Antioxidant traits and protective impact of *Moringa oleifera* leaf extract against diclofenac sodium-induced liver toxicity in rats

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**Abstract**

*Moringa oleifera* gained importance as a medicinal plant. The current study assesses *Moringa* leaf ethanol extracts (MLE) against experimentally diclofenac sodium (DcNa)-induced liver toxicity in male rats. Leaves were extracted with different solvents differing in polarity. Assessment involved total phenolic compounds, total flavonoids and radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH·). HPLC was performed for identifying phenolic compounds, wherein ethyl vanillin (1,205 mg/kg), 3-OH-tyrosol (812.2 mg/kg), benzoic acid (273.8 mg/kg), salicylic acid (240.0 mg/kg), chlorogenic acid (233.3 mg/kg) and 3,4,5-methoxy-cinnamic acid (172.5 mg/kg) were measured. Fifty animals (each treatment group consisted of 10 rats) were subjected to five treatments and the experiment lasted for 4 weeks. Animals were exposed to DcNa (100 mg/kg) and two doses of MLE as well as silymarin (an antioxidant flavonoid C25H22O10) for 4 weeks. Liver marker enzymes, including alkaline phosphatase, alanine transaminase, and aspartate transaminase as well as urea, uric acid, and creatinine were increased. Serum albumin and total protein decreased in DcNa-treated rats. Homogenates nitric oxide increased in liver tissue of the DcNa-treated rats, while the activity of each of glutathione peroxidase, glutathione-S-transferase, glutathione, and catalase decreased. It could be concluded that MLE in both doses and silymarin are considerably hepatoprotective with antioxidant activity (AOA) against DcNa-induced hepatotoxicity in rats.

**Practical applications**

Administration of MLE caused improvements in kidney functions and acted as antioxidant enzymes as compared with silymarin (as a reference drug). AOA was exhibited by MLE in vivo, and this would have a positive effect against oxidative liver damage caused by DcNa. Plasma membrane was protected and the regenerative and reparative capacity of liver increased by phenolics in the MLE. The study demonstrated the MLE hepatoprotective activity and recommends using *M. oleifera* leaves for the treatment of liver disorders.

**Keywords**

hepatoprotective, hepatotoxicity, kidney, nonsteroidal anti-inflammatory drugs, phenolic compounds
1 | INTRODUCTION

The moringa cruciferous plant has nutritional and medicinal properties (Cuellar-Nuñez et al., 2018; Leone et al., 2015). It has a high economic value due to its medicinal applications, livestock forage, and nutritional value (Mohammed & Abd Monan, 2015). It belongs to the mono-generic family of Moringaceae, which is a native to the sub-Himalayan regions of India, Bangladesh, Pakistan, and Afghanistan (Kumar, Pandey, Mohan, & Singh, 2012). All parts of moringa are edible. The plant is called “miracle vegetable” because it has medicinal values and is used as a functional food (Mona, 2013). The leaves have high contents of protein and are the main protein source in many countries (Gopalakrishnan, Doriya, & Kumar, 2016). Its leaf extracts have anti-hypertensive, anti-inflammatory, and anti-ulcer effects (Pal, Mukherjee, & Saham, 1995). The extracts possess cytotoxic properties, chemopreventive traits, antiradical activity against free radicals, prevent oxidative damage to major biomolecules and protect against oxidative damage (Charoensin, 2014; Cuellar-Nuñez et al., 2018). The leaves have high contents of flavonoids glucosinolates, isothiocyanates, and phenolic acids (Brunelli et al., 2010). Quercetin, kaempferol, and chlorogenic acid, are the main phenolic compounds found in moringa’s methanol or water extracts (Vongsak et al., 2013). The plant has quercetin and kaempferol flavonoids, which decrease oxidative stress, and have antiproliferative, anti-inflammatory, and antihypertensive properties (Coppin et al., 2013; Cuellar-Nuñez et al., 2018; Tiloke, Phuludkaree, & Chuturgoon, 2013). Antiproliferative effect of its methanol extract on HCT-116 human colorectal carcinoma cells showed a cytotoxic effect due to components of such as astragalin and isoquercetin (Tragulpakseerojn et al., 2017). Aqueous extracts showed cytotoxicity on colon cancer cell lines HTC116, HCT116P53 and Caco2 (Reda, Borjac, Fakhouri, & Usta, 2017).

