Hepatorenal Effect of Single or Combined Moringa Oleifera and Citrullus Colocynthis on Carbon Tetrachloride-Induced Injury in Spurge Dawely Rat

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Abstract: Medicinal plants consider a source of potential drug, adjuvant or protective agent against hepatorenal toxic chemicals. Liver and kidney diseases of different domestic animals resulted from different agents, antibiotics, plants toxin and even post vaccinal reaction. To better understand the potential of single or combined Moringa Oleifera and Citrullus Colocynthis on carbon tetrachloride-induced injury in 70 male spurge dawely rat. Carbon tetrachloride at dose of 0.5ml/kg (10% in olive oil) for 8 weeks increased significantly level of creatinine while reduced significantly level of blood area and non-significantly reduced level of total protein and albumin. Additionally, Moringa oleifera at dose of 250 and 500 mg/kg increased significantly level of glucose and total protein and albumin while retained creatinine to quite similar level to control group. Additionally, Citrullus colocynthis at dose of 12.5 and 25 mg/kg increased significantly level of blood area and total protein while non-significantly increased of creatinine level and reduced significantly level of glucose. Kidney and liver of rats treated with combined moringa oleifera at dose of 250 mg/kg and citrullus colocynthis at dose of 12.5 mg/kg with carbon tetrachloride at dose of 0.5ml/kg (10% in olive oil) retain liver and kidney weight and structure to quite like control group even fail to retain creatinine level to basal level due to present of infiltrate or cast blocked renal tubules.

Keywords: moringa Oleifera, Citrullus colocynthis, hepatorenal toxicity, rats, SMA

1. INTRODUCTION

Liver and kidney diseases were reported in different domestic animals resulted from different agents, antibiotics, plants toxin and even post vaccinal reaction. Medicinal plant considers a source of potential drug or protective agent against hepatorenal toxic chemicals.

The pharmacological and nutritive potentials of extract of Moringa Oleifera (Biswas et al. (2012) and Citrullus colocynthis (Dallak 2011) has been frequent studied, they aimed to provide a chance for the future scientists to discover a new drug molecule for curing animal or human diseases. The potent pharmacological effects of this medicinal plant as it possesses anti-inflammatory, antioxidant, antimicrobial, anti-hepatorenal toxicity (Nanjappaiah and Hugar 2012, Sachan ,2012, Kumar et al. 2013, Olatosin et al. ,2014 and Mansour et al. 2014), anti-cancer, antiulcer (Patel et al. 2012), hypotensive, and anti-hyperlipidimic activities (Ouedraogo et al., 2011, Saalu et al. 2012 and Yadav et al. 2013).

The antioxidant and hypolipidemic effects of Citrullus Colocynthis extract in alloxan induced diabetic rats investigated by Dallak (2011) and the analysis showed significant decreases in phospholipids, total cholesterol, triglycerides, free fatty acids levels of diabetic rats treated with Citrullus Colocynthis as compared to diabetic untreated rats, Colocynthis had a potent antioxidant and hypolipidemic effects in alloxan induced diabetic rats (Benariba et al., 2013). Additionally, Shokrzadeh et al. (2013) investigated the protective effect of Citrullus Colocynthis extract against genotoxicity in bone marrow induced by cyclophosphamide which was evaluated by micronucleus assay as an index of chromosomal damaged occurred through its antioxidant feature (Nessa and Khan 2014). Also,
the protective effect of Citrullus Colocynthis oil and olive oil against oxidative stress induced by cadmium (Amamou et al. (2015) or paracetamol induced hepatotoxicity in the liver of rats Vakiloddin et al. (2015).

The rationale of this study to provide the hepatorenal effect of single or combined treatment of Moringa and Citrullus in Spurge dawely rat.

2. MATERIAL AND METHODS

2.1. Medicinal Plants

2.1.1. Preparation of Moringa Oleifera Leaves Ethanolic Extract

Moringa Oleifera leaves ethanolic extract was prepared and described earlier (Fathy et al., 2017).

