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FULL ARTICLE

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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 $C_{25}H_{22}O_{10}$) 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, Phulukdaree, & 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 chemo-protection activity, and decreases aberrant crypt foci (ACF) number found in colon cancer-induced ICR-mice (Budda et al., 2011; Promkum, Kupradinun, Tuntipopipat, & Butryee, 2010). Anticancer properties of methanol extracts of moringa leaves showed an increase upto 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 $C_{14}H_{10}C_{l2}NNaO_2$. It is a nonsteroidal anti-inflammatory drug (NSAID) with an anti-inflammatory, analgesic and antipyretic effects (Husna, Sumera, 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 (Agúndez, 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-Ciocalteu and standards of phenolic compounds (Sigma Chemical Co., St. Louis, MO, USA), diagnostic kits (Bio Meriêuex 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-Ciocalteu and gallic acid being the standard. One milliliter of diluted extract (1 mg/ml) was mixed with 1 ml Folin-Ciocalteu and 1 ml of Na_2CO_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^{-5} 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:

$$\text{DPPH discoloration} = 100 \times \left(A_1 - A_2/A_2\right)$$

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-IOA (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, C_{18} 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 (λ_{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 patron 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, Meiattini, 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 ethanol 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 1 The yield of extracts, total phenolic content (TPC), total flavonoid content, and antioxidant activity of different *Moringa oleifera* leaf extracts

	Total extract (g/100 g)	TPC (mg GAE/g extract)	Total flavonoid content (mg QE/kg extract)	DPPH· scavenging activity (%)
Ether extract	11.85 ± 0.36	128.1 ± 2.02	2.47 ± 0.14	36.91 ± 5.92
Ethyl acetate extract	1.60 ± 0.22	91.77 ± 6.37	3.54 ± 0.47	43.64 ± 2.94
Ethanol extract (MLE)	15.14 ± 0.87	380.3 ± 81.1	4.76 ± 1.01	92.94 ± 0.43
Water extract	13.09 ± 1.39	109.1 ± 4.12	0.66 ± 0.10	79.13 ± 0.28

TABLE 2 Phenolic compounds (mg/kg) in MLE analyzed by HPLC

Compound	mg/kg
Gallic acid	9.43
Pyrogallol	80.52
3-OH-tyrosol	812.2
4-Amino-benzoic acid	21.51
Protocatechuic acid	49.13
Chlorogenic acid	233.3
Catechol	59.04
Catechin	128.7
Caffeine	138.3
p-hydroxybenzoic acid	54.15
Caffeic acid	14.52
Vanillic acid	7.33
Ferulic acid	103.2
Iso-ferulic acid	115.0
Ethyl vanillin	1,205
Resveratrol	100.52
Ellagic acid	65.67
α-coumaric acid	55.58
Benzoic acid	273.8
3,4,5-methoxy-cinnamic acid	172.5
Salicylic acid	240.0
Coumarin	22.29
p-coumaric acid	13.80
Cinnamic acid	3.45

Gepdiremen, & Boyer, 2006; Mohdaly, Mahmoud, Roby, Smetanska, & Ramadan, 2015). 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-Maati, 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 antioxidant and healt-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 bioactives 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

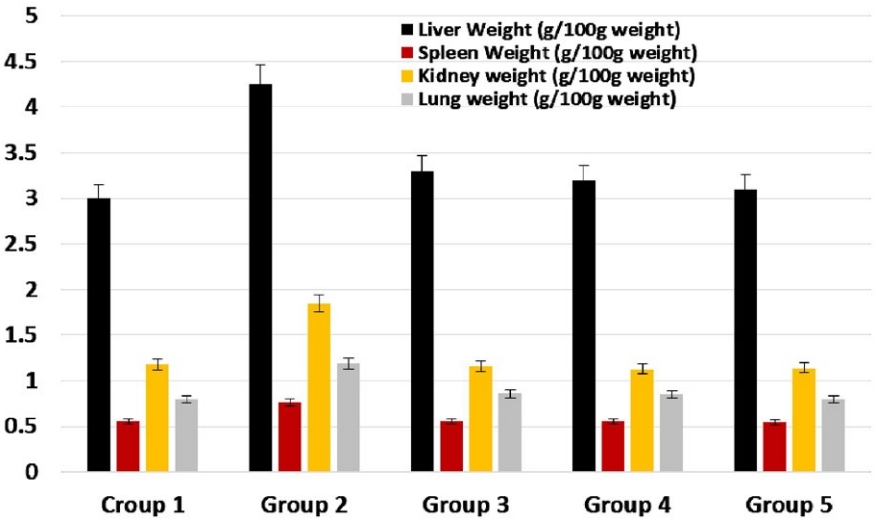

FIGURE 1 Effects of MLE on body weight gain and organs weights as affected by hepatic damage by DcNa