Moringa possesses chemoprotection activity, and decreases aberrant crypt foci (ACF) number found in colon cancer-induced ICR-mice (Budda et al., 2011; Promkum, Kupradinun, Tuntipopipat, & Butyree, 2010). Anticancer properties of methanol extracts of moringa leaves showed an increase up to 30%-62% in median survival time of adult C57BL/X mice with transplanted B16F10 mouse melanoma (Purwal, Pathak, & Jain, 2010). The orally administered dose of 400-6,400 mg/kg did not lead to mortality but the high doses of 3,200 and 6,400 mg/kg caused decreased locomotion in animals following 2 hr after administration (Awodele, Oreagba, Odoma, Teixeira Da Silva, & Osunkalu, 2012). Chemo-protective activity was shown by moringa freeze-dried pods administered to cancer-induced ICR-mice causing a decrease in their ACF (Budda et al., 2011; Promkum et al., 2010).

Diclofenac sodium (DcNa) is a benzene acetic acid which is a derivative related to meclofenamic acid and Na-meclofenamate called 2-[2,6-dichlorophenyl]amino] benzene acetic acid, mono-sodium salt with a molecular weight of 318.14; its molecular formula is \( \text{C}_{14}\text{H}_{10}\text{Cl}_{2}\text{NaO}_{2} \). It is a nonsteroidal anti-inflammatory drug (NSAID) with an anti-inflammatory, analgesic and antipyretic effects (Husna, Sumeria, Laiba, & Anam, 2017). The mechanism of action of DcNa, like that of other NSAIDs, is not completely understood but may be related to prostaglandin synthetase inhibition (Taha, Rabah, Shaker, & Mograby, 2015). Lauer, Tuschi, King, and Mueller (2009) illustrated the mechanism of DcNa hepatotoxicity, where CYP450 enzyme oxidizes DcNa to form reactive metabolites (5-OH and 4-OH DcNa) which are oxidized the benzoquinone iminem, which in turn is detoxified by a reduced glutathione (GSH) conjugation and moved out of cells. Thus, elimination of an elevated dose of DcNa may lead to GSH depletion reflected in liver injury.

Presence of NSAIDs can increase the risk of thrombotic cardiovascular state, myocardial infarction and stroke, which would lead to death. All such states can increase with time of duration particularly with patients with cardiovascular disease; also, NSAIDs may increase serious gastrointestinal dangers including bleeding, ulceration, stomach or intestine perforation, renal papillary injury and necrosis (Agündez, Lucena, & Martínez, 2011; Husna et al., 2017; Mahalakshmi, Rajesh, Ramesh, Balasubramanian, & Kannan, 2010; Tarasankar, Ahmad, Pahari, & Gangu-li, 2012).

The current study is aimed to (a) assess different moringa leaf extracts in their antioxidant activity (AOA) extracts, and (b) investigate the possible hepato-protective effect of ethanol extract against DcNa-induced hepatotoxicity in albino rats.

2 | MATERIALS AND METHODS

2.1 | Materials

Moringa leaves (fresh) were obtained from the Faculty of Agriculture farm, Benha University, Egypt. Reagents used in the study include 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau and standards of phenolic compounds (Sigma Chemical Co., St. Louis, MO, USA), diagnostic kits (Bio Merieux Laboratory Reagents and Products, France) as well as DcNa and silymarin (Sedico Pharmaceutical Company, Egypt).

2.2 | Methods

2.2.1 | Preparation of leaf extracts

One kilogram fresh leaves were air-dried in the shade for one week and samples were taken and powdered using a mill. Powdered samples were extracted by solvents with different polarities including petroleum ether, ethyl acetate, ethanol, and water for 15 hr for each solvent, using Soxhlet extractor. The extracts were filtered through Buchner funnel and evaporated under vacuum using a rotary evaporator (N-N series, EYELA, Japan) at 40°C in the dark.

