as means (M) ± standard error of mean (SE) and analysed with Graph Pad Prism 6.0 (Graph Pad Software, San Diego, California). Data obtained were checked for normal distribution using Shapiro-Wilk test. One-way analysis of variance (ANOVA) with Tukey multiple comparison post-hoc test was used to compare number of Kupffer cells and the concentrations of AST, ALP, Urea and Creatinine in the sera. Comparison between histopathological lesions in the liver and kidney were made using a Kruskal-Wallis (non-parametric) test. A value of $p<0.05$ was considered significant.

3. Results

3.1. Biochemical findings

The results of AST, ALP, Urea and creatinine activities in the serum from all the groups are summarized in Figures 1.A and B. The level of AST activity was elevated in the T0 group ($p<0.05$), while T1 was unchanged when compared to the control. On the other hand, AST activity was reduced in the T2 and T3 groups. Similarly, ALP activity was significantly higher in T0 group compared to group T3. There were no significant changes in T1 and T2 groups. The concentration of urea was significantly higher in T0 and T1 groups compared to the Control ($p<0.05$). However, T3 had low level of urea when compared to other treatment groups and control group ($p<0.05$) (Figure 1.C). On day 35, there was no significant difference between the treatment groups in creatinine concentration (Figure 1.D).

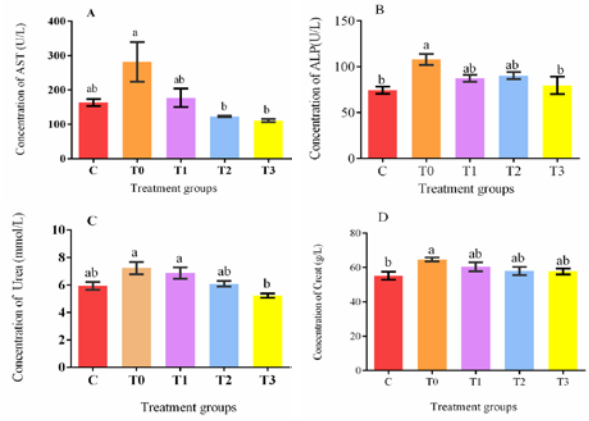

Figure 1. Effect of EBN on the serum biochemistry of LA exposed rats. AST = Aspartate aminotransferase; ALP = alkaline phosphatase; Creat = creatinine. Data are expressed as means ± S.E.M. Different letters a and b denotes significant difference at $p < 0.05$. Note: C = control; T0 = positive control, LA (10mg/Kg BW); T1= EBN (30mg/Kg BW) + LA (10mg/Kg BW); T2 = EBN (60mg/Kg BW) + LA (10mg/Kg BW); T3= EBN (120mg/Kg BW) + LA (10mg/Kg BW).

3.2. . Histomorphological findings in the livers and kidneys of rats exposed to lead acetate and supplemented with EBN

There were no apparent gross pathological lesions found in the liver and kidney of the rats. Nevertheless, histomorphological inspection of liver and kidney of the different groups except C and T3, showed lead-induced histopathological alterations, which involves portal and central veins dilatation and congestion evidenced by hepatocyte necrosis and degeneration, increased infiltration of inflammatory cells, and damaged tubular system of kidney. However, T3 supplemented with the highest EBN dose showed normal hepatic and renal structures as in the control group (Figures 2 and 3).

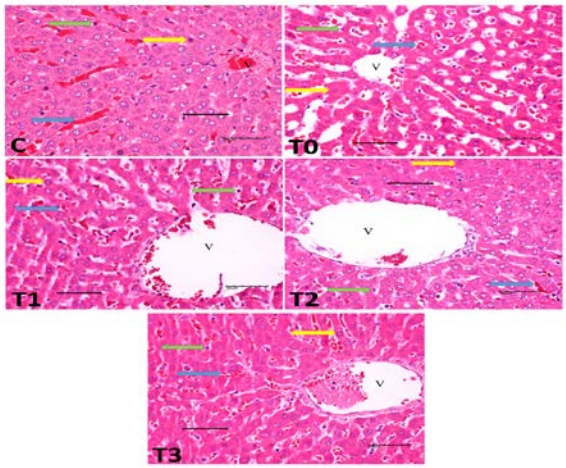

Figure 2. Histomorphological sections of the liver showing the effects of EBN in LA exposed female rats. T0 and T1 showing moderate vacuolation of the hepatocytes (black arrows) and pyknotic hepatocytes cells (yellow arrows) with a congested central vein (V). Notice Kupffer cells (green arrows); vascularization (blue arrows); C=control; T0= positive control, LA .(10mg/Kg BW); T1= EBN (30mg/Kg BW) + LA (10mg/Kg BW); T2= EBN (60mg/Kg BW) + LA (10mg/Kg BW); T3= EBN (120mg/Kg BW) + LA (10mg/Kg BW) (measure of scale bar = 50 µm, H & E 40 x magnification).