TABLE 3 Effect of MLE on rats liver functions as affected by hepatic damage induced by DcNa

G	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dL)		
					Total	Direct	Indirect
1	Negative control	78 ± 1.13^{c}	46 ± 0.89^{c}	250.1 ± 4.24^{c}	0.37 ± 0.02^{c}	0.10 ± 0.01^{cd}	0.27 ± 0.02^{c}
2	Positive control (DcNa 100 mg/kg)	196.5 ± 1.13^{a}	71.03 ± 0.89^{a}	473.0 ± 4.24^{a}	0.84 ± 0.02^{a}	0.29 ± 0.01^{a}	0.55 ± 0.02^{a}
3	Silymarin (50 mg/kg) + DcNa (100 mg/kg)	104.9 ± 1.13^{b}	52.59 ± 0.89^{b}	265.4 ± 4.24^{b}	0.42 ± 0.02^{b}	0.12 ± 0.01^{c}	0.30 ± 0.02^{b}
4	MLE (150 mg/kg) + DcNa (100 mg/kg)	82.31 ± 1.13^{c}	48.96 ± 0.89^{c}	249.8 ± 4.24^{c}	0.39 ± 0.02^{c}	0.14 ± 0.01^{b}	0.25 ± 0.01^{d}
5	MLE (300 mg/kg) + DcNa (100 mg/kg)	72.76 ± 1.13^{d}	45.43 ± 0.89^{c}	241.7 ± 4.24^{d}	0.37 ± 0.02^{c}	0.12 ± 0.01^{c}	0.25 ± 0.01^{d}

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

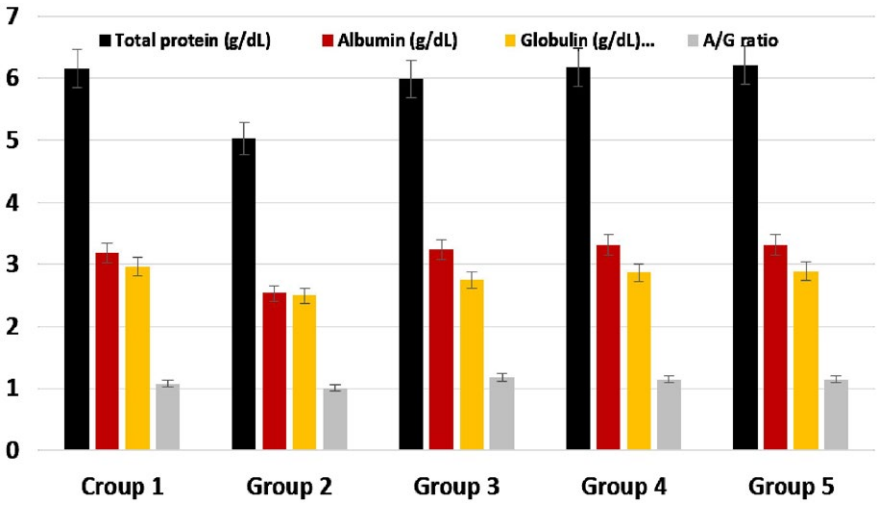

FIGURE 2 Effect of MLE on serum protein profile in rats as affected by hepatic damage induced by DcNa

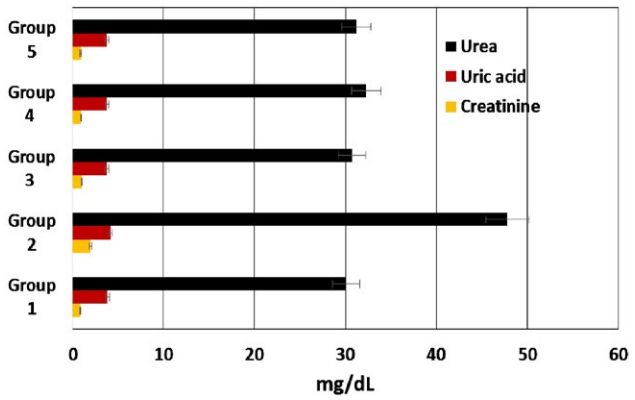

FIGURE 3 Effect of MLE on kidney function of rats as affected by hepatic damage induced by DcNa

