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Research Article

The Therapeutic Effects of Moringa Pterygosperma Extract on Type 2 Diabetes: Modulation of TNF-α, Oxidative Stress, and Liver Function Biomarkers

Asia Ali Hamza
Department of Clinical laboratory sciences, College of Pharmacy, AlKafeel University, Iraq

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Corresponding Author
Asia Ali Hamza,
Department of Clinical laboratory sciences, College of Pharmacy, AlKafeel University, Iraq

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Abstract
The Type 2 diabetes mellitus is considered as a metabolic disorder which included chronic hyperglycemia, insulin resistance, and systemic inflammation, oxidative stress and liver dysfunction. Natural plant therapies, as Moringa pterygosperma (*Moringa oleifera*) have gained attention for their potential role in managing diabetes which is rich to phytochemical and medicinal properties. This present study aimed to investigate the protective and important role of Moringa pteridosperms extracts in reducing oxidative stress, inflammation, and hepatic dysfunction against the alloxan, which induced diabetic rats, by evaluating the levels of, Tumor Necrosis Factor-alpha, Malondialdehyde, Total Antioxidant Capacity, Alanine Aminotransferase, and Aspartate Aminotransferase. The study included forty adult male albino Wistar rats was divided into equal four groups included: a control group (C), a Diabetic (N) indicated by alloxan injection, a Treatment Group (T1) indicated by diabetic rats treated with 100 mg/kg Moringa extract, and Treatment Group (T2) indicated by the diabetic rats treated with 200 mg/kg Moringa extract. Blood glucose levels were monitored, and biochemical markers were analyzed using ELISA and spectrophotometric assays. Moringa extraction supplementation showed a significant decrease in blood glucose levels and reduced TNF-α and MDA levels, indicating decreased inflammation and oxidative stress compared with induction group (N). Additionally, the T1 and T2 showed an increase in TAC levels, which illustrated the improved antioxidant defense and liver function. The present study results proved that Moringa pterygosperma extract plays a critical role in maintaining diabetic, oxidant stress, and protecting the hepatic cell. In additionally the results illustrated the ability of use the Moringa as a natural protective for diabetes, in animal and humans.

INTRODUCTION

The Type 2 diabetes mellitus is considered as a metabolic disorder which included chronic hyperglycemia, insulin resistance, and systemic inflammation, oxidative stress and liver dysfunction[1]. In T2DM, hyperglycemia leads to an increase in oxidative stress and inflammatory interaction, which leads to exacerbation in metabolic disorders. The tumor necrosis factor-alpha (TNF-a) is one of the most important markers of inflammation in diabetes since it has an important role in the development of insulin resistance and ßcell dysfunction, which both cause difficulties with glucose metabolism. The elevated levels in malondialdehyde (MDA) lead to an increase in the adverse lipid peroxidation, indicating to increased oxidative stress and cellular membrane damage due to diabetes mellitus side effect. Additionally, diabetes is associated with a decrease in the total antioxidative capacity (TAC), furthermore, that causes a reduction in the antioxidant against oxidative stress and accelerates the of diabetic complications[2].

Liver dysfunction is considered an indicator of T2DM, in which the liver plays a critical role in glucose homeostasis and lipid metabolism; moreover, the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are biomarkers of liver health since the elevated in the levels of both that indicating hepatic injury and metabolic stress. The previous studies illustrated that hyperglycemia induced the oxidative stress and inflammation contribute and sequentially cause liver damage by increasing ALT and AST levels in diabetic patients[3].

Natural plant-based therapies have gained attention for their potential in managing diabetes and its associated complications. Moringa pterigosperma known as the drumstick tree, is a medicinal plant widely recognized for its rich phytochemical composition, including flavonoids, polyphenols, vitamins, and essential amino. The Moringa pterigosperma has demonstrated significant anti-inflammatory, antioxidant, and hepatoprotective properties, considering it as a candidate for diabetes management. The latest research showed that Moringa extract reduces oxidative stress markers, modulates inflammatory cytokines like TNF-α, and improves hepatic function by lowering ALT and AST levels. Furthermore, enhanced the TAC due to potential to combat oxidative stress- related damage in diabetic patients[4].

Thus the present study aimed to investigate the protective and important role of Moringa pteridosperms extracts in reducing oxidative stress, inflammation, and hepatic dysfunction against the alloxan, which induced diabetic rats, by evaluating the levels of, Tumor Necrosis Factor-alpha, Malondialdehyde, Total Antioxidant Capacity, Alanine Aminotransferase, and Aspartate Aminotransferase[5].

MATERIALS AND METHODS

Animals and Housing: The present study used forty male albino Wistar rats, that weighing between 150–200 g, which obtained from the Abo Grab Animal House in Baghdad, Iraq, and housed in standard laboratory cages at the Faculty of Science, University of Kufa, Iraq. The housing conditions were maintained under controlled environmental settings, with a temperature range of 25–30 °C, relative humidity of 50–60%, and a 12-hour light/dark cycle to simulate natural conditions. The rats were provided with a standard pellet diet and had free access to water and libitum to ensure optimal nutrition and hydration throughout the study.