2.2.2 | Total phenolic content

The total phenolic content (TPC) was determined according to Singleton and Rossi (1965) using Folin-Ciocalteau and gallic acid being the standard. One milliliter of diluted extract (1 mg/ml) was mixed with 1 ml Folin-Ciocalteau and 1 ml of \( \text{Na}_{2}\text{CO}_{3} \) (20% w/w), then incubated at room temperature for 30 min. TPC measurement was done at 765 nm using UV-1800 spectrophotometer (TOMOS, Italy) and the contents were expressed as mg of gallic acid equivalents.
2.2.3 | Total flavonoid content

Total flavonoid content (TFC) of all extracts was determined according to Meda, Lamien, Romito, Millogo, and Nacoulma (2005), with a slight modification. An aliquot of 0.5 ml diluted extract was mixed with 0.5 ml methanol, 50 µl of 1 M K-acetate and 1.4 ml of distilled water then incubated for 30 min at room temperature. The measurement was done at 415 nm and TFC was calculated using quercetin as a standard.

2.2.4 | Determination of AOA

Determination of the AOA expressed as the free radical activity of the extracts was done using DPPH- according to Lee, Kim, Kim, and Jang (2002). A solution of DPPH- in ethanol (1 × 10⁻³ M) was prepared by mixing 0.5 ml diluted sample with 3.5 ml of DPPH- solution, then incubated for 30 min at room temperature. The measurement was done at 517 nm absorbance using UV-Vis spectrophotometer (Jenway, UK) against blank ethanol and control absorbance of the DPPH radical, according to the following:

\[ \text{DPPH discolouration} = 100 \times \left( A_1 - A_2 / A_2 \right) \]

where \( A_1 \): absorbance of the control and \( A_2 \): absorbance in presence of the extract.

2.2.5 | HPLC analysis of phenolic compounds

The determination was done according to Prakash, Singh, and Upadhyay (2007). The ethanol extract (MLE) was dissolved in HPLC-grade methanol (1.0 mg/ml), filtered through sterile 0.22 µm Millipore filter and subjected to qualitative and quantitative analysis using Shimadzu LC-10A (Kyoto, Japan) HPLC instrument. The instrument was supplied with a binary dual-pump LC-1 OAT (Shimadzu, Kyoto, Japan) HPLC, an SPD-10A UV detector SPD-10A (Shimadzu, Kyoto, Japan) and a Phenomenex Luna RP, C₁₈ column (4.6 s 250 mm). Data were integrated by Shimadzu Class VP software (Shimadzu, Kyoto, Japan). Separation was done with an acetonitrile: water having 1% acetic acid linear gradient program, starting with 18% acetonitrile then changing to 32% in 15 min and finally to 50% in 40 min. Results were obtained by comparison of peak areas (\( \lambda_{\text{max}} = 254 \) nm) of the samples (mg g⁻¹ dry extract) with that of the standards.

2.2.6 | Experimental animals

The animals were adult albino male rats (Wister Strain), healthy of the same age, (each weighing 120–140 g) purchased from the farm of the Biological Products and Vaccines Organization (Egypt). Experimental procedures were executed according to the guidelines of care and use of laboratory animals of the WHO (World Health Organization). The rats were kept under 25°C ambient temperature, 50% relative humidity and a 12-hr light–dark cycle and were allowed free access to water and a standard diet (Reeves, Nielsen, & Fahey, 1993).

2.2.7 | Acute toxicity

The acute toxicity test for MLE was estimated to evaluate any possible toxicity. The test was performed according to Organisation for Economic Cooperation and Development (OECD) 423 guidelines (OECD, 2001). Five adult albino male rats were fasted overnight with free access to drinking water then given MLE at graded doses up to 4,000 mg/kg. The dosing pattern was 500, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, and 4,000 mg/kg body weight for MLE, while the control group received only the normal saline. Animals were closely monitored for 24 hr and daily for 14 days until early signs of toxicity and/or mortality were observed. Death of half of the examined animals was observed at 3,000 mg/kg. Therefore, 300 mg/kg (1/10 of 3,000 mg/kg) was selected as the maximum safety dose.