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 CCl_4 (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 hepato-protective 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

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

G	Treatment	Glutathione peroxidase (U/g tissue)	Glutathione-S-transferase (U/g tissue)	Glutathione reduced (μmol/g tissue)	Catalase (μM H_2O_2/Sec/g wt tissue)	Nitric oxide (μmol/L)
1	Negative control	182.6 ± 0.07^{d}	5.21 ± 0.02^{a}	88.60 ± 0.09^{c}	560.8 ± 0.53^{d}	14.40 ± 0.06^{e}
2	Positive control (DcNa 100 mg/kg)	160.4 ± 0.07^{e}	4.00 ± 0.02^{b}	50.10 ± 0.09^{d}	544.0 ± 0.53^{e}	36.90 ± 0.06^{a}
3	Silymarin (50 mg/kg) + DcNa (100 mg/kg)	219.2 ± 0.07^{c}	5.11 ± 0.02^{a}	128.4 ± 0.09^{b}	630.0 ± 0.53^{c}	26.40 ± 0.06^{b}
4	MLE (150 mg/kg) + DcNa (100 mg/kg)	234.7 ± 0.07^{b}	5.22 ± 0.02^{a}	130.2 ± 0.09^{b}	701.3 ± 0.53^{b}	20.60 ± 0.06^{c}
5	MLE (300 mg/kg) + DcNa (100 mg/kg)	245.1 ± 0.07^{a}	5.27 ± 0.02^{a}	138.4 ± 0.09^{a}	730.8 ± 0.53^{a}	17.90 ± 0.06^{d}

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

alteration in mitochondrial function and inhibiting protein synthesis, all of which are due to liver damage through induction of lipid peroxidation. Treatment with MLE and silymarin can cause an ameliorating effect on the function of serum liver enzymes. MLE possess antioxidants, such as phenolic and flavonoid compounds, that play vital roles in protection against reactive oxygen species (ROS). Taha et al. (2015) reported that DcNa administration resulted in hepatocellular damage and alterations in the membrane permeability.

The present study showed increases in creatinine, urea and uric acid in rats treated with DcNa. The elevation of kidney function may be due to glomerular sufficiency as well as an increase in oxidative stress. Treating rats with silymarin and MLE decreased kidney functions, which revealed that MLE could prevent kidney damage. A marked decrease occurred in contents of antioxidant enzymes (GSH, GST, catalase, and GPx) and nitric oxide in the liver by DcNa. The MLE or silymarin treatment decreased in the effect of DcNa on liver enzymes and lipid peroxidation with increased hepatic antioxidant enzymes as well as a reduction in elevated nitric oxide (Onah et al., 2016).

Agúndez et al. (2011) and Husna et al. (2017) reported that NSAIDs are responsible for 10% of the cases of drug-induced hepatotoxicity. The mechanism of action was reported to occur *via* inhibition of the cyclooxygenase (COX), causing inhibition of prostaglandin synthesis leading to anti-inflammatory action (Mahalakshmi et al., 2010). NSAIDs induce liver damage due to the formation of ROS. Studies by Tarasankar et al. (2012) and Husna et al. (2017) showed that administration of NSAIDs increased lipid oxidation by decreasing the glutathione contents, leading to induced hepatotoxicity due to the generation of free radicals.

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 and sulfate; and the main constitutive P_{450} form in diclofenac hydroxylation in man is cytochrome P_{450}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.

5 | CONCLUSION

The studied moringa extracts showed differences in total yield, TPC, total flavonoids, and antiradical 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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REFERENCES

Abo El-Maati, M. F., Mahgoub, S. A., Labib, S. M., Al-Gaby, A. M. A., & Ramadan, M. F. (2016). Phenolic extracts of clove (*Syzygium aromaticum*) with novel antioxidant and antibacterial activities. *European Journal of Integrative Medicine*, *8*, 494–504.

Agúndez, J. A., Lucena, M. I., & Martínez, C. (2011). Assessment of non-steroidal anti-inflammatory drug-induced hepatotoxicity. *Expert Opinion on Drug Metabolism & Toxicology*, *7*, 817–828.