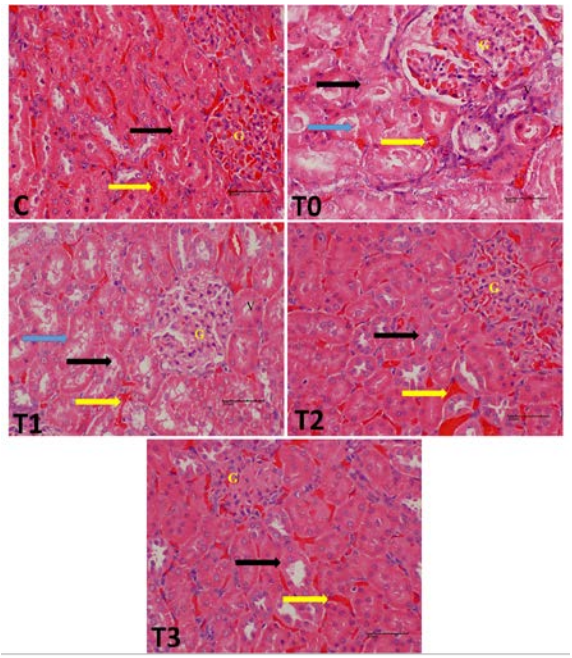

Figure 3. Histomorphological sections of the kidney showing the effects of EBN in LA exposed female rats. Notice degenerative changes of tubular systems (blue arrows) at T0 and T1. Renal structure of tubular systems (black arrows) and glomeruli (G) of nephrons and vascularization (yellow arrows). Note: C = control; T0 = positive control, LA (10mg/Kg BW); T1= EBN (30mg/Kg BW) + LA (10mg/Kg BW); T2= EBN (60mg/Kg BW) + LA (10mg/Kg BW); T3= EBN (120mg/Kg BW) + LA (10mg/Kg BW) (measure of scale bar = 50 µm, H & E 40 x magnification).

The results of histopathological lesion scores of the liver are presented in Figure 4. The results showed a statistically significant difference in the hepatocyte lesions with vacuolations and pyknotic hepatocytes among the experimental groups ($p<0.05$). The hepatocyte vacuolations and pyknotic hepatocytes were higher in T0 and lowest in T3 group (Figure 4). Kupffer cell proliferation had the lowest value at 120 mg/kg body weight of EBN (T3) compared to T0. The number of Kupffer cells found to decrease in a dose dependent manner with EBN. T2 had comparable with T1 and T3 (Figure 5).

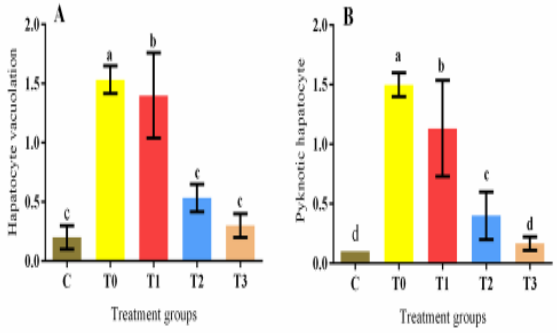

Figure 4. Effects of EBN on histopathological lesion scores in LA exposed livers of rats. C = control; T0 = positive control LA (10mg/Kg BW); T1= EBN (30mg/Kg BW) + LA (10mg/Kg BW); T2= EBN (60mg/Kg BW) + LA (10mg/Kg BW); T3= EBN (120mg/Kg BW) + LA (10mg/Kg BW) (positive control magnification).

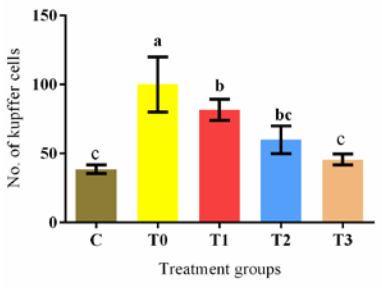

Figure 5. Effects of EBN on histomorphometric parameters in LA exposed liver of rats. Notice the greater quantity of Kupffer cells in T0 and T1, than the other groups. Data are expressed as means ± standard error (SE). Error bars with different alphabets (a, b, and c) denotes significant difference at $p < 0.05$.

