**Ipomoea aquatica** Extract Reduces Hepatotoxicity by Antioxidative Properties following Dichlorvos Administration in Rats

Mohammad Reza Salahshoor¹, Amir Abdolmaleki¹, Ahmad Shabanizadeh², Amir Jalali², Shiva Roshankhah*¹

¹Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran, ²Department of Anatomical Sciences, Immunology of Infectious Diseases Research Center, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran, ³Department of Nursing, Kermanshah University of Medical Sciences, Kermanshah, Iran

**Abstract**

*Ipomoea aquatica* (IA) with antioxidant properties is used in therapeutic trends. An organophosphate, dichlorvos (Dich), is a common insecticide with various side effects on living tissues. This study examines the role of IA on Dich-induced hepatotoxicity in male rats. Sixty-four male rats were divided into eight groups including sham, Dich (4 mg/kg/day, intraperitoneally), IA 1, 2, and 3 (250, 500, and 1000 mg/kg/day, respectively, orally), and Dich + IA 1, 2, and 3. All treatments were applied daily for 60 days. At the end of the treatment, the animals were sacrificed. The histopathological changes, leukocyte infiltration, and apoptosis were assessed by light and fluorescent microscopy. The serum levels of hepatic enzymes, nitrite oxide (NO), and total antioxidant capacity (TAC) were evaluated biochemically. Dich statistically significantly increased the NO level, hepatic enzyme activity, apoptosis, leukocyte infiltration, the mean diameter of hepatocytes (DHs), and central hepatic vein diameter (CHVD) and also decreased the TAC, mean weight of liver, and the total weight of rats compared to the sham group (*P* < 0.01). In all IA and Dich + IA groups, a statistically significant decrease was detected in apoptosis, leukocyte infiltration, hepatic enzyme activity, NO level, mean DH, and CHVD, whereas an increase in TAC level, mean liver weight, and total weight was detected compared to the Dich group (*P* < 0.01). IA, due to the antioxidant property, recovers the Dich-related catastrophic changes in liver.

**Keywords:** Antioxidative, dichlorvos, hepatotoxicity, *Ipomoea aquatica*

**INTRODUCTION**

The biochemical structure of organophosphates has pesticide effects on harmful insect attacks. The classification of organophosphates based on WHO protocol is in Class B as highly toxic chemicals. These substances are known as the third cause of poisoning in Iran.² Three molecular pathologic mechanisms related to organophosphate are phosphorylation of cholinesterase active site and the cell membranes damaged by disruption of antioxidant system.³ Dichlorvos (Dich) (C₄H₇Cl₂O₄P) was discovered in 1940 and commercialized in 1972.⁴ Other names of Dich are Nuvan, Dedevap, Phosvit, and Nogos.⁵ Dich is rapidly absorbed by the gastrointestinal system, skin, and respiratory tracts and undergoes through the liver metabolism cycle.⁶ The mechanism of organophosphorus operation is conducted by the inhibition of acetylcholinesterase and enzymatic degeneration of acetylcholine.⁷ According to the scientific findings, Dich reduces the activities of antioxidant enzymes such as superoxide dismutase which induces the mitochondrial apoptosis pathway in liver tissues.⁸ Dich is a potential cause of cancer generation.⁹ Ten. Contamination or exposure to Dich leads to hepatic histological and biochemical changes, including ulcers, hepatocyte irritation, sinusoidal space increment, necrosis, and nuclear hypertrophy of the

---

*Address for correspondence: Dr. Shiva Roshankhah, Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Daneshgah Ave., Taghbostan, Kermanshah, Iran. E-mail: roshankhah@yahoo.com*