Ak, T., & Gülçin, I. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions*, *174*, 27–37.

Amaglo, N. K., Bennett, R. N., LoCurto, R. B., Rosa, E. A. S., LoTurco, V., Giuffrid, A., … Timpo, G. M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. *Food Chemistry*, *122*, 1047–1054.

Awodele, O., Oreagba, I. A., Odoma, S., Teixeira Da Silva, J. A., & Osunkalu, V. O. (2012). Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). *Journal of Ethnopharmacology*, *139*, 330–336.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity occurrence and potential uses. *Food Chemistry*, *99*, 191–203.

Baravalia, Y., Vaghasiya, Y., & Chanda, S. (2011). Hepatoprotective effect of *Woodfordia fruticosa* Kurz flowers on diclofenac sodium-induced liver toxicity in rats. *Asian Pacific Journal of Tropical Medicine*, *4*, 342–346. https://doi.org/10.1016/S1995-7645(11)60100-4

Bock, P. P., Karmer, R., & Paverka, M. (1980). A simple assay for catalase determination. *Cell Biology Monographs*, *7*, 44–74.

Brunelli, D., Tavecchio, M., Falcioni, C., Frapolli, R., Erba, E., Iori, R., & D'Incalci, M. (2010). The isothiocyanate produced from glucomoringin inhibits NF-kB and reduces myeloma growth in nude mice in vivo. *Biochemical Pharmacology*, *79*, 1141–1148.

Budda, S., Butryee, C., Tuntipopipat, S., Rungsipipat, A., Wangnaithum, S., Lee, J. S., & Kupradinun, P. (2011). Suppressive effects of *Moringa oleifera* Lam pod against mouse colon carcinogenesis induced by azoxymethane and dextran sodium sulfate. *Asian Pacific Journal of Cancer Prevention*, *12*, 3221–3228.

Charoensin, S. (2014). Antioxidant and anticancer activities of *Moringa oleifera* leaves. *Journal of Medicinal Plants Research*, *8*, 318–325.

Cheung, L. M., Cheung, P. C. K., & Ooi, V. E. (2003). Antioxidant activity and total phenolic of edible mushroom extracts. *Food Chemistry*, *81*, 249–255.

Cho, A. S., Jeon, S. M., Kim, M. J., Yeo, J., Seo, K. I., Choi, M. S., & Lee, M. K. (2010). Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. *Food and Chemical Toxicology*, *48*, 937–943.

Coppin, J. P., Xu, Y., Chen, H., Pan, M. H., Ho, C. T., Juliani, R., & Wu, Q. (2013). Determination of flavonoids by LC/MS and anti-inflammatory activity in *Moringa oleifera*. *Journal of Functional Foods*, *5*, 1892–1899.

Cuellar-Nuñez, M. L., Luzardo-Ocampo, I., Campos-Vega, R., Gallegos-Corona, M. A., González de Mejía, E., & Loarca-Piña, G. (2018). Physicochemical and nutraceutical properties of moringa (*Moringa oleifera*) leaves and their effects in an in vivo AOM/DSS-induced colorectal carcinogenesis mode. *Food Research International*, *105*, 159–168.

Doumas, B. T. (1975). Standard methods of protein determination. *Clinical Chemistry*, *7*, 175–188.

Doumas, B. T., Biggs, H. G., Arends, R. L., & Pinto, P. V. C. (1971). Albumin standard and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta*, *31*, 87–95.

El-Hadary, A. E., & Ramadan, M. F. (2016). Hepatoprotective effect of cold-pressed *Syzygium aromaticum* oil against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. *Pharmaceutical Biology*, *54*, 1364–1372. https://doi.org/10.3109/13880209.2015.1078381

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*, *5*, 1–8.

Gülçin, I. (2010). Antioxidant properties of resveratrol: A structure–activity insight. *Innovative Food Science and Emerging Technologies*, *11*, 210–218.

Gülçin, I. (2012). Antioxidant activity of food constituents: An overview. *Archives of Toxicology*, *86*, 345–391.

Gülçin, İ., Elias, R., Gepdiremen, A., & Boyer, L. (2006). Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). *European Food Research and Technology*, *223*, 759–767.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, *249*, 7130–7139.