2.1.2. Preparation of Citrullus Colocynthis Ethanolic Extract

Citrullus Colocynthis fruits ethanolic extract was prepared and described earlier (mahmoud et al., 2019)

2.1.3. Pharmacological Evaluation (Determination of Maximum Ultraviolet Absorbance (Amax) and Construction of the Calibration Curves of both Plants Extracts)

The maximum ultraviolet absorbance ($\lambda_{max}$) and construction of the calibration curves of both plants extracts were carried out inside the pharmaceutics laboratory, faculty of pharmacy, Mansoura University was done to confirm that each 1 gm of the obtained extracts contained an exact 1 gm of the plants extract substance to avoid the miss calculation of the administrated doses of each plant (mahmoud et al., 2019).

2.2. Animals and Treatments

70 males’ spurge dawely rats weighted from 130 to 150 gm were kept in the laboratory under constant conditions of temperature (24 ± 2 C) for at least one week before and through the experimental work, being maintained on a standard diet and water were available ad libitum. The animals were maintained in accordance with the guidelines prescribed by the Faculty of Science and the study was approved by the Animal Ethics Committee of the University of Mansoura, Egypt.

The experimental rats were divided into seven groups:

Group 1: Animals were fed on the standard diet and were served as control group.

Group 2: Rats were injected intraperitoneally with 1.0 ml/kg b.w. of 10% CCl₄ dissolved in olive oil twice a week for eight weeks (Abdelaziz and Ali, 2014).

Group 3: Rats were injected with 1.0 ml/kg b.w. of 10% CCl₄ followed by oral administration of 250 mg/kg b.w. ethanolic extract of Moringa oleifera (Fathy et al., 2017), twice a week for eight weeks.

Group 4: Rats were injected with 1.0 ml/kg b.w. of 10% CCl₄ followed by oral administration of 500 mg/kg b.w. ethanolic extract of Moringa oleifera, twice a week for eight weeks.

Group 5: Rats were injected with 1.0 ml/kg b.w. of 10% CCl₄ followed by oral administration of 12.5 mg/kg b.w. (1/8of the LD50) ethanolic extract of Citrullus Colocynthis, twice a week for eight weeks (Mahmoud et al., 2019).

Group 6: Rats were injected with 1.0 ml/kg b.w. of 10% CCl₄ followed by oral administration of 25 mg/kg b.w. (1/4 of the LD50) ethanolic extract of Citrullus Colocynthis, twice a week for eight weeks.

Group 7: Rats were injected with 1.0 ml/kg b.w. followed by oral administration of 12.5 mg/kg b.w. ethanolic extract of Citrullus Colocynthis and 250 mg/kg b.w. ethanolic extract of Moringa Oleifera, twice a week for eight weeks.

2.3. Histopathological Examination

The treated animals and their controls were sacrificed by decapitation after 8 weeks of treatment. Their kidneys were removed and fixed in 10% neutral formalin. Fixed materials were embedded in paraffin wax and sections of 4 micrometer thickness were cut. Slides were stained with hematoxylin and eosin for histological examination.

2.4. Immunohistochemical Localization of A-SMA

Fixed kidney tissue was embedded in paraffin and then sectioned at 4–5 mm. The sections were deparaffinized, rehydrated, and heated (using a microwave oven) to boiling 0.01 M citrate buffer (pH 6.0). Upon boiling, the sections were subsequently heated (low heat setting) for an additional 15 min. The sections were then blocked in 1.5% normal serum for 10 min at room temperature. The following antisera (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used SMA. The kidney sections were incubated with antisera overnight at room temperature using the following dilutions for 1:200. then washed 3 times and incubate for secondary antibody Immunoreactivity (as
identified by brown color) to the respective protein was then visualized using the DAKO Staining System following the manufacturer’s protocol. Sections were counterstained with Mayer’s hematoxylin. The sections were then photographed using digital camera.

