Research Article

Prophylactic Action of *Moringa oleifera* against Cyclophosphamide-Induced Harmful Effects in Male Mice

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Abstract | The current study examined how *Moringa oleifera* protects male mice's liver, kidney, and blood against the effects of cyclophosphamide (CP). Forty male Swiss albino mice were divided evenly into four groups (Gp1, control group; Gp2, *Moringa oleifera* group; Gp3, CP group; Gp4, CP+*Moringa oleifera*). The CP group had lower serum ALT, AST, and ALP levels than the control and *Moringa oleifera* groups. In contrast, the CP group's blood albumin levels significantly increased in comparison to the control and *Moringa oleifera* groups. The CP group, on the other hand, experienced a significant increase in ALT, AST, and ALP levels and a drop in albumin level as a result of *Moringa oleifera* treatment. The CP group had lower levels of serum urea and creatinine than the *Moringa oleifera* and control groups. However, as compared to the control group, the group that received *Moringa oleifera* treatment had significantly higher blood urea and creatinine levels. Blood potassium and sodium ion levels were lower in the CP group than in the *Moringa oleifera* and control groups. However, the *Moringa oleifera*-treated CP group exhibited noticeably higher blood levels of potassium and sodium ions than the CP group. The CP group's RBC and WBC levels and HB% were significantly lower than those of the control and *Moringa oleifera* groups; however, these changes are regulated and improved by *Moringa oleifera* treatment. These results exhibited that moringa has a potential to modify the liver and kidney biomarkers in CP-treated mice.

Keywords | Cyclophosphamide, *Moringa oleifera*, mice, CBC, liver Functions, kidney Functions

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INTRODUCTION

Moringa oleifera is a rapidly growing tree that was first employed in traditional medicine in sub-Himalayan regions of Bangladesh, Pakistan, India, and Afghanistan. Among the ailments that the herbs are used to cure include nerve diseases (including hysteria, headaches, epilepsy, and muscular spasmodic (Fahey, 2005). According to earlier reports, *Moringa oleifera* leaves have nootropic properties that can improve memory (Mohan *et al.*, 2005) most likely via changing the electrical activity and monoamine levels in the brain (Ganguly and Guha *et al.*, 2008). Furthermore, it has been shown that the methanol extract from the leaves provides protection against seizures caused by pentylenetetrazole and convulsions induced by the maximum electroshock seizure test (Amrutia *et al.*, 2011). Moringa leaves are abundant in vitamins (especially C and E), proteins, calcium, iron, potassium, β-carotene, and bioactive and antioxidant substances such tannins, saponins, glucosinolates and isothiocyanates, flavonoids, and phenolic acids. Thus, the many pharmacological qualities ascribed to *Moringa oleifera* leaves appear to have

originated from Moringa leaves that were illuminated in many animal species (Chachar *et al*., 2024). Flavonoids and polyphenols are referred to be natural antioxidants in this context. The cycle of lipid peroxidation can be inhibited or broken by polyphenols and flavonoids because they may interact directly with lipid peroxyl radicals and superoxide anions (Tuorkey, 2016). cyclophosphamide (CP) is a well-researched teratogen that mostly causes skeletal and central nervous system abnormalities in humans, rats, mice, rabbits, and primates. Moreover, CP is among the antienoplastic substances that have been examined the most. According to recent research employing *in vitro* mouse embryo culture, CP has to be bioactivated in order to be teratogenic (Mirkes, 1985).

DNA cross-linking appears to be a key factor in the antineoplastic characteristics of CP, despite the fact that the nature of the DNA lesions that cause its teratogenic, mutagenic, and antineoplastic actions are not well known (Emadi *et al*., 2009). Cyclophosphamide that was injected quickly spread to 64% of body weight, and in individuals who had never taken the medication before, its half-life in plasma was 6.5 hours. At all dosage levels, no more than 20% of the injected cyclophosphamide was eliminated intact in the urine (De Jonge *et al*., 2005; Bagley *et al*., 1973). The aim of our study is to investigate the protective effect of *Moringa oleifera* against the toxic effect caused by cyclophosphamide in male mice.

MATERIALS AND METHODS

Animals

Each of the 40 male Swiss albino mice weight between 20 and 25 grams were assigned at random to rooms with relative humidity and a 12-hour light/dark cycle, and an ambient temperature of 22 to 25 degrees Celsius. The animals were fed a commercial meal and unrestricted water for two weeks.

Experimental design and animal groups

Four groups of ten mice each were formed with equal numbers of animals: G1 – Control, G2 – *Moringa oleifera*, G3 – Cyclophosphamide (CP), and G4 – Cyclophosphamide (CP) + *Moringa oleifera*. A dry extract of *Moringa oleifera*, diluted in distilled water, was administered orally at a dosage of 250 mg/kg body weight daily for 14 days (Bakre *et al*., 2013). Cyclophosphamide (100 mg/kg body weight) was administered intraperitoneally as a single dose (Pavin *et al*., 2018). In the G4 group, CP was administered one hour after the final dose of the *Moringa oleifera* extract on the 14th day.