2.2.8 | Experimental design

The experimental design was completely randomized, with ten replicates. Fifty rats were used, divided into five groups, each group consisted of 10 rats. Group 1 (a negative control) received a basal diet. Group 2 (a positive control) received DcNa (thrice a week) at 100 mg/kg bw. Group 3 received DcNa at 100 mg/kg bw, with simultaneous administration of silymarin (50 mg/kg bw) given orally through gastric gavages (thrice a week) for 30 days. Group 4 received DcNa at 100 mg/kg bw, with simultaneous administration of MLE (150 mg/kg bw) given orally through gastric gavages (thrice a week) for 30 days. Group 5 received DcNa at 100 mg/kg bw, with simultaneous administration of MLE (300 mg/kg bw) given orally through gastric gavages (thrice a week) for 30 days. The experiment lasted for 4 weeks.

2.2.9 | Blood biochemical analysis

Samples of blood at end of the experiment were taken from the retro-orbital plexus veins by fine capillary heparinized tubes, and were allowed to clot. Serum was separated by centrifuging for 15 min at 3,000 rpm. The biochemical analysis included liver enzyme activities of alanine transaminase (ALT) aspartate transaminase (AST), and alkaline phosphatase (ALP), serum protein and albumin contents (Doumas, 1975; Doumas, Biggs, Arends, & Pinto, 1971; Reitman & Frankel, 1957; Tietz, 1983). Globulin was estimated by calculation subtracting albumin from serum total protein, whereas kidney function parameters including urea, uric acid, and creatinine were determined chemically (Tabacco, Meliattini, Moda, & Tarlip., 1979).

2.2.10 | Assessment of hepatic oxidative stress biomarkers

Tissue samples of liver were washed with an ice-cold saline solution to remove excess blood, then homogenized in cold 0.1 M potassium phosphate solution (pH 7.4); extraction ratio of 1:9 (w/v), then centrifuged for 10 min at 5,000 rpm at 4°C. The supernatant was analyzed for antioxidant markers while glutathione peroxidase (GPx) was
determined spectrophotometrically using Ellman’s reagent (Moron, Depierre, & Mannervik, 1979). The activity of glutathione-S-transferase (GST) was done according to Habig, Pabst, and Jakoby (1974) using the aromatic substrate by monitoring the change in absorbance due to thioether formation. Glutathione reduction was measured according to Prins and Loos (1969) and catalase activity was measured according to Bock, Karmer, and Paverka (1980). Nitric oxide was determined according to Montgomery and Dymock (1961).

2.2.11 Statistical analysis

Data were subjected to ANOVA (SAS, 1996) and statistical analyses using the statistical software SPSS 11.0 (SPSS Ltd., Surrey, UK).

3 | RESULTS

3.1 Yield and AOA

Results of TPC, TFCs, and AOA of leaf extracts (Table 1) show the highest yield was in the ethanol extract (15.14 mg/L) followed by water extract (13.09). TPC (mg gallic acid equivalent g⁻¹) ranged from 91.77 in the ethyl acetate extract to 380.30 in the ethanol extract. Total flavonoids (mg QE kg⁻¹) ranged from 0.66 in water extract to 4.76 in ethanol extract. The extracts exhibited strong scavenging activity against DPPH· radicals. Extracts with low TPC showed lower antiradical activity. Radical scavenging activity of phenolic compounds are largely affected by the number of hydroxyl groups on the aromatic ring (the higher the number of hydroxyl groups, the greater is the antiradical activity). The ethanolic extract showed the strongest antiradical activity (92.9%) followed by water extract (79.1%) then the ethyl acetate extract (43.6%). The lowest was given by the ether extract (36.9%).