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

**How to cite this article:** Salahshoor MR, Abdolmaleki A, Shabanizadeh A, Jalali A, Roshankhah S. *Ipomoea aquatica* extract reduces hepatotoxicity by antioxidative properties following dichlorvos administration in rats. Chin J Physiol 2020:63:77-84.
Vegetables with a high amount of fiber and low energy content reduce appetite, but they are rich in nutrients such as vitamins, minerals, proteins, and fibers. It is estimated that 70% of the world population uses medicinal plants. Medicinal plants have biochemical substances with potential therapeutic effects. Ipomoea aquatica (IA) is of this category, which belongs to the Convolvulaceae family. The plant grows in the wild and is also cultivated in Asia. Due to its rapid growth, it is common in rice paddies, fish ponds, and drainage canals. IA is a useful natural herb used in traditional medicine. Its traditional use and pharmacologic activities have been supported by in-vivo and in-vitro studies. Furthermore, the practical features of IA confirmed the presence of essential and active phytochemicals. The leaf of IA increases the antioxidant level, leading to the prevention of oxidative stress production, and also protects the DNA against oxidative damages. Due to the numerous antioxidant properties of IA and the poor findings regarding its beneficial effects on liver, this study was designed to assess the probable impacts of IA against Dich hepatotoxicity model in rats.

**Materials and Methods**

**Experimental design and animal groups**

Sixty-four male Wistar rats were divided into eight groups including sham (Group 1, received 0.09% normal saline), Dich (Group 2, received 4 mg/kg Dich, intraperitoneally [i.p.]), IA (Groups of 3, 4, and 5 received 250, 500, and 1000 mg/kg IA, respectively, orally), and Dich + IA (Groups of 6, 7, and 8 received 4 mg/kg Dich, i.p. + 250, 500, and 1000 mg/kg IA, respectively, orally). In all Dich groups, the rats were treated with a single dose (4 mg/kg), but in IA groups, they were treated with triple doses (250, 500, and 1000 mg/kg). All experimental interventions were applied at 9 a.m. for 60 consecutive days.

**Animals**

Animals were purchased from the Pasteur Institute (Tehran, Iran) and placed in the animal house of Medical School. The animals were kept under standard conditions (12 h light/12 h dark, 22°C ± 2°C, humidity of 50%–60%) in special cages and on a straw bed. Water and standard food plates were freely available. All animals were treated in accordance with guidelines of National Institute of Health for the Care and Use of Laboratory Animals approved by Research Deputy at Kermansh University of Medical Sciences based on WMA Declaration Ethic of Helsinki (Ethic number: IR.KUMS.REC.1398.0161).

**Ipomoea aquatica extract preparation**

IA plant was obtained from the Center of Medicinal Plants. A botanist evaluated the accuracy and viability. The plant was purged and the leaves and stems were desiccated in shadow for 5 days and subsequently, they were grounded. For preparation of ethanolic solution, 100 g of the plant powder was added to 70% ethanol, and it was reserved in a warm water bath (36°C) in a dark environment. The solution was progressively poured on a Buchner funnel filter paper and cleaned by a vacuum pump, and then it was transferred to a rotary device in order to obtain the extra solvent. This process was continued until the concentrated extract was obtained which was finally dissolved in distilled water (1.5 cc).

**Animal weighing and sampling**

In the two stages of treatments, the rats were weighted using a digital scale with 0.001 g accuracy (Precisa 125A, Chicago, USA), including at the first and a day after the last treatment. The animals were anesthetized by ketamine HCl (70 mg/kg) and xylazine (20 mg/kg) through intramuscular injection. Immediately, the animals were sacrificed by cervical dislocation. Small liver fragments and 5 cc blood samples were prepared. The blood serum was isolated with a centrifuge (1500 g, 15 min) and kept at −80°C for future biochemical assessments including hepatic enzyme, nitrite oxide (NO), and total antioxidant capacity (TAC) levels. The weight of liver specimens was recorded and then, they were preserved in 20% buffered formaldehyde for apoptosis and quantitative histopathological assessment.

**Histopathological examination of liver and measurement of leukocyte infiltration**

The process of tissue preparation was applied by automatic tissue processors through routine histological method: 5-μm histological sections (five sectional intervals) were prepared and stained by hematoxylin and eosin. The diameter of hepatocyte (DH) and central hepatic vein diameter (CHVD) used in liver morphometric analysis were calculated by a zigzag form of monitoring. This method was done by double-blinded observers, on the five fields of each section (thirty fields/animal). For each hepatocyte, the full cellular area, the hepatocyte outline, and the maximum and minimum axes (for mean axis) were measured. At least fifty cells from each zone were measured in each liver fragment. All quantitative measurements were done by Motic camera (CF-4; Motic, Washington, USA) attached to a light microscope (×200 magnification) (CM-3; Motic USA) with the use of appropriate software, based on randomly selected captured pictures. To analyze the rate of leukocyte infiltration, the process was carried out in the following order: number of leukocytes was counted in 30 microscopic fields (0.15 mm²), the average number was calculated, and, finally, it was used to estimate the quantity of each field.