Sample collection

At the end of the experiment, the mice having fasted overnight were anesthetized, and two ocular blood samples were collected from each animal. To prevent clot formation, the first portion was drawn into a heparinized tube and mixed thoroughly before analysis for complete blood counts (CBCs). The second portion was centrifuged at 3000 rpm for 10 minutes at room temperature. The resulting serum was separated and stored at –20°C in sterile stoppered vials until analysis. The serum samples were used to assess kidney function and electrolyte levels.

Complete blood counts (CBC)

After being transferred to CBC tubes containing EDTA as an anticoagulant and kept in a cool box with ice packs, the blood samples were delivered directly to the lab for processing according to Hasan *et al*. (2022).

Biochemical assays

Serum creatinine and urea levels in mice were determined according to the method of Patton and Crouch (1977). Blood electrolyte levels, including potassium and sodium, were measured using pre-calibrated setups (Sensa Core Electrolyte Analyzer, India), following the procedures described by Hasan *et al*. (2024), Ezz *et al*. (2023), and Hameed *et al*. (2023). The activities of serum aspartate transaminase (AST) and alanine transaminase (ALT) were assessed using the method of Reitman and Frankel (1957), as reported by Hasan *et al*. (2024) and Al-Khuzaay *et al*. (2024). Serum albumin levels were determined according to the procedure described by Hasan *et al*. (2021).

Statistical analysis

The analysis was carried out using the Statistical Package for the Social Sciences (SPSS software version 16). The Least Significant Difference (LSD) tests and one-way ANOVA (Analysis of Variance) were used to statistically assess the data. The mean± standard error of mean (SEM) was used to present the data. The threshold for statistical significance was set at $P<0.05$. Group differences were assessed for significance using LSD comparisons.

RESULTS

Effect of *Moringa oleifera*, and cyclophosphamide (CP) on liver function parameters in male mice

The data presented in Table 1 indicate that rats treated with cyclophosphamide exhibited a significant decrease ($P < 0.05$) in serum ALT, AST, and ALP levels, while liver albumin levels were significantly increased compared to the control group. However, co-administration of *Moringa oleifera* with cyclophosphamide helped maintain these parameters closer to normal values.

Table 1: Variations in liver function parameters among the study groups.

Groups	ALT (U/L)	AST (U/L)	Alb (g/dL)	ALP (U/L)
G1	43.4^{b} ± 3.16	138.4^{b} ± 3.13	5. 66^{b} ± 0.08	130.1^{b} ± 3.25
G2	39.7^{b} ± 3.75	135.2^{b} ± 4.50	5.50^{b} ± 0.16	125.4^{b} ± 5.22
G3	55.3^{a} ± 2.77	177.3^{a} ± 4.22	3.33^{a} ± 0.22	150.1^{a} ± 8.63
G4	47.4^{b}±2.30	160.1^{ab} ± 3.55	4.11^{b} ± 0.30	136.7^{ab}±6.33

Data are expressed as mean ± S.E.M of 8 observations. G1: Control; G2: *Moringa oleifera*; G3: Cyclophosphamide (CP); G4: Cyclophosphamide + *Moringa oleifera;* ALT: Alanine transaminase; AST: Aspartate transaminase; Alb: Albumin; ALP: Alkaline phosphatase. ^{a}notable distinction from the control group. ^{b}Notable distinction from the CP group.

Effect of *Moringa oleifera*, and cyclophosphamide (CP) on Kidney Functions and Electrolytes in Male Mice

Data present in Table 2 showed that rats treated with cyclophosphamide caused significant decrease ($P < 0.05$) in serum urea, creatinine and Na+ while K+ in kidney was significantly increased as compared with control. The presence of *Moringa oleifera* with cyclophosphamide maintained the levels of the measured parameters closer to the normal values.

Table 2: Variations in the electrolyte and renal function parameters among the study groups.

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Na+ (mmol/L)	K+ (mmol/L)
G1	26.1^{b}± 2.02	0.42^{b} ± 0.11	133.3^{b}± 5.11	6.30^{b}±0.22
G2	24.1^{b} ± 2.32	0.41 ^{b}± 0.01	131.9^{b}± 5.23	4.21^{b}±0.13
G3	33.3^{a}± 1.03	0.55^{a} ± 0.04	139.0^{a} ± 6.11	3.11^{a}±0.11
G4	29.7^{ab}± 1.21	0.47^{b} ± 0.02	136.9^{b}± 5.11	4.60^{ab}±0.02

Data are expressed as mean ± S.E.M of 8 observations. G1: Control; G2: *Moringa oleifera*; G3: Cyclophosphamide (CP); G4: Cyclophosphamide + *Moringa oleifera*. ^{a}notable distinction from the control group. ^{b}Notable distinction from the CP group.