3.2 Identification of phenolic compounds in (MLE) using ethanol extract

Data presented in Table 2 show the identified phenolic compounds in MLE analyzed by HPLC. The tabulated data reveal the presence of 24 phenolic compounds in MLE. The main identified compounds were ethyl vanillin (1.205 mg/kg), 3-OH-tyrosol (812.2 mg/kg), benzoic acid (273.8 mg/kg), salicylic acid (240.0 mg/kg), chlorogenic acid (233.3 mg/kg), and 3,4,5-methoxy-cinnamic acid (172.5 mg/kg).

3.3 Effect of MLE administration on hepatic function and kidney function tests

Effect of MLE on body weight (bw) gain and organs weight as affected by hepatic damage by DcNa is shown in Figure 1. The final weight (g) for groups 1, 2, 3, 4, and 5 were 144, 109, 147, 151, and 153, respectively. The DNa-induced hepatotoxicity group (group 2) recorded the lowest body weight gain and the highest organ weights. Table 3 shows the effect of MLE on liver function exhibited by the hepatic damage induced by DcNa. Figure 2 presents the effect of MLE on serum protein profile as affected by hepatic damage induced by DcNa. There was a significant increase in ALT, AST, ALP, total bilirubin and direct bilirubin (Table 3) due to DcNa-induced hepatotoxicity (group 2) accompanied by a marked decrease in globulin, total protein and albumin (Figure 2). In the MLE-groups, there were marked decreases in total bilirubin, ALT, AST, ALP, and direct bilirubin accompanied by increases in total protein, albumin, and globulin. These results agree with the results reported by Taha et al. (2015) on the DcNa-induced hepatic injury. The effect of MLE on kidney function due to DcNa (Figure 3) indicates a considerable increase in DcNa-induced hepatotoxicity (group 2), while MLE-groups showed a decrease in contents of creatinine, uric acid, and urea.

3.4 Effect of MLE administration on antioxidant enzymes

Table 4 shows the effect of MLE on antioxidant markers as affected by hepatic damage induced by DcNa. There was an increase in nitric oxide and a decrease in each of GSH, GST, GPx, and catalase enzyme activity due to DcNa-induced hepatotoxicity. The MLE-groups showed a significant decrease in nitric oxide and increases in the activity of each of GSH, GST, GPx, and catalase compared with the DcNa group.

4 | DISCUSSION

Being secondary metabolites produced by the plants, phenolic compounds are responsible for antioxidants’ activity. Plant phenolics exhibit physiologic traits which include anti-allergic, anti-inflammatory, antimicrobial effects along with cardio-protective ones (Ak & Gülçin, 2008; Balasundram, Sundram, & Samman, 2006; Gülçin, Elias,

<table>
<thead>
<tr>
<th>Extract Type</th>
<th>Total Extract (g/100 g)</th>
<th>TPC (mg GAE/g extract)</th>
<th>Total Flavonoid Content (mg QE/kg extract)</th>
<th>DPPH· Scavenging Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether extract</td>
<td>11.85 ± 0.36</td>
<td>128.1 ± 2.02</td>
<td>2.47 ± 0.14</td>
<td>36.91 ± 5.92</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>1.60 ± 0.22</td>
<td>91.77 ± 6.37</td>
<td>3.54 ± 0.47</td>
<td>43.64 ± 2.94</td>
</tr>
<tr>
<td>Ethanol extract (MLE)</td>
<td>15.14 ± 0.87</td>
<td>380.3 ± 81.1</td>
<td>4.76 ± 1.01</td>
<td>92.94 ± 0.43</td>
</tr>
<tr>
<td>Water extract</td>
<td>13.09 ± 1.39</td>
<td>109.1 ± 4.12</td>
<td>0.66 ± 0.10</td>
<td>79.13 ± 0.28</td>
</tr>
</tbody>
</table>
Selection of plant extract is important for obtaining phenolics and other antioxidants with acceptable yields. The kind of extract and the method of extraction are vital for separation of antioxidants in reasonable yields and economic viability. Several extracts and extraction methods for phenolics and other bioactive compounds from plants using a solvent such as ether, ethyl acetate, ethanol, and water were reported by Cheung, Cheung, and Ooi (2003) and Abo El-Maat, Mahgoub, Labib, Al-Gaby, and Ramadan (2016). The antioxidants reduce or prevent oxidation and contribute in scavenging free radicals and inhibiting their effect thus protecting against several infections and diseases, including cancer, heart, and degenerative diseases (Gülçin, 2010, 2012). They remove radicals and inhibit their initiation, and break their chain reaction; they also reduce hydrogen peroxides and oxygen singlets (Sreelatha & Padma, 2009). Results of the coherent study indicate that the AOA of MLE may be due to the presence of phenolics, which donate the electrons and react with free radicals converting them into the more stable product and end the chain reaction of free radicals.