Husna, M., Sumera, S., Laiba, S., & Anam, A. (2017). The effect of crude *Nigella sativa* oil against the acute toxicity of diclofenac sodium and ibuprofen on the liver of albino mice. *Slovenian Veterinary Research*, *54*, 21–27.

Karthikesan, K., Pari, L., & Menon, V. P. (2010). Combined treatment of tetrahydrocurcumin and chlorogenic acid exerts potential antihyperglycemic effect on streptozotocin-nicotinamide-induced diabetic rats. *General Physiology and Biophysics*, *29*, 23–30.

Khan, W., Parveen, R., Chester, K., Parveen, S., & Ahmad, S. (2017). Hypoglycemic potential of aqueous extract of *Moringa oleifera* leaf and *in vivo* GC-MS metabolomics. *Frontiers in Pharmacology*, *8*, 577.

Kumar, V., Pandey, N., Mohan, N., & Singh, R. P. (2012). Antibacterial and antioxidant activity of different extract of *Moringa oleifera* leaves—An in vitro study. *International Journal of Pharmaceutical Sciences Review and Research*, *12*, 89–94.

Lauer, B., Tuschi, G., King, M., & Mueller, S. O. (2009). Species-specific toxicity of diclofenac and troglitazone in primary humane and rat hepatocytes. *Chemico-Biological Interactions*, *179*, 17–24.

Lee, J. C., Kim, H. R., Kim, J., & Jang, Y. S. (2002). Antioxidant property of an ethanol of the stem of *Opuntia ficu-indica* var. Saboten. *Journal of Agricultural and Food Chemistry*, *50*, 6490–6496.

Leone, A., Fiorillo, G., Criscuoli, F., Ravasenghi, S., Santagostini, L., Fico, G., & Bertoli, S. (2015). Nutritional characterization and phenolic profiling of *Moringa oleifera* leaves grown in Chad, Sahrawi refugee camps, and Haiti. *International Journal of Molecular Sciences*, *16*, 18923–18937.

Mahalakshmi, R., Rajesh, P., Ramesh, N., Balasubramanian, V., & Kannan, V. R. (2010). Hepatoprotective activity on *Vitexnegundo* Linn. (Verbenaceae) by using wistar albino rats in ibuprofen induced model. *International Journal of Pharmacology*, *6*, 658–663.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina faso honey, as well as their radical scavenging activity. *Food Chemistry*, *91*, 571–577.

Mohammed, S., & Abd Manan, F. (2015). Analysis of total phenolics, tannins and flavonoids from *moringa olifera* seed extract. *Journal of Chemical and Pharmaceutical Research*, *7*, 132–135.

Mohdaly, A. A. A., Mahmoud, A. A., Roby, M. H. H., Smetanska, I., & Ramadan, M. F. (2015). Phenolic extract from propolis and bee pollen: Composition, antioxidant, and antibacterial activities. *Journal of Food Biochemistry*, *39*, 538–547.

Mona, M. A. (2013). The potential of *Moringa olifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria* subsp. Sativa) plants. *International Journal of Plant Physiology and Biochemistry*, *5*, 42–49.

Montgomery, H. A. C., & Dymock, J. F. (1961). Colorimetric determination of nitric oxide. *Analyst*, *86*, 414–417.

Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of GSH, GR and GST activities in rat lung and liver. *Biochimica et Biophysica Acta*, *582*, 67–78.

Organisation for Economic Cooperation and Development (OECD). (2001). *OECD guideline for testing of chemicals. Acute oral toxicity-acute toxic class method. OECD 420. Acute oral toxicity-fixed dose procedure*. Paris, France: Author.

Onah, C. E., Meludu, S. C., Dioka, C. E., Onuegbu, A. J., Onah, C. F., Ajaghaku, D. L., … Ejeatuluchukwe, O. (2016). Amelioratory effect of methanolic leaf extract of *Moringa oleifera* on some liver and kidney function and oxidative stress markers in lead- intoxicated rats. *European Journal of Medicinal Plants*, *12*, 1–12.

Pal, S. K., Mukherjee, P. K., & Saham, B. P. (1995). Studies on the antiulcer activity of *M.oleifera* leaf extracts on gastric ulcer models in rats. *Phytotherapy Research*, *9*, 463–465.

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, *2*, 270–278.

