Moringa oleifera-based diet protects against nickel-induced hepatotoxicity in rats

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Abstract

Multiple health-promoting effects have been attributed to the consumption of Moringa oleifera leaves, as part of diet without adequate scientific credence. This study evaluated the effect of M. oleifera-based diets on nickel (Ni)-induced hepatotoxicity in rats. Male rats assigned into six groups were given oral administration of 20 mg/kg body weight nickel sulfate in normal saline and either fed normal diet or M. oleifera-based diets for 21 days. All animals were sacrificed under anesthesia 24 hours after the last treatment. Ni exposure elevated the rat plasma activities of alanine transaminase, aspartate transaminase and alkaline phosphatase significantly. Ni exposure also raised the levels of triglyceride, total cholesterol and low-density lipoprotein cholesterol while depleting the high-density lipoprotein cholesterol concentration. Further, Ni exposure raised rat plasma malondialdehyde but depleted reduced glutathione concentrations. The histopathological presentations revealed inflammation and cellular degeneration caused by Ni exposure. We show evidence that M. oleifera-based diets protected against Ni-induced hepatotoxicity by improving the rat liver function indices, lipid profile as well as restoring cellular architecture and integrity. Study lends credence to the health-promoting value of M. oleifera as well as underscores its potential to attenuate hepatic injury.

Keywords: diet supplement, hepatoprotective, metal toxicity, Moringa oleifera

Introduction

Moringa oleifera (M. oleifera) Lam is a perennial plant belonging to the Moringaceae family. The M. oleifera is an edible plant with impressive range of medicinal uses and high nutritional value[1]. M. oleifera is a promising medicinal plant found in almost every country and can grow even in harsh conditions[2]. It is native to tropical Africa but also widely distributed in India, Ceylon and Madagascar[3]. The medicinal and nutritional values of M. oleifera have been well documented[4–5]. In folk medicine, M. oleifera is used extensively in the treatment of ascites, rheumatism, heart damage, venomous bites and as a cardiac and circulatory stimulant[6]. While every part of the plant has been shown to possess therapeutic properties[7], the leaves have been used to treat various ailments like obesity, cancer, liver cirrhosis and kidney damage[4,8]. The leaves of M. oleifera can be eaten raw, cooked or

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air-dried and stored over a long period of time without losing its therapeutic properties\(^9\). In terms of phytochemistry, the *M. oleifera* plant has been implicated for richness in potassium, calcium, phosphorus, iron, vitamins A and D, essential amino acids, as well as antioxidants such as \(\beta\)-carotene, vitamin C and flavonoids\(^{10-12}\). Moreover, studies have demonstrated the relative safety of the plant\(^{13-14}\).

Perhaps, the numerous medicinal and nutritional significances of *M. oleifera*\(^{4,8,9,15-16}\) are fueling the dietary consumption of *M. oleifera* for personal health preservation and/or self-medication. Meanwhile, there is growing advocacy for the addition of *M. oleifera* to diet as an adjunct to therapy for personal health improvement\(^{9,17}\). In light of the many health-promoting benefits attributed to the consumption of *M. oleifera*, this study investigated the protective effect of *M. oleifera*-based diets on Ni-induced hepatotoxicity in rats.

**Materials and methods**

**Chemicals and reagents**

All chemicals and reagents used were of analytical grade. Commercial reagent kits for the assay of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferases (ALT), total cholesterol (TC), total protein (TP), triglyceride (TAG), high density lipoprotein cholesterol (HDL-C) were supplied by Randox Diagnostics, Crumlin, UK.

**Experimental animals**

Thirty-six male rats of Wistar strain weighing between (190-210) g were obtained from the Experimental Animal Farm at the University of Ilorin, Ilorin, Nigeria. The Wistar rats were placed in plastic animal cages and housed in a well-ventilated experimental room. The rats were allowed to acclimatize for a period of 14 days before the commencement of treatments. The animals were maintained at (22\(^\circ\)C5)\(^\circ\)C with humidity control with automatic dark and light cycle of 12 hours. The animals were given standard rat feed and clean drinkable water \textit{ad libitum}. Handling of animals was in accordance with relevant local institutional and ethical guidelines as approved for scientific study.