2.5. Biochemical Assays

For biochemical study serum were obtained by centrifugation of the blood samples and stored at 20°C until assayed for the biochemical parameters as following methods; Creatinine (Bowers et al., 1980), urea (Patton and Crouch, 1977), glucose (Kaplan et al., 1984), total protein (Vassault et al., 1986), albumin (Doumas et al., 1981) and globulin levels (Doumas et al., 1972).

2.6. Statistical Analysis

The obtained data in this study were statistically analyzed for variance by One Way ANOVA (Snedecor et al., 1989) by using computerized SPSS program (1996) version 13.0.

3. RESULTS

Both plants treated group reduced body weight non-significantly when compared with ccl4 treated as control positive for nephropathy. Notably, group treated with both Moringa oleifera and Citrullus colocynthis retain body weight to normal level.

In the current study, kidney and liver weight was reduced significantly in group treated with only ccl4 or combined with Moringa oleifera at doses of 250 or 500 mg/kg. But Moringa oleifera and Citrullus colocynthis treated group retain kidney weight slightly little than normal level. Moreover, Renal or liver to body weight ratio of all treated group was reduced non-significantly when compared with control group.

Table 1. Shows Effect of single treatment of Moringa oleifera and Citrullus colocynthis on body weight, kidney weight and renal to body weight ratio when given with carbon tetrachloride

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight</th>
<th>Kidney weight</th>
<th>Renal to body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>260±12.4</td>
<td>1.9±0.07</td>
<td>0.74±0.01</td>
</tr>
<tr>
<td>2</td>
<td>240±19</td>
<td>1.68b1.7±0.09</td>
<td>0.71±0.03</td>
</tr>
<tr>
<td>3</td>
<td>206b±12</td>
<td>1.44b1.7±0.09</td>
<td>0.69±0.03</td>
</tr>
<tr>
<td>4</td>
<td>214b1.7±20</td>
<td>1.5b1.7±0.1</td>
<td>0.71±0.02</td>
</tr>
<tr>
<td>5</td>
<td>218b1.7±9.0</td>
<td>1.60b1.7±0.80</td>
<td>0.74±0.03</td>
</tr>
<tr>
<td>6</td>
<td>197b1.7±11.0</td>
<td>1.30b1.7±0.80</td>
<td>0.71±0.03</td>
</tr>
<tr>
<td>7</td>
<td>267±15</td>
<td>1.8±0.1</td>
<td>0.69±0.02</td>
</tr>
</tbody>
</table>

B1.2 significantly at ≤ 0.05

Both group treated with Citrullus colocynthis at 12.5 and 25 mg/kg reduced body weight of treated male rats significantly when compared with control group while only treated group at dose of 25 mg/kg reduced significantly when compared with carbon tetrachloride treated animals as control positive for nephropathy. Notably, group treated with both Moringa oleifera and Citrullus colocynthis retain body weight to normal level. In the current study, kidney weight was reduced significantly in group treated with only carbon tetrachloride or combined with Citrullus colocynthis at doses of 12.5 and 25 mg/kg. But Moringa oleifera and Citrullus colocynthis treated group retain kidney weight slightly little than normal level. Moreover, group treated with 25mg/kg of Citrullus colocynthis retain Renal to body weight ratio to basal level while other treated groups was reduced non-significantly when compared with control group.

In the current study, carbon tetrachloride increased significantly level of creatinine while reduced significantly level of blood urea and non-significantly reduced level of total protein, albumin. Additionally, Moringa oleifera at dose of 250 and 500 mg/kg increased significantly level of glucose and total protein and albumin while retained creatinine to quite similar level to control group. Additionally, Citrullus colocynthis at dose of 12.5 and 25 mg/kg increased significantly level of blood urea and total protein while non-significantly increased of creatinine. Moreover, Citrullus colocynthis reduced significantly reduced level of glucose. In contrast, combined treatment of Moringa oleifera at dose of 250 mg/kg and Citrullus colocynthis at 12.5 mg/kg increased significantly level of creatinine, glucose and total protein.
Table 2. Effect of single treatment of Moringa oleifera and Citrullus colocynthis on serum biochemical parameters on induced nephropathy model by carbon tetrachloride.