Pandu, S. M., Cherupanalli, R., & Muthukumar, S. P. (2018). Effects of bound phenolic from defatted *Moringa oleifera* seed flour on diet-induced hypercholesterolemic mice. *Journal of Food Biochemistry*. https://doi.org/10.1111/jfbc.12553

Prakash, D., Singh, B. N., & Upadhyay, G. (2007). Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chemistry*, *102*, 1389–1393.

Prins, H. K., & Loos, J. A. (1969). Determination of energy-rich phosphate, 2, 3-diphosphoglycerate, lactate, and glutathione in small amounts of blood cells. *Advances in Autoanalysis*, *1*, 285–291.

Promkum, C., Kupradinun, P., Tuntipopipat, S., & Butryee, C. (2010). Nutritive evaluation and effect of *Moringa oleifera* pod on clastogenic potential in the mouse. *Asian Pacific Journal of Cancer Prevention*, *11*, 627–632.

Purwal, L., Pathak, A. K., & Jain, U. K. (2010). In vivo anticancer activity of the leaves and fruits of *Moringa oleifera* on mouse melanoma. *Pharmacologyonline*, *1*, 655–665.

Reda, F., Borjac, J., Fakhouri, R., & Usta, J. (2017). Cytotoxic effect of *Moringa oleifera* on colon cancer cell lines. *Acta Horticulturae*, *1158*, 269–278.

Reeves, P. G., Nielsen, F. H., & Fahey, G. C. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition*, *123*, 1939–1951.

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, *22l*, 56.

Sanmugapriya, E., & Venkataraman, S. (2006). Studies on hepatoprotective and antioxidant actions of *Strychnos potatorum* Linn. Seeds on CCl_4-induced acute hepatic injury in experimental rats. *Journal of Ethnopharmacology*, *105*, 154–160. https://doi.org/10.1016/j.jep.2005.10.028

SAS. (1996). *SAS procedure guide*. "Version 6.12 Ed". Cary, NC: SAS Institute Inc.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Sreelatha, S., & Padma, P. R. (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition*, *64*, 303–311. https://doi.org/10.1007/s11130-009-0141-0

Subramanian, S. (2009). Diclofenac induced toxic manifestations on adjuvant induced arthritic rats pheripheral and reproductive organ of male wistar rats rattus norvegicus. *Journal of Toxicology and Environmental Health Sciences*, *1*, 12–21.

Tabacco, A., Meiattini, F., Moda, E., & Tarlip, P. (1979). Simplified enzymitic colorimetric serum urea nitrogen determination. *Clinical Chemistry*, *25*, 336–337.

Taha, N. R., Rabah, O. S., Shaker, A. S., & Mograby, M. M. (2015). Effect of *Moringa oleifera* leaves on diclofinc sodium-induced hepatic injury in albino rats: Ultrastructureal and immunohistochemical studies. *Journal of Cytology & Histology*, *6*, 315–322.

Tarasankar, M., Ahmad, A., Pahari, N., & Gangu-li, S. (2012). Hepatoprotective activity of *Mikania scandes* (L.) wild against diclofenac sodium induced liver toxicity in rats. *Asian Journal of Pharmaceutical Sciences*, *5*, 185–189.

Tietz, N. M. (1983). *Textbook of clinical chemistry*. Philadelphia, PA: W. B. Sunders Co.

Tiloke, C., Phulukdaree, A., & Chuturgoon, A. A. (2013). The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. *BMC Complementary Alternative Medicine*, *13*, 226.

Tragulpakseerojn, J., Yamaguchi, N., Pamonsinlapatham, P., Wetwitayaklung, P., Yoneyama, T., Ishikawa, N., & Apirakaramwong, A. (2017). Anti-proliferative effect of *Moringa oleifera* Lam (Moringaceae) leaf extract on human colon cancer HCT116 cell line. *Tropical Journal of Pharmaceutical Research*, *16*, 371–378. https://doi.org/10.4314/tjpr.v16i2.16

Vergara-Jimenez, M., Almatrafi, M. M., & Fernandez, M. L. (2017). Bioactive components in *Moringa oleifera* leaves protect against chronic disease. *Antioxidants*, *6*, 91. https://doi.org/10.3390/antiox6040091

Verma, S., Singh, A., & Mishra, A. (2013). Gallic acid: Molecular rival of cancer. *Environmental Toxicology and Pharmacology*, *35*, 473–485.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., & Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Industrial Crops and Products*, *44*, 566–571.

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