**M. Oleifera**

The leaves of *M. Oleifera* were harvested at the Landmark University Farm, Omu-Aran, Nigeria. The leaves were identified and authenticated by Mr Bolu Ajayi at the Herbarium Unit, Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The specimen voucher number is VIH 001/1008. Leaves were air-dried and ground into powder. The powdered leaf material was stored at room temperature in cool dry environment until required for use.

**Table 1  Formulation of experimental diets**

| Feed Components | Normal Feed (g) | 5% *Moringa oleifera* (g) | 10% *Moringa oleifera* (g) | 15% *Moringa oleifera* (g) |
|-----------------|-----------------|---------------------------|---------------------------|---------------------------|
| Sucrose         | 100             | 100                       | 100                       | 100                       |
| Cellulose       | 40              | 40                        | 40                        | 40                        |
| Soybean         | 250             | 250                       | 250                       | 250                       |
| *Moringa oleifera* | -              | 50                        | 100                       | 150                       |
| Vitamin Mix     | 50              | 50                        | 50                        | 50                        |
| D-methionine    | 4               | 4                         | 4                         | 4                         |
| Corn starch     | 506             | 456                       | 406                       | 356                       |
| Soybean oil     | 50              | 50                        | 50                        | 50                        |
| Total           | 1,000           | 1,000                     | 1,000                     | 1,000                     |

**Table 2  Proximate composition (%) of experimental diets**

| *M. oleifera* | Ash content | Carbohydrate | Crude fat | Crude fibre | Protein | Moisture content |
|---------------|-------------|--------------|-----------|-------------|---------|-----------------|
| 0 (control)   | 5.9±0.32    | 43.01±0.61   | 6.5±0.74  | 11.35±0.10  | 25.54±0.52 | 8.5±0.12        |
| 5%            | 6.4±0.11    | 30.34±0.13*  | 8.25±0.25 | 13.03±0.06  | 32.88±0.49* | 9.1±0.31        |
| 10%           | 7.12±0.03   | 28.02±0.05*  | 10.5±0.72 | 12.4±0.90   | 32.24±0.38* | 9.72±0.04       |
| 15%           | 8.5±0.42    | 27.22±0.39*  | 10.8±0.30 | 11±0.15     | 31.58±0.68* | 11.5±0.29       |

Note: Data are presented as mean values±standard error of mean (SEM), \(n=3\). * vs. control at \(P<0.05\).
Preparation of *M. oleifera*-based diet

*M. oleifera*-based diets were prepared as previously described[15]. Briefly, the feed composition consisted of sucrose, cellulose, soybean, vitamin mix, D-methionine, corn starch and soybean oil. The *M. oleifera* leaves were added in various concentrations to yield 5%, 10% and 15% supplementation. Additional details are as presented in Table 1.

Determination of proximate composition

Protein content, crude fat and fiber, moisture content, carbohydrate and ash content of diets were determined following established protocols as described by the Association of Official Analytical Chemists[18].

Experimental design

Thirty-six Wistar rats were randomly assigned to receive NiSO₄ and normal rat diet lacking *M. oleifera* (Group 1), NiSO₄ and 5% *M. oleifera*-based diet (Group 2), NiSO₄ and 10% *M. oleifera*-based diet (Group 3), NiSO₄ and fed with 15% *M. oleifera*-based diet (Group 4), normal saline and fed with 15% *M. oleifera*-based diet (Group 5) and normal saline and fed with normal rat diet lacking *M. oleifera* (Group 6).

The rats were given daily administration of NiSO₄ at 20 mg/kg bodyweight by oral gavage. The rats were fed with either the normal diet or *M. oleifera*-based diets daily. Treatments lasted for 21 days. Handling of animals was consistent with relevant guidelines on the care and use of laboratory animals[19].

Necroscopy

All animals were fasted overnight and sacrificed under anesthesia in slight diethyl ether, 24 hours after the last treatments. Blood samples were obtained by cardiac puncture into clean EDTA bottles. The blood samples were centrifuged at 4,000 g for 10 minutes in a refrigerated centrifuge (Anke TDL-5000B, Shanghai, China) to yield plasma for biochemical determinations. The liver from each animal was excised into iced isotonic solution (pH 7.4) and weighed immediately. The liver samples were then fixed in 10% buffered neutral formalin and used for histopathologic examinations.