**Measurement of liver enzymes available in plasma**

The fragments of the liver were changed into homogenate mixture. This solution was centrifuged twice (1500 rpm, 20 min), and the supernatant was separated to biological enzyme assessments. The activity of two important enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was analyzed by the Reitman and Frankel method. The alkaline phosphatase (ALP) protocols were determined according to the procedure, which was set out in a practical laboratory manual.
Evaluation of nitrite oxide serum levels
The serum level of NO was measured by the Griess procedure. 10 mg powder of zinc sulfate was mixed with 500-μl serum samples and vortexed for 2 min to eliminate the serum protein. The samples were centrifuged (15,000 rpm, 4°C, 10 min), and the obtained supernatant was used to measure the NO level. Briefly, 100 μl of the sample was added to 200 μl of the reagent (Sigma; St. Louis, USA), and the reaction mixture was incubated for about 20 min at room temperature. The sample optical density was measured by enzyme-linked immunosorbent assay reader (Hyperion; Washington, USA) at a wavelength of 540 nm according to the manufacturer’s protocol.[23]

Total antioxidant capacity measurement
The TAC value was assessed via TEAC and Randox kits based on the formation of ABTS + 3, 3′-azinobis (4-ethylbenzothiazoline-5-sulfonic acid) action and then inhibited by antioxidant compounds of the sample. In fact, the reduction in the absorption of green water chromophore is inversely related to the antioxidant content of the samples. Trolox as the control antioxidant is an analog of hydrophilic vitamin A.[24]

Apoptosis
For apoptotic cell detection, the TUNEL assay was used according to the procedures in kit (Roche, Basel, Switzerland). Extra tissues attached to the liver fragments were dissected, and paraffin-embedded blocks were prepared. Coronal histological thin sections (5 μm) were cut by a microtome (Leica RM 2235, Leica Microsystems Nussloch GmbH, Munich, Germany), and five sections for each animal were chosen. After routine deparaffinization and blocking of endogenous peroxidase with 0.3% hydrogen peroxide in methanol, the incubation with 50 μg/mL proteinase K (Sigma, USA) was performed. The sections were exposed to terminal deoxynucleotidyl transferase with digoxigenin-12-dUTP. Counterstaining was performed by adding 0.2% methyl green solution. Apoptotic index (AI) was obtained by the observation of 200 cross-sectional areas from different areas of the liver.[25]

Statistical analysis
The data were presented as mean ± standard error of the mean. One-way ANOVA was used for multiple group comparisons followed by Turkey’s test using SPSS software (Version 16, IBM, New York, USA). P < 0.05 was considered statistically significant.

Results
Total body and liver weights
The mean weight of liver and total weight of animals revealed a statistically significant decrease in the Dich group compared to the sham group (P < 0.01). The mean of weights also indicated statistically significant increase in all IA and Dich + IA groups in comparison with the Dich group (P < 0.01). The weights in IA and Dich + IA groups displayed no statistically significant changes compared to the sham group (P > 0.05) [Table 1].

Diameter of central vein and mean diameter of hepatocyte
As shown in Figure 1, a statistically significant increase in CHVD and DH was detected in the Dich group compared to the sham group (P < 0.01). The CHVD and DH also indicated statistically significant reductions in all IA and Dich + IA groups compared to the Dich group (P < 0.01). IA: Ipomoea aquatica, Dich: Dichlorvos, CHVD: Central hepatic vein diameter, DH: Diameter of hepatocyte.