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>BUN:Creatinine (BCR)</th>
<th>T.P (mg/dL)</th>
<th>Albumin (g/dL)</th>
<th>Globulin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.1</td>
<td>220±6.4</td>
<td>0.5±0.03</td>
<td>50.6±3.9</td>
<td>101.2</td>
<td>7±0.29</td>
<td>3.9±0.1</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>Gr.2</td>
<td>224±1.6</td>
<td>0.64±0.02</td>
<td>36.8±1.8</td>
<td>57.59</td>
<td>6.8±0.6</td>
<td>3.8±0.24</td>
<td>3.06±0.1</td>
</tr>
<tr>
<td>Gr.3</td>
<td>278±2.2</td>
<td>0.55±0.05</td>
<td>40.7±2.1</td>
<td>74.12</td>
<td>8.19±0.2</td>
<td>4.4±0.37</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>Gr.4</td>
<td>282±2.4</td>
<td>0.57±0.02</td>
<td>46.5±4.6</td>
<td>81.51,2,3</td>
<td>9.99±0.7</td>
<td>4.6±0.34</td>
<td>5.41,2,3±0.3</td>
</tr>
<tr>
<td>Gr.5</td>
<td>206±1.7</td>
<td>0.77b±0.06</td>
<td>82.7b1,2,3,4,5.1</td>
<td>107.4b1,2,3,4,5.1</td>
<td>8.79±0.2</td>
<td>3.2±0.09</td>
<td>5.5b1,2,3±0.2</td>
</tr>
<tr>
<td>Gr.6</td>
<td>209±1.2</td>
<td>0.67b1±0.02</td>
<td>79.9b1,2,3,4,5.1</td>
<td>118.9b1,2,3,4,5.1</td>
<td>7.89±0.6</td>
<td>3.2±0.47</td>
<td>4.6b1,2,3±0.2</td>
</tr>
<tr>
<td>Gr.7</td>
<td>251b1±2.5</td>
<td>0.88b1,2,3,4,5.6±0.06</td>
<td>57b2,5,6±3.6</td>
<td>101.2</td>
<td>9.19b1±1.3</td>
<td>3b1±0.24</td>
<td>6.1b1,2,3±0.2</td>
</tr>
</tbody>
</table>

B1,2 significantly at ≤ 0.05

**BUN:** Creatinine ratio (BCR) is interesting tools for Glomerular filtration function of kidney, but only used in patient rather than experimental research, in the current study carbon tetrachloride reduce significantly the BCR level when compared to control group. While Citrullus colocynthis and combined treatments improve the BCR quite to normal than single treatment of moringa Olivera.

Notably, nephropathy model induced by carbon tetra chloride at dose 0.5 ml/kg of 10% (v/v) show interstitial fibrosis, mild inflammatory infiltrate and thick-walled blood vessels. Kidney of rat treated with moringa olifera at dose of 250 mg/kg combined with carbon tetrachloride show focal interstitial fibrosis and chronic inflammation. Kidney of rat treated with Moringa oleifera at dose of 250 mg/kg combined with carbon tetrachloride show moderate staining collagen stained with mallory trichrome stain. Moreover, kidney of rats treated with Moringa oleifera at dose of 500 mg/kg combined with carbon tetrachloride show moderate focal interstitial fibrosis stained with mallory trichrome stain.

![Fig1a](normal kidney architecture). ![Fig1b](kidney of rats treated with ccl4 shown interstitial fibrosis, mild inflammatory infiltrate and thick walled blood vessels). ![Fig1c](focal interstitial fibrosis and chronic inflammation (Moringa Olivera 250mg/kg)). ![Fig1d](collagen deposition in kidney evidence by Mallory trichrome (Moringa Olivera 250mg/kg, 500mg/kg respectively). ![Fig1e](normal liver histology). ![Fig1f](massive liver fibrosis (ccl4 treatment)). ![Fig1g](focal lytic necrosis ((Moringa Olivera 250mg/kg)). ![Fig1h](collagen deposition in liver evidence by Mallory trichrome (Moringa Olivera 250mg/kg, 500mg/kg respectively).