Biochemical assays

The biochemical indices were determined in rat plasma using a UV/Vis spectrophotometer (Jenway, Staffordshire, UK) where applicable. The levels of rat plasma TP, AST (EC: 2.6.1.1), ALT (EC: 2.6.1.2), ALP (EC: 3.1.3.1), lipid profile including TC, TAG, and HDL-C were determined using Randox assay kits (Crumlin, UK). Low-density lipoprotein cholesterol (LDL-C) was estimated according to the Friedewald formula[20]. Reduced glutathione level (GSH) was determined as described by Ellman[21] and Akanji et al.[22]. Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) using the method described by Niehaus and Samuelson[23].

Histopathologic examination

The rat liver was fixed in 10% buffered neutral formalin immediately following excision from animals. Fixed tissues were subsequently processed for histopathologic examinations as previously described[24]. Capture and scoring for morphological changes were done by a pathologist blind to the treatments, at the University of Ilorin Teaching Hospital, Ilorin, Nigeria. The photomicrographs were captured at ×100 using the software Presto Image Folio package.

Data analysis

Data were analyzed using the one-way ANOVA (GraphPad Software Inc., San Diego, CA, USA) and presented as the mean±standard error of mean (SEM). Differences among the group means were determined by the Tukey’s test. Mean values at *P*<0.05 were considered to be significant.

![Fig. 1](image-url) **Effect of nickel exposure and *Moringa oleifera*-based diets.** A: Average rat weight; B: rat liver weight; C: liver-to-bodyweight ratio. Data are presented as mean value±standard error of mean (SEM), *n* = 6. Relative to nickel only group (control), *P*<0.05, **P**<0.01 and ***P***<0.001.
Fig. 2  Effect of nickel exposure and *Moringa oleifera*-based diets. A: Rat plasma protein concentration. B: Rat plasma alanine transaminase activity. C: Rat plasma aspartate transaminase activity. D: Rat plasma alkaline phosphatase activity. Data are presented as mean value±standard error of mean (SEM), n = 6. ***P < 0.001 relative to nickel only group (control).

Fig. 3  Effect of nickel exposure and *Moringa oleifera*-based diets. A: Rat plasma total cholesterol concentration; B: rat plasma tryglyceride concentration; C: rat plasma high density lipoprotein-cholesterol (HDL-C) concentration; D: rat plasma low density lipoprotein-cholesterol (LDL-C) concentration. Data are presented as mean value±standard error of mean (SEM), ***P < 0.001 relative to nickel only group (control).
RESULTS

Proximate composition

The experimental diets were evaluated for proximate composition using the AOAC protocols. The contents of crude fat, fiber, moisture and ash in the normal diet and *M. oleifera*-based diets showed no significant difference (Table 2). The carbohydrate content of *M. oleifera* supplemented diets decreased with increasing concentration of *M. oleifera* relative to the normal diet. Conversely, *M. oleifera*-based diets had higher protein content.

Body and liver weights

Ni exposure caused reduction in rat body and liver weights (Fig. 1). Ni exposure also altered the liver-to-bodyweight ratio. However, feeding rats with *M. oleifera*-based diets appreciably improved the rat weight indices.

Plasma protein and liver marker enzymes

To evaluate for liver function, some biochemical indices including ALT, AST, ALP and TP were determined in rat plasma. The rat plasma protein was depleted following daily exposure to Ni. However, Ni-induced reduction in plasma protein was mitigated by the *M. oleifera*-based diet (Fig. 2). Ni exposure caused significant elevation of rat plasma ALT, AST and ALP, but these increases were averted in groups sustained on *M. oleifera*-based diets.

![Fig. 4 Effect of nickel exposure and *Moringa oleifera*-based diets. A: Atherogenic index (AI) in rats; B: rat plasma malondialdehyde (MDA) concentration; C: rat plasma reduced glutathione (GSH) concentration. Data are presented as mean value±standard error of mean (SEM), n = 6. ***P<0.001 relative to nickel only group (control).](image)