Table 1 The effect of Dich and Ipomoea aquatica on AI, body weight and liver weight in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver weight (g)</th>
<th>Body weight (g)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>9.68±1.02</td>
<td>250.19±4.2</td>
<td>1.2±0.01</td>
</tr>
<tr>
<td>Dich</td>
<td>3.54±0.05**</td>
<td>161.3±4.3**</td>
<td>3.18±1.3**</td>
</tr>
<tr>
<td>IA1</td>
<td>9.04±1.3s</td>
<td>259.1±6.1s</td>
<td>1.3±0.01s</td>
</tr>
<tr>
<td>IA2</td>
<td>9.72±0.94ss</td>
<td>258.2±4.7ss</td>
<td>1.3±0.06ss</td>
</tr>
<tr>
<td>IA3</td>
<td>9.5±0.95ss</td>
<td>251.3±4.4ss</td>
<td>1.4±0.05ss</td>
</tr>
<tr>
<td>Dich + IA1</td>
<td>7.42±0.34ss</td>
<td>172.8±6.9ss</td>
<td>2.02±0.04ss</td>
</tr>
<tr>
<td>Dich + IA2</td>
<td>7.31±1.17ss</td>
<td>187.7±4.4ss</td>
<td>1.91±0.02ss</td>
</tr>
<tr>
<td>Dich + IA3</td>
<td>7.91±1.76ss</td>
<td>199.0±3.3ss</td>
<td>1.94±0.02ss</td>
</tr>
</tbody>
</table>

The data were presented as mean±SEM **P<0.01, compared to sham group; *P<0.01 compared to Dich group. Dich: Dichlorvos, IA: Ipomoea aquatica, AI: Apoptotic index, IA1, 2, 3: IA at a dose of 250, 500, 1000 mg/kg respectively.

Figure 1: Effect of Dich, IA, and IA + Dich administration on the CHVD (a) and DH (b). **Statistically significant difference compared to the sham group (P < 0.01). ††Statistically significant difference compared to the Dich group (P < 0.01). ††Statistically significant difference compared to Dich group (P < 0.01). IA: Ipomoea aquatica, Dich: Dichlorvos, CHVD: Central hepatic vein diameter, DH: Diameter of hepatocyte.
Salahshoor, et al.: Ipomoea aquatica and dichlorvos effects on liver

in comparison with Dich group ($P < 0.01$). The CHVD and DH in IA and Dich + IA groups showed no statistically significant differences compared to the sham group ($P > 0.05$) [Figure 1].

**Leukocyte infiltration rate**
Following Dich administration, the lymphocyte infiltration from the liver vessels increased significantly in Dich group compared to the sham group ($P < 0.01$). The mean infiltrated lymphocytes decreased statistically significantly in IA-treated animals in IA and Dich + IA groups compared to the Dich group ($P < 0.01$). The lymphocyte infiltration showed no statistically significant trend in IA groups (IA1, IA2, IA3) compared to the sham group ($P > 0.05$) [Figure 2].

**Estimation of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase**
Dich caused a significant increase in enzymes in comparison with the sham group ($P < 0.01$). The mean concentration of enzymes showed no statistically significant alteration in IA groups compared to the sham group ($P > 0.05$). Furthermore, in all IA and Dich + IA groups, statistically significant reduction was recorded in the mean of ALT, AST, and ALP enzymes in comparison with the Dich group ($P < 0.01$) [Table 2].

**Nitrite oxide serum levels**
A statistically significant increase in mean serum NO was found in Dich group compared to the sham group ($P < 0.01$). This value showed no statistically significant differences in all IA groups compared to the sham group ($P > 0.05$). In addition, the mean of NO in serum was statistically significantly reduced in all IA and Dich + IA groups compared to the Dich group ($P < 0.01$) [Figure 3].

**Serum total antioxidant capacity levels**
A statistically significant decrease in CHVD and DH was observed in the Dich group compared to the sham group ($P < 0.01$). The TAC also indicated statistically significant increases in all IA and Dich + IA groups in comparison with Dich group ($P < 0.01$). The TAC value in IA and Dich + IA groups represented no statistically significant alterations compared to the sham group ($P > 0.05$) [Figure 4].

**Number of apoptotic cells**
The AI was statistically significantly higher in Dich group compared to the sham and other treatment groups ($P < 0.01$), whereas there were no statistically significant differences between Dich + IA and sham groups ($P > 0.05$) [Table 1 and Figure 5].