Effect of *Moringa oleifera*, and cyclophosphamide (CP) on Hematological Parameters in Male Mice

Data present in Table 3 showed that rats treated with cyclophosphamide exhibited significant decline ($P < 0.05$) in serum RBCs ,WBCs while Hb in kidney was non affected as compared with control. The presence of *Moringa oleifera* with cyclophosphamide maintained the levels of the measured parameters closer to the normal values.

DISCUSSION

Moringa oleifera has not been shown to aid in weight loss or significantly alter illness risk factors in overweight individuals. However, in this study, the administered dosage of *Moringa oleifera* did not produce any adverse effects in experimental animals, as also reported by Fakurazi *et al.* (2008), Onah *et al.* (2016), Priyadarshani and Verma (2014), and Alia *et al.* (2019).

Table 3: Variations in RBCs, Hb%, and WBCs levels among the various research groups.

Groups	RBCs (10^{6}/ml)	Hb (g/dL)	WBCs (10^{3}/ml)
G1	7.59^{b} ± 0.12	14.2^{b} ± 1.11	7. 61^{b}± 0.11
G2	6.62^{b} ± 0.02	14.2^{b} ± 0.03	7.77^{b} ± 0.20
G3	9.80^{a}± 0.11	11.1^{a} ± 0.12	10.11^{a} ± 0.19
G4	6.16^{ab}±0.23	12.1^{ab} ± 1.02	7.23^{b} ± 0.09

Data are expressed as mean ± S.E.M of 8 observations. G1: Control; G2: *Moringa oleifera*; G3: Cyclophosphamide (CP); G4: Cyclophosphamide + *Moringa oleifera*; RBCs: Red blood cells; Hb: Hemoglobin; WBCs: White blood cells. ^{a}notable distinction from the control group. ^{b}hardly any difference from the CP group.

According to the current investigation, cyclophosphamide (CP)-induced hepatic dysfunction was evidenced by a significant decrease in serum albumin levels and increased activities of serum ALT, AST, and ALP. These findings confirm that CP causes liver impairment, consistent with previous studies by Juma (1984) and Shokrzadeh *et al.* (2015). However, co-treatment with *Moringa oleifera* appeared to improve liver function, as reflected by more normalized levels of these parameters. Our results align with those of Nurhayati *et al.* (2024) and Aja *et al.* (2015), who also reported hepatoprotective effects of *Moringa oleifera*.

In terms of renal function, CP administration resulted in elevated levels of urea, creatinine, and potassium ions (K^{+}), alongside decreased levels of sodium (Na^{+}) and calcium ions (Ca^{2+}). These disturbances suggest renal dysfunction, likely due to CP-induced damage to renal tissues. This outcome supports the findings of Defronzo *et al.* (1974), Dobrek *et al.* (2017), and Bhat *et al.* (2018), who similarly observed elevated creatinine, urea, and potassium levels following CP exposure. Notably, treatment with *Moringa oleifera* improved renal function and helped restore electrolyte balance, consistent with the observations of Wen *et al.* (2022) and Saleh and Sarhat (2019).

Additionally, CP treatment significantly reduced hemoglobin percentage (Hb%) and red blood cell (RBC) counts compared to the control group. However, administration of *Moringa oleifera* effectively moderated these hematological changes. While white blood cell (WBC) counts were significantly elevated in the CP group compared to both the control and *Moringa oleifera* groups, WBC levels were lower in the *Moringa oleifera*-treated group compared to the CP-only group. These observations

are supported by findings from Idoko *et al*. (2024) and Ufelle *et al*. (2018).

CONCLUSIONS

The experimental animals did not exhibit any adverse effects from the administered dosage of *Moringa oleifera*. Overall, *Moringa oleifera* demonstrated a protective effect against cyclophosphamide-induced hematological and biochemical alterations in mice.

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NOVELTY STATEMENT

The study investigated the efficacy of Moringa oleifera at a dose of 250 mg/kg body weight against toxicity caused by Cyclophosphamide in male mice.

AUTHOR'S CONTRIBUTION

Ahmed Flayyih Hasan ,Haneen Mushtaq Hameed: They prepared the plan, designed the study, prepared the materials, photographed and performed the statistical analysis.Zainab Haytham Razooki,Sameer Abed Mohammed ,Hany M. El-Wahsh: They wrote, organized the images and references, and also helped answer reviewers' comments.

Ethical approval

The study protocol was approved by our Institutional Animal Care and Use Committee. Animals were housed at the Biotechnology Research Center, Al-Nahrain University, under randomly assigned conditions that included a 12-hour light/dark cycle, relative humidity, an ambient temperature of 22–25 °C, and *ad libitum* access to commercial food and water for two weeks prior to experimentation.

Data availability

Whenever necessary, the appropriate author can provide the data supporting the findings of our study.

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Conflict of interest

The authors have declared no conflict of interest.

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