Representative photomicrographs showing tubular degenerations and the histological sections of the kidneys of all experimental groups as well as their differences in scores are shown in Figure 6. Tubular degeneration score in the kidney was significantly higher in T0 group than in the T1 group ($p<0.05$).

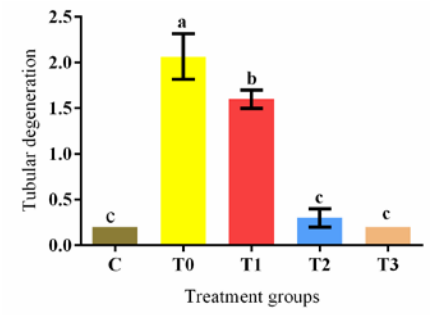

Figure 6. Effects of EBN on histopathological lesion scores in LA exposed kidneys of rats. C = control; T0 = positive control, LA (10mg/Kg BW); T1= EBN (30mg/Kg BW) + LA (10mg/Kg BW); T2= EBN (60mg/Kg BW) + LA (10mg/Kg BW); T3= EBN (120mg/Kg BW) + LA (10mg/Kg BW).

4. Discussion

The liver is a vital organ for storage, biotransformation and detoxification of toxic substances, particularly absorbed heavy metal materials (Ohkawa et al., 1979). In the present study, although laboratory findings have shown different alterations in the physiological functions of the animals, LA showed no gross pathological lesions in the liver and kidney. Examination of the tissue sections of the liver exposed to LA revealed that the portal and central veins underwent dilatation and congestion as evidenced by hepatocyte necrosis and degeneration. Kupffer cells represent 15% of the total liver cells and cover inside endothelial walls where they protrude and are found predominantly in the periportal region, which may represent the best location for the cell to exhibit their endocytic role for blood-born materials entering the liver (Arii and Imamura, 2000). The phagocytic capacity of Kupffer cells is characterized by very high, and numerous phagocytic structures and a large number of different types of very well developed lysosomes (Arii and Imamura, 2000). The liver can be damaged by different injuries such as chemical substances, toxins and pharmacological agents (Winwood and Arthur, 1993; Su, 2002). The immediate resulting effects of liver injuries are increased hepatocellular necrosis, which is one of the principal sources of Kupffer cells activation (Kolios et al., 2006). This is in agreement with the present study, which confirmed LA effect on the number of Kupffer cells. Another pertinent finding observed in the present study was the elevated levels of ALS and ALP in T0 group. The ALT and AST are two important enzymes that signify liver function in animals. An increased level of these enzymes indicates possible hepatocellular damage or injury (Assi et al., 2017a).

Prior studies have revealed that lead toxicity resulted in elevated serum ALT, AST, liver homogenate tumor necrosis factor-a (TNF-a), caspase-3, malondialdehyde (MDA), nitric oxide (NO) levels and a significant decline of total serum proteins, liver homogenate reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity (El-Tantawy, 2016). On the other hand, the administration of LA could have generated a high level of oxidative stress which can cause defects in mitochondrial membrane permeability, resulting in outflow of free radicals and cytochrome-c from the mitochondria to bind with another protein called apoptotic protease activating factor-1 (Apaf-1), which stimulates the activation of caspase 3, leading to cell death (Liu *et al.*, 2005: Liu *et al*., 2012). Omobowale *et al.* (2014) undertook investigation of Pb-induced oxidative stress in the rat liver. Animals were administered with different doses for six weeks and then were not treated for another six weeks. The result revealed disturbances in homaginized liver ALT, AST, ALP, MDA and H_2O_2 concentrations, and a decrease in antioxidant enzymes SOD, catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) activities and lower concentration of GSH. According to the observations of Omobowale *et al.* (2014), in the liver, Pb caused the stimulation of MDA levels and lipid peroxidation that is considered as source of the modification of membrane integrity and fatty acid composition (Liu *et al*., 2012). In a similar finding, Pb-induced oxidative stress increase in liver (measured by MDA levels) in dose and time-dependent manner, and a consequence of the oxidative stress and apoptosis leading to influence mitogen-activated protein kinases in hepatocytes were reported (Mujaibel and Kilarkaje, 2015). Interestingly, histological examination of the livers of EBN treated groups in the present study showed significant protection. EBN supplemented groups especially T3 showed normal hepatic structure as in the control despite exposure to LA toxicity. Furthermore, it was observed that powdered EBN acted as a regulator for the levels of AST and ALP in treated rats. In previous studies, there was evidence of decreased GSH and SOD enzyme levels in rats exposed to LA (Assi *et al*., 2017 b); however, it is a limitation of the present study that these parameters were not assessed.