**Histopathological changes**
Histological analysis showed normal liver structure in the sham and IA treatment groups. After treatment with Dich (in Dich group), the liver showed visible anomalous changes including the increment in the number of white blood cells

---

**Table 2 The effect of Dich and IA on the liver enzymes in rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (ng/mL)</th>
<th>ALT (ng/mL)</th>
<th>ALP (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>29.3±2.3</td>
<td>58.9±3.2</td>
<td>161.09±3.3</td>
</tr>
<tr>
<td>Dich</td>
<td>88.1±2.9**</td>
<td>99.4±3.8**</td>
<td>253.61±5.1**</td>
</tr>
<tr>
<td>IA1</td>
<td>31±2.6**</td>
<td>58.3±3.8**</td>
<td>164.15±1.7**</td>
</tr>
<tr>
<td>IA2</td>
<td>31.9±1.6**</td>
<td>60.5±4.6**</td>
<td>167.65±3.6**</td>
</tr>
<tr>
<td>IA3</td>
<td>32.7±1.4**</td>
<td>59.7±2.7**</td>
<td>169.78±3.1**</td>
</tr>
<tr>
<td>Dich + IA1</td>
<td>50.2±1.99**</td>
<td>70.3±3.9**</td>
<td>188.9±3.1**</td>
</tr>
<tr>
<td>Dich + IA2</td>
<td>53.8±5.26**</td>
<td>75.46±4.2**</td>
<td>194.45±5.1**</td>
</tr>
<tr>
<td>Dich + IA3</td>
<td>47.6±3.55**</td>
<td>78.27±3.75**</td>
<td>195.15±5.43**</td>
</tr>
</tbody>
</table>

The data were presented as mean±SEM. **$P < 0.01$, compared to sham group; $P < 0.01$ compared to Dich group. Dich: Dichlorvos, IA: Ipomoea aquatica, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase. IA1, 2, 3: IA at a dose of 250, 500, 1000 mg/kg, respectively.**

---

**Figure 2:** Mean of leukocyte infiltration per mm². **Statistically significant difference compared to the sham group ($P < 0.01$). ††Statistically significant difference compared to Dich group ($P < 0.01$). †Statistically significant difference compared to Dich group ($P < 0.01$). IA: Ipomoea aquatica, Dich: Dichlorvos.

**Figure 3:** Effects of Dich, IA, and IA + Dich on mean levels of NO. **Statistically significant difference compared to the sham group ($P < 0.01$). †Statistically significant difference compared to Dich group ($P < 0.01$). ††Statistically significant difference compared to Dich group ($P < 0.01$). IA: Ipomoea aquatica, Dich: Dichlorvos, NO: Nitrite oxide.
Salahshoor, et al.: Ipomoea aquatica and dichlorvos effects on liver

(inflammation), increased irregularities, sinusoidal dilatation, and hepatocyte vacuolization (necrosis). Treatment with Dich + IA in all treated groups reduced the liver damage caused by the Dich toxicity [Figure 5].

**DISCUSSION**

The histological and biochemical results of this study represent the recovery property of IA following the destructive effects of Dich contamination. Due to the easy access to organophosphates, the human tissue injury and suicide are common.\[^{26}\] As stated in the results of this study, the liver and total body weights in Dich group showed a significant decrease compared to the sham group, whereas Dich + IA 1, 2, and 3 groups showed a significantly increasing trend compared to the Dich recipient group. As mentioned in the study of Edem et al., 4-week contamination (respiratory or inhalable) of pregnant mice to Dich reduced the weight of pregnant mice and also their infants significantly.\[^{27}\] In addition, Sharma et al. have shown that a period of 45-day of Dich administration in albino male rats could reduce the weight of sexual organs such as prostate, testicular tissue, epididymis, and seminal vesicles, which is in line with the results of this study.\[^{28}\] These findings indicate the adverse effects of Dich on endocrine system activity.\[^{29}\] The results of some studies have emphasized adrenocorticotropic hormone (ACTH) increment following the destructive role of organophosphorus administration that finally amplifies the protein catabolism and weight loss.\[^{30}\] IA has substantial biocompatible mixture such as sugars, fatty acids, minerals, and vitamins.\[^{17}\] One of the most considerable results is the presence of equilibrium in liver enzyme activity which was