*Moringa oleifera* is commonly applied in folk medicine via its anti-oxidant and health-promoting properties. Yet, its biological activities are not limited to the antioxidant capacity (Pandu, Cherupanalli, & Muthukumar, 2018). *M. oleifera* leaves are a great source of bio-actives and phenol compounds, such as flavonoids and phenolic acids. Flavonoids have been shown to protect against chronic diseases linked with oxidative stress, including cancer and cardiovascular disease (Pandey & Rizvi, 2009; Vergara-Jimenez, Almatrafi, & Fernandez, 2017). In dried *M. oleifera* leaves, chlorogenic acid was found as one of the main phenolic acids (Amaglo et al., 2010). Chlorogenic acid has a role in glucose metabolism. It inhibits glucose-6-phosphate translocase in the animal liver, reducing hepatic glycogenolysis and gluconeogenesis (Karthikesan, Pari, & Menon, 2010). In addition, chlorogenic acid had anti-dyslipidemic traits, as it reduces plasma cholesterol and triglycerides in obese Zucker animals fed a high fat diet (Cho et al., 2010) and reverses STZ-induced dyslipidemia in diabetic animals (Vergara-Jimenez et al., 2017; Verma, Singh, & Mishra, 2013). Recently, Khan, Parveen, Chester, Parveen, and Ahmad (2017) reported that aqueous extract of *M. oleifera* leaf protects pancreas against ROS-mediated damage by...
enhancing cellular antioxidant defenses and minimizing hyperglycemia in STZ-induced diabetes, which might be due to the glucose uptake enhancement in skeletal muscle, insulin secretion stimulation, and α-amylase and α-glucosidase inhibition.

Since the liver acts as a regulator of metabolic functions, its damage is associated with distortion of such functions. In absence of allopathic medical effective liver protective drugs, medicinal plants may offer a reliable management of liver disorder (Baravalia, Vaghasiya, & Chanda, 2011). Liver fibrosis is caused by hepatocyte damage due to actions of the hepatitis virus, bile duct obstruction, cholesterol overload, and chemicals such as CCl₄ (El-Hadary & Ramadan, 2016). Although incidences of chronic fibrosis are prevalent, there are no satisfactory effective agents with little side effects. Therefore, obtaining effective therapies to inhibit liver fibrosis and prevent cirrhosis is extremely vital. The ability of a hepatoprotective drug to alleviate the injurious effects or preserve the normal physiological mechanisms of the liver can be disturbed by hepatotoxic agents (Baravalia et al., 2011; Sanmugapriya & Venkataraman, 2006).

The current study shows that DcNa elevated serum ALT, AST, ALP, total bilirubin and direct bilirubin indicating chronic hepatotoxicity with a hazardous injury to hepatic cell membranes and liberation of enzymes into circulation. Increases in cytosolic enzymes in blood are requisites for markers of liver damage. The decrease in protein, albumin and globulin caused by toxicity of DcNa leading to
The DcNa is metabolized in the liver (Baravalia et al., 2011) and the main pathways are hydroxylation in the 4- and 5-positions; and (to a less extent) by forming 3’-hydroxy-(humans) and 4’,5-dihydroxy diclofenac. Diclofenac and its metabolites conjugate with glucuronic acid in sulfate; and the main constitutive P450 form in diclofenac hydroxylation in man is cytochrome P450 2C9 (the human orthologous of rat 2C11); diclofenac forms selective proteins in mice liver (Subramanian, 2009). This is caused by a transacylation reaction of its glucuronide conjugate; a mechanism proposed by Baravalia et al. (2011) to explain the allergic and intrinsic hepatotoxicity of drugs.