Experimental Design: This study aimed to investigate the therapeutic potential of Moringa pterigosperma extract on endocrine and metabolic parameters in alloxan-induced diabetic rats. A total of forty male albino Wistar rats were randomly assigned into four groups (n = 10 per group): the Control Group (C), which received no treatment; the Negative Control Group (N), where diabetes was induced by administering alloxan monohydrate (100 mg/kg body weight, intraperitoneally) on the first day without further treatment; Treatment Group 1 (T1), which received alloxan and was treated with 100 mg/kg body weight of Moringa pterigosperma aqueous extract via gavage daily for 10 days; and Treatment Group 2 (T2), which received alloxan and a higher dose of Moringa pterigosperma extract (200 mg/kg body weight) via gavage daily for 10 days. Glucose levels were measured at Week 1 and Week 2 to assess the impact of the treatment on glycemic control. The experiment lasted 41 days, after which all animals were sacrificed for the collection of blood samples to evaluate biochemical markers, including inflammatory and oxidative stress parameters.

Induction of Diabetes: The diabetes mellitus was induced 3 groups (n = 30) following a 24-hour fasting period which each rat injection intraperitoneal alloxan monohydrate at a dose of (100 mg/kg) body weight. After six hours, the animals were provided with a 5% sucrose solution to prevent acute hypoglycemia then blood glucose levels were measured after three days using a glucometer to confirm diabetes induction. Over the following three weeks, blood glucose levels were monitored once per week using a glucometer, with blood samples collected via a small puncture in the tail.

Sample Collection: At the end of the experimental period (41 days), all rats were sacrificed under ethical and humane conditions. Blood samples were collected via cardiac puncture to analyze serum concentrations of Tumor Necrosis Factor-alpha (TNF-α), Malondialdehyde (MDA), Total Antioxidative Capacity (TAC), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST). The liver and pancreas were excised for histological examination to assess tissue integrity and the potential protective effects of Moringa pterigosperma extract on these vital organs.

Biochemical Analyses:

Tumor Necrosis Factor-alpha (TNF-α): Serum TNF-a levels were quantified using a specific ELISA kit (Cat No: EKRAT- 0419) from Melsin Company, China. This assay employs the enzyme-linked immunosorbent assay technique to detect and measure TNF-a concentrations in biological samples.

Total Antioxidant Capacity (TAC): The total antioxidant capacity in serum samples was assessed using a colorimetric assay kit (Cat No: BC1315) provided by Nalondi Company, Iran. The assay measures the cumulative action of all antioxidants present in the sample, providing an integrated parameter rather than the simple sum of measurable antioxidants.

Malondialdehyde (MDA): Lipid peroxidation levels were determined by measuring malondialdehyde concentrations using a spectrophotometric assay kit (Cat No: BC0025) from Nalondi Company, Iran. This method is based on the reaction of MDA with thiobarbituric acid, forming a colored complex that can be quantified spectrophotometrically.

Liver Function Test: The Alanine Transaminase Activity Assay Kit (Colorimetric) from Ray Biotech (Cat No: MA-ALT) was used to evaluate the level of serum ALT activity in blood. The present it detects ALT activity by recording the drop in absorbance at 340 nm, which correlates with the enzyme activity in the sample. The Aspartate Aminotransferase Activity Assay Kit (Colorimetric) from Sigma-Aldrich (Cat No: MAK055) was used to evaluate serum level of AST activity then measured calorimetrically at 450 nm.

Statistics: All statistical analyses were using GraphPad Prism 9 which the Data are expressed as mean ± standard error (SE) then used the One-way analysis of variance (ANOVA) by Tukey's post hoc to compare between groups. The statistical significance levels were denoted as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (***), while non-significant differences were indicated as "ns."

RESULTS AND DISCUSSIONS

The present study evaluated the therapeutic effects of Moringa pterigosperma extract on oxidative stress, inflammation, and liver function in alloxan-induced diabetic rats[6].

The Figure 1 presents the levels of MDA in different experimental groups, showing significant variations among them. The negative control group (N), which received alloxan without treatment, exhibited the highest MDA level (0.3701), indicating severe oxidative stress in diabetic rats. In contrast, the control group (C) had a significantly lower MDA level (0.1466), confirming normal oxidative status in healthy rats. The treatment groups, T1 and T2, which received Moringa pterigosperma extract at 100 mg/kg and 200 mg/kg, respectively, demonstrated a reduction in MDA levels compared to the negative control. Specifically, T1 showed an MDA level of 0.1716, while T2 had an even lower value of 0.1195. The statistical analysis revealed highly significant differences (****, $p < 0.0001$) between the negative control (N) and the control (C), as well as between the negative control (N) and both treatment groups (T1 and T2). However, there was no significant difference (ns) between the control (C) and T2, suggesting that a higher dose of Moringa extract (200 mg/kg) effectively reduced oxidative stress to near-normal levels[2].

Figure 2 presents the TAC levels in different experimental groups, highlighting significant variations. The N group, which received alloxan without treatment, exhibited the lowest TAC level (1.6449), indicating severe oxidative stress and reduced antioxidant defense in diabetic rats. In contrast, the C group had a significantly higher TAC level (3.3618), demonstrating the normal antioxidant capacity in healthy rats[7].

The treatment groups, T1 and T2, improved TAC levels compared to the negative control. Specifically, T1 exhibited a TAC level of 3.3044, while T2 showed a slightly lower level of 2.9966. Statistical analysis revealed highly significant differences (****, $p < 0.0001$) between the N group and all other groups, confirming the damaging effect of diabetes on antioxidant capacity. However, there was no significant difference between the C and T1, suggesting that the lower dose of Moringa pterigosperma (100 mg/kg) was effective in restoring antioxidant capacity close to normal levels. Meanwhile, T2 also showed a significant improvement compared to the negative control but remained slightly lower than T1.