![Figure 4: Comparison of TAC in sham, Dich, IA, and Dich + IA groups. *Statistically significant difference compared to the sham group (P < 0.01). ††Statistically significant difference compared to the Dich group (P < 0.01). ‡‡Statistically significant difference compared to the Dich group (P < 0.01). IA: Ipomoea aquatica, Dich: Dichlorvos, TAC: Total antioxidant capacity.](image)

![Figure 5: Microscopic images of liver tissue in different groups (4-μm-thick sections, H and E staining, magnification ×100, ×200, and × 400) and apoptosis induction following Dich and the IA3 usage (×400, TUNEL staining). Liver section in the sham group (a and f), normal liver structure. Liver section of Dich group (b, e, h, and k), increased white blood cells (inflammation) (black arrows and triangle sign), and central hepatic vein dilatation (star), the vacuolization hepatocyte (necrosis) (square sign), sinusoidal dilatation (red arrow), and hyperemia (circle), due to the oxidative stress caused by Dich. Liver section in IA group (1000 mg/kg) (c and g), normal liver structure. Micrograph of the liver section in IA + Dich (1000 mg/kg) group (d, i and l), normal liver structure (scale bars: black = 1 μm). (j) Left: cytoplasm staining, middle: nuclei staining, right: merge. The yellow arrows refer to the shiny green nuclei of apoptotic cells. IA: Ipomoea aquatica.](image)
Salahshoor, et al.: Ipomoea aquatica and dichlorvos effects on liver