![Fig. 5 Photomicrographs of rat liver following exposure to nickel and sustenance on either a normal diet or *Moringa oleifera*-based diets. H&E staining (×400). A: Nickel group (1) but sustained on normal diet showing inflammation and cellular degeneration. B: Nickel group (2) but sustained on 5% *Moringa oleifera* diet indicating cellular restoration. C: Nickel group (3) but sustained on 10% *Moringa oleifera* diet shows improvement of cellular architecture. D: Nickel group (4) but sustained on 15% *Moringa oleifera* diet shows restoration of cellular architecture. E: Group (5) sustained only on 15% *Moringa oleifera* diet shows intact cellular architecture. F: Group (6) sustained only on normal diet shows intact cellular architecture.](image)
Lipid profile

Ni exposure raised ($P<0.05$) the rat TC, TAG and LDL-C. In contrast, rat plasma HDL-C was depleted following Ni exposure. The *M. oleifera*-based diets reversed Ni-induced lipid profile alterations (Fig. 3).

Atherogenic index (AI), lipid peroxidation and reduced glutathione

Furthermore, Ni exposure raised the rat AI and increased the plasma concentration of MDA while decreasing the concentration of GSH. However, feeding *M. oleifera*-based diets to rats lowered the AI, reduced the level of MDA and raised the GSH concentration (Fig. 4).

Histopathologic changes

The histopathological presentations revealed inimical cellular alterations including inflammation caused by Ni exposure. However, feeding *M. oleifera*-based diets to rats restored the cellular architecture and integrity (Fig. 5).

DISCUSSION

This study investigated the protective effect of *M. oleifera*-based diets on Ni-induced hepatotoxicity. The proximate composition of diets revealed high protein but low carbohydrate content for *M. oleifera*-based diets. This is consistent with previous findings, and may indicate relevance for nutritional management of metabolic related disorder.

Earlier studies showed that change to body or organ weights after chemical exposure may indicate toxicity. In the present study, Ni exposure caused rat weight loss as well as alterations in vital organ weights in manners that may suggest ensuing adverse effect. However, *M. oleifera*-based diets improved Ni-induced rat weight loss, thus ameliorating the effect of Ni-induced toxicity. Further, Ni exposure caused liver injury evidenced by the elevated plasma activities of ALT, AST and ALP. Elevated liver function indices have been shown to implicate cellular damage. Conversely, the *M. oleifera*-based diets demonstrated protection against Ni assault on rat by improving the liver function indices. Our findings are in consonance with previous reports which demonstrated the relative safety, and hepatoprotective potential of *M. oleifera*.

Furthermore, the addition of *M. oleifera* to diets protected rats against Ni-induced alteration of lipid profile by elevating the HDL-C while reducing the AI. This agrees with previous findings, which demonstrated the anti-lipidemic and/or cardiovascular-promoting effect of *M. oleifera*. The elevation of HDL-C by *M. oleifera*-based diets resulted in the decrease of atherogenic index (AI) and may indicate that *M. oleifera* has potential for cardiovascular health-promoting effect.

Also, we determined the concentration of GSH and MDA in rat plasma as early indicators of oxidative stress. Ni exposure raised the rat plasma MDA but lowered GSH concentrations. This may indicate the presence of lipid peroxidation as well as ensuing oxidative stress caused by Ni exposure. However, the *M. oleifera*-based diets elevated plasma GSH concentration while decreasing the MDA concentration in clear demonstration of protective capacity against Ni-induced oxidative cellular damage. The findings support earlier reports which showed the capability of *M. oleifera* to ameliorate oxidative stress. In like manner, separate studies have demonstrated the potential of *M. oleifera* to ameliorate oxidative stress. Further, the histopathology revealed Ni-induced inflammatory processes and cellular degeneration. However, these cellular lesions were conspicuously absent in the groups sustained on *M. oleifera*-based diets, thus reinforcing the hepatoprotective potential of *M. oleifera*-based diets. These findings are further evidence of the nutritional and medicinal value of *M. oleifera*. According to existing reports, *M. oleifera* leaves have high polyphenolic contents among other phyto-constituents which could be responsible for several of the medicinal potential.

The protective effect of *M. oleifera* on indices of lipid profile, liver function and oxidative stress may substantiate its inclusion as part of diet for personal health improvement. In summary, the addition of *M. oleifera* as part of diet holds prospects which may be explored in several significant ways; e.g. for workers in the mining industries or other related sectors where the frequency of exposure to heavy metals is high, consumption of diet containing *M. oleifera* may help protect against occupational health risks.

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