The EBN has been used as a hormonal replacement prophylactic agent in various conditions such as in the form of anti-aging, anti-cancer, and immunity enhancement. The protective role of the EBN supplement observed might be attributed to its biological properties such as cellular proliferation. The composition of EBN reported to consists of reproductive hormones including testosterone, estradiol (E2), progesterone (P4), LH, FSH and prolactin (Ma and Liu, 2012; Albishtue *et al.*, 2019a). EBN contains sialic acid which has some molecular mechanisms of causing cell proliferation being regulated

by the hormone E2. EBN is also known to contain VEGF and IL-6 which prevent apoptosis of embryonic neurons by interfering with the activation of caspase three leading to suppression of apoptosis in cells (Jin *et al*., 2001). A previous study had suggested that there is a synergistic relationship between VEGF, FSH and estradiol in preventing apoptosis, inhibiting caspase 3 activation and stimulating proliferation (Vitale *et al*., 2002; Bussmann *et al*., 2006; Irusta *et al*.,2010). EBN has Epidermal Growth Factor (EGF) – like activity (Kong *et al*., 1987), resulting in its stimulatory effect on cell growth and regeneration (Ng *et al*., 1986; Kong *et al*., 1989; Zhiping *et al*., 2015). Oxidative stress created by LA exposure is a major disorder contributor, which causes the development of toxicity that indicates significant pathological lesions on the vital organs (Oyagbemi *et al*., 2015). EBN has been shown to regulate antioxidant and inflammatory genes (Yida *et al*., 2015) and have many therapeutic benefits.

Presence of LA in the hepatocytes has been reported to induce necrosis, degeneration, and fatty change in a dose-dependent manner (Mudipalli, 2007). Although the present study has a limitation to provide evidence as to what happen to the oxidative stress and anti-oxidant biomarkers, we have demonstrated a similar positive effect of EBN on oxidative stress in our previous study (Albishtue et al., 2019b). Hence, based on the information on histo-morphological findings, it can be deduced that EBN prevents damage to the hepatocytes possibly through modulation of oxidative stress, and this may imply a strong ameliorative effect of EBN against LA toxicity on the hepatic tissues through an integrated mechanism.

The present study has shown that LA exposure for extended time stimulates gradual loss of membrane integrity and changes in tubular cells. Moreover, the toxicity results in the present study showed that urea and creatinine levels in LA alone administered groups were significantly higher than those in other groups supplemented with EBN. Laboratory findings revealed that concentration levels of urea and creatinine were higher in rats exposed to LA compared to the control. According to most authors, renal toxicity of LA can be characterised with evidence of degenerations of the tubular epithelium associated with nuclear bodies, which contain lead-protein complexes, and impaired tubular transport (Assi *et al*., 2017a). Most of the authors agreed that LA induces production of lipid peroxidation. Sharma and Singh (2014) have administered 10 and 150 mg/kg BW LA for 24 h and stimulated increased in renal TBARS levels and reduce SOD and CAT activities in the kidney of Balb-c mice. Interestingly, histological examination of the kidney of EBN treated groups in our study showed significant protection. Moreover, despite exposure to LA toxicity, EBN supplemented groups (especially T3) showed normal renal structure similar to the control. Furthermore, it was also observed that powdered EBN acted as a regulator for the levels of urea and creatinine in treated rats. Assessment of additional parameters such as fatty degeneration and intranuclear inclusion rates in kidneys in future might further strengthen the understanding of the mechanism of EBN by which it protects the kidney tissues. Similar to EBN, there has been an increase of interest in the consumption of other natural products that serve as antioxidants like seeds of nigella sativa, fruits and vegetables as a strategy to prevent oxidative damage in various health disorders with oxidative stress (Assi *et al*., 2017; Khaki *et al*., 2013). In previous reports, EBN was also praised for its effect of increasing SOD enzyme levels in rats exposed to LA (Albishtue *et al*., 2019b).

5. Conclusion

The present study suggested that pre-treatment of EBN significantly and preventively attenuated LA-induced detrimental effects on the liver and kidney as evidenced by decreased concentrations of toxicity indicators in the sera and maintenance of histomorphological integrity.

Acknowledgement

The authors would like to thank UPM for providing fund for this project (Grant Putra IPS/9641900).

Competing Interests

None

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