exacerbated by the destructive effects of Dich. These enzymes can be released into the blood flow due to cell membrane damages.[12] The findings of Alkiyumi et al. confirmed the outcome of this study, indicating that IA administration in rats caused a significant decrease in the serum levels of AST, ALT, creatinine, and weight.[22] It seems that Dich can induce the disorganization of mitochondrial membrane by inhibition of 1–4 respiratory chain complexes.[13] According to other articles, the beneficial effects of IA are related to the antioxidant properties, as well as increased potential of the body’s defense system against the activity of free radicals.[15–17] IA appears to stabilize the cell membranes and prevent the enzyme leakage by the prevention of lipid peroxidation.[20] Further, the results are in agreement with the findings of Dewanjee et al.’s survey, which reported that IA administration attenuates doxorubicin-induced liver injury via restoration of damaged oxidative system, which significantly leads to increase in the values of weight, catalase, and superoxide dismutase and also decrease in the levels of hepatic enzymes.[19] The enhancement of liver function indicates hepatic injury in this study. It seems that Dich inhibits the complexes of I–IV in respiratory chain, leading to cell membrane destruction.[22] IA by an arrest in lipid peroxidation preserves cell membrane integrity and prevents leakage of enzymes.[17] In fact, the main fundamental role of IA in the removal of toxicity available in the animal body is related to the optimization of liver functional enzymes achieved by the antioxidant property of IA. IA expands antioxidant and anti-inflammatory features by the activation of antioxidant enzyme function, lipid metabolism adjustment, and lipid peroxidation reduction.[17] In this study, Dich showed an increasing trend in CHVD and HD values which were both recovered by IA administration. It seems that necrosis in hepatic parenchymal cells occurs following the accumulation of free radicals in this organ.[7] The necrotic hepatic cells can blunt extensive inflammation in the liver and hepatic damage by single-nuclei inflammatory cells. The damaged cells exacerbate liver injury by the release of pro-inflammatory mediators in extracellular matrix. In this inflamed tissue, the macrophages release inflammatory factors such as alpha tumor necrosis, interleukin-1, and NO. The copper cells, as the macrophages in hepatic tissue, accumulate and secrete toxic mediators causing toxicity and necrosis in liver.[25] In this regard, it should be noted that the production of free radicals and subsequent oxidative stress can be one of the most critical and essential causes of the liver cell death.[33] Free radical production following Dich administration initiates the necrosis process in hepatocytes, which provokes the extensive inflammatory responses in this organ by the production of pro-inflammatory mediators. Dich induces generation of active oxygen species such as hydroperoxides, singlet oxygen, hydrogen peroxide, and superoxide. These substances lead to DNA, protein, and intracellular lipid destruction in hepatocytes.[12] The results confirm the findings of Heikal et al., in which methemyl as an organophosphate increases the expression of caspase 3, 9, TP53, and the level of lipid peroxidation; besides, it inhibits the expression of Bcl2, as well as decreases the levels of superoxide dismutase, glutathione, and catalase, and finally, all of these destructive factors together cause cell death.[34] IA has a protective effect against liver fibrogenesis due to polyphenol capacity, inhibitory feature of stellate cells' activity, irregularity in signal transduction pathways, and expression of the cell cycle proteins.[20] It is estimated that IA has various effects including the inhibitory effects on p38 mitogen-activated protein kinase (MAPK) phosphorylation in activated lipopolysaccharide (LPS) in microglia, anti-inflammatory effects on nuclear factor kappa beta (NF-κβ), inhibitory effects on NF-κβ by reduction in H2O2 production, inhibitory feature on IKKβ kinase, and arrest in p65 phosphorylation.[20] These abovementioned phenomena are responsible for apoptosis induction just similar to the results of this study. Moreover, the surveys by Gu et al. confirmed the results of the current study that Dich reduced cell survival rate, increased the expression of Caspase 3, inhibited Bcl2, and induced oxidative stress in HepaRG cells.[35] Studies around IA administration have found a reduction in hepatotoxicity status and oxidative stress caused by toxic compounds such as doxorubicin, paracetamol, and cisplatin.[16,32] In the present study, it was found that the increased serum levels of NO in the Dich group demonstrated a detrimental effect of Dich in the reduction of antioxidant capacity, which confirms this theory that the nature of Dich is a main cause of oxidative stress induction. Mitochondrial dysfunction by the consumption of high amount of oxygen may increase the production of free radicals such as NO and induce hepatic injury due to the oxidative and nitrative stresses.[1] Obviously, the organophosphate-related toxicity induced by the inhibition of cholinesterase enzyme is inevitable.[24] Elelaimy et al. in an in-vivo study concluded that administration of chlorpyrifos, a type of organophosphate, increased NO levels in male rats.[36] Numerous findings such as the study of Jalili et al. indicated that the administration of malathion may increase the hepatic levels of NO.[1] The results are in agreement with the findings of the study of Beydilli et al., in which the administration of diazimon in albino rats could increase the hepatic NO levels.[17] According to previous findings, the lipid peroxidation available in cell membrane and subsequent induction of ROS formation resulted in the generation of NO. Increased levels of NO play a crucial role in regulating oxidative stress and tissue degradation.[12] On the other hand, antioxidants can disrupt all aspects of NO system such as enzymes, substrates, and cofactors, hence reduce NO production.[19] The results are in accord with those of recent studies, in which it was stated that taxol, as one of the chemotherapy drugs, damages hepatocytes, reduces antioxidant capacity, and elevates serum levels of liver enzymes and NO by activation of oxidative stress. The study of Shī et al. showed that IA administration in mice resulted in a decrease in NO level, which is in line with the results of this study.[19] On the contrary, IA with antioxidant property can reduce the destructive effects of organophosphates. This study also confirms that IA increases antioxidant capacity in liver, causing a reduction in oxidative stress.
CONCLUSION

The most apparent finding in this study is that Dich induces oxidative stress. Another destructive effect of Dich returns to the inhibition of the action of endocrine hormones, leading to a reduction in total body and organ weights such as liver weight. Hepatic oxidative stress caused by Dich administration is noticeably based on the arrest of hepatic enzymatic action, increased NO levels, and reduced serum level of TAC. This catastrophic consequence of Dich application induces hepatocyte apoptosis. IA, as a potent antioxidant substance, can reduce the dangerous effects of Dich administration from oxidation/antioxidant imbalance.

Financial support and sponsorship

We gratefully acknowledge the financial support of Kermanshah University of Medical Science (Grant no. 980161).

Conflicts of interest

There are no conflicts of interest.

REFERENCES


33. Husain R, Husain R, Adhami VM, Seth PK. Behavioral, neurochemical,
Salahshoor, et al.: Ipomoea aquatica and dichlorvos effects on liver