Citrullus colocynthis at dose of 12.5 mg/kg show moderate improvement of kidney architecture while rats treated with Citrullus colocynthis at dose of 12.5 mg/kg combined with carbon tetrachloride show focal staining of collagen with mallory trichrome stain while kidney of rats treated with Citrullus colocynthis at dose of 25 mg/kg combined with carbon tetrachloride show reduction of focal staining of collagen with mallory trichrome stain.
Hepatorenal Effect of Single or Combined Moringa Olivera and Citrullus Colocynthis on Carbon Tetrachloride-Induced Injury in Spurge Dawely Rat

Fig2. a, b collagen deposition in kidney evidence by Mallory trichrome (Citrullus 12.5mg/kg, 25 mg/kg respectively). c. renal tissue show complete absence of collagen evidence by Mallory trichrome stain in combined treatments of moth moringa and Citrullus against ccl4 treatment. d, e collagen deposition in liver evidence by Mallory trichrome (Citrullus 12.5mg/kg, 25 mg/kg respectively). f. few leucocytic infiltration in combined treatments of moth moringa and Citrullus against ccl4 treatment.

Interestingly, kidney of rats treated with both Moringa oleifera at dose of 250 mg/kg and Citrullus colocynthis at dose of 12.5 mg/kg combined with carbon tetrachloride show negative staining of mallory trichrome stain or no evidence of collagen fiber infiltration.

Notably, moderate reduction of S.M.A treatment of both moringa oleifera at dose of 250 mg/kg and Citrullus colocynthis at dose of 12.5 mg/kg show moderate reaction against SMA Monoclonal antibody. While moringa Olivera treatment at of 500 mg/kg (fig 3 e) and (fig 3 f) colocynthis at dose of 25 mg/kg shown little staining of SMA. Finally, combination treatment of moringa oleifera at dose of 250 mg/kg and Citrullus colocynthis at dose of 12.5 mg/kg (fig 3 g) reduction of S.M.A staining reaction.

Fig3. show level of SMA (smooth muscle actin), (a) Negative staining of SMA in treated negative control group. (b) Massive staining of SMA induced by carbon tetrachloride. (c) moderate reduction of SMA by treatment of moringa oleifera at dose of 250 mg/kg. (d) little staining of SMA by moringa oleifera treatment at of 500 mg/kg (e) Citrullus colocynthis at dose of 12.5 mg/kg shown little SMA expression (f) moderate SMA expression by treatment of Citrullus colocynthis at dose of 25 mg/kg (g) reduction of SMA staining by combination treatment of moringa oleifera at dose of 250 mg/kg and Citrullus colocynthis at dose of 12.5 mg/kg.
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4. DISCUSSION

Many agents like heavy metal, drugs, pesticide, environmental agents and poisonous plant consider hazard factors for hepatorenal toxicity. Moreover, hepatorenal toxicity can occur due to another disease like diabetes enhanced agents, so our world scientist still do the best effort to search new medical cure for diseases affect liver and kidney with advanced technology.

In the present study, both Moringa oleifera and Citrullus colocynthis when each was given alone along with carbon tetrachloride reduced body weight when compared with carbon tetrachloride treated as control positive for nephropathy or control group. Notably, group treated with both Moringa oleifera and Citrullus colocynthis along with carbon tetrachloride retain body weight to normal level. This result agrees with Adewole (2007) who found that the administration of carbon tetrachloride significantly decreased body weight when compared with the control.