The studied moringa extracts showed differences in total yield, TPC, total flavonoids, and antioxidant activity due to various extractions. Administration of MLE improved liver and kidney functions as well as antioxidant enzymes in comparison with silymarin. It could be concluded that MLE, in the tested doses, exhibited in vivo AOA, which could have a beneficial effect against oxidative liver damage induced by DcNa. Bioactive phenolics in MLE protected plasma membrane and increased liver’s regenerative and reparative capacity. The beneficial MLE-effect may be due to the presence of phenolics causing membrane-stabilizing effects. The study demonstrates the hepatoprotective activity of MLE and thus supports the use of M. oleifera leaves as a traditional medicine for treating liver disorders. The hepatoprotective mechanisms of M. oleifera leave constituents are remain to be elucidated.

**TABLE 4 Effects of MLE on antioxidant markers in rats as affected by hepatic damage induced by DcNa**

<table>
<thead>
<tr>
<th>G</th>
<th>Treatment</th>
<th>Glutathione peroxidase (U/g tissue)</th>
<th>Glutathione-S-transferase (U/g tissue)</th>
<th>Glutathione reduced (µmol/g tissue)</th>
<th>Catalase (µM H₂O₂/Sec/g wt tissue)</th>
<th>Nitric oxide (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>182.6 ± 0.07</td>
<td>5.21 ± 0.02</td>
<td>88.60 ± 0.09</td>
<td>560.8 ± 0.53</td>
<td>14.40 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>Positive control (DcNa 100 mg/kg)</td>
<td>160.4 ± 0.07</td>
<td>4.00 ± 0.02</td>
<td>50.10 ± 0.09</td>
<td>544.0 ± 0.53</td>
<td>36.90 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>Silymarin (50 mg/kg) + DcNa (100 mg/kg)</td>
<td>219.2 ± 0.07</td>
<td>5.11 ± 0.02</td>
<td>128.4 ± 0.09</td>
<td>630.0 ± 0.53</td>
<td>26.40 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>MLE (150 mg/kg) + DcNa (100 mg/kg)</td>
<td>234.7 ± 0.07</td>
<td>5.22 ± 0.02</td>
<td>130.2 ± 0.09</td>
<td>701.3 ± 0.53</td>
<td>20.60 ± 0.06</td>
</tr>
<tr>
<td>5</td>
<td>MLE (300 mg/kg) + DcNa (100 mg/kg)</td>
<td>245.1 ± 0.07</td>
<td>5.27 ± 0.02</td>
<td>138.4 ± 0.09</td>
<td>730.8 ± 0.53</td>
<td>17.90 ± 0.06</td>
</tr>
</tbody>
</table>

Note. There is no significant difference (p > 0.05) between any two means with the same superscript letter in each column.

5 | CONCLUSION

The studied moringa extracts showed differences in total yield, TPC, total flavonoids, and antioxidant activity due to various extractions. Administration of MLE improved liver and kidney functions as well as antioxidant enzymes in comparison with silymarin. It could be concluded that MLE, in the tested doses, exhibited in vivo AOA, which could have a beneficial effect against oxidative liver damage induced by DcNa. Bioactive phenolics in MLE protected plasma membrane and increased liver’s regenerative and reparative capacity. The beneficial MLE-effect may be due to the presence of phenolics causing membrane-stabilizing effects. The study demonstrates the hepatoprotective activity of MLE and thus supports the use of M. oleifera leaves as a traditional medicine for treating liver disorders. The hepatoprotective mechanisms of M. oleifera leave constituents are remain to be elucidated.

**COMPLIANCE WITH ETHICS REQUIREMENTS**

This article does not contain any studies with human or animal subjects.

**CONFLICT OF INTEREST**

The authors declared that they have no conflict of interest.

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