Kidney weight was reduced significantly in group treated with only carbon tetrachloride or combined with Moringa oleifera at doses of 250 or 500 mg/kg. But Moringa oleifera and Citrullus colocynthis treated group retain kidney weight slightly little than normal level. This result disagrees with Adewole (2007) who found that carbon tetrachloride significantly increased kidney weight. Additionally, Soufane et al (2013) who found that group sacrificed after 10 days of treatment of Citrullus colocynthis has presented a significant reduction in the relative weights of the kidney when compared to control group. In the current study, carbon tetrachloride increased significantly levels of creatinine and reduced significantly levels of blood urea and non-significantly reduced level of total protein and albumin.

In the current study, Moringa oleifera at dose of 250 and 500 mg/kg when given with carbon tetrachloride increased significantly levels of glucose, total protein and albumin while retained creatinine to quite similar level to control group. These results agree with Ouédraogo et al. (2011) who found that the Moringa oleifera at dose of 150 and 300 mg/kg plus gentamicin treated groups reduced serum urea and creatinine levels. On pathological examinations, kidney of intoxicated rabbits’ groups which received Moringa oleifera extract showed reparative tendencies. Combined treatment of Moringa oleifera and gentamicin group showed a highly significant depletion in lipid peroxidation (LPO) level. Moreover Al-Malki and El Rabey (2015) found that treatment of diabetic rats with 50 or 100 mg/kg Moringa oleifera seeds powder ameliorated the levels of renal parameters approaching the negative control values and restored the normal histology of both kidney and pancreas compared with that of the diabetic positive control group.

Notably in the presented study, combined treatment of Moringa oleifera at dose of 250 mg/kg and Citrullus colocynthis at 12.5 mg/kg when combined with carbon tetrachloride increased significantly levels of creatinine, glucose, total protein. Moreover, in other study reported by Omayma et al. (2013) who found that Citrullus colocynthis administration to diabetic rat, improved hyperglycemia, impaired kidney, dyslipidemia and attenuates the states of antioxidant enzyme and oxidative stress induced by diabetes mellitus. Additionally, El-Baky and Amin (2011) reported that the medicinal plant Citrullus colocynthis fruit may have protective effect on the kidney functions and tissues. As it may play a role in prevent nephropathy as one of micro vascular complications of diabetes mellitus. Notably, Efiong E E and Ebong P E (2014) recorded that the efficacy of the combined extracts of Ocimum Gratissimum and Moringa Oleifera ameliorates diabetic nephropathy at better manner in a synergistic
pattern when compared with the single extracts and standard drugs insulin and glibenclamide.

Interestingly, Kidney and liver of rats treated with both moringa oleifera at dose of 250 mg/kg and citrullus colocynthis at dose of 12.5 mg/kg combined with carbon tetrachloride retain renal weight and renal histology to quite like control group even fail to retain creatinine level to basal level due to present of infiltrate or cast blocked renal tubules. These results agree with Iwara et al. (2013) investigated the protective effect of combined extracts of Moringa oleifera and Vernonia Amygdalina on STZ induced kidney damage in rats. Also, Rajan et al. (2016) who found that the combination of Moringa oleifera seeds and cannabidiol was more effective than treatment with single constituents in the anti-inflammatory, anti-apoptotic and ant oxidative effects.

In the current study, moringa oleifera at 2 successful doses reduced collagen infiltration by mallory trichrom stain when treated with carbon tetrachloride. These results agree partially with Al-Malki and El Rabey (2015) who found that treated group with two low doses of Moringa oleifera seed powder (50 and 100 mg/kg body weight) in the diet restored the normal histology of both kidney when combined treated with streptozotocin

5. CONCLUSIONS

Administration of both plants together has been failed to decrease creatinine level while improve BUN/creatinine ratio and improved the kidney and liver structure in carbon tetrachloride model of toxicity.

REFERENCES


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[15] Mahmoud M. Elalfy¹, Amany Farag², Ahmed A. Helmy³, Zein Elaabdeen Metwaly⁴, and Fathy Radwan Hematological, Biochemical and Cytotoxic Effect of Ethanolic Raw Extract of Egyptian Citrullus Colocynthis In Spurge Dawley Rat. Enzyme Engineering journal volume 8, issue 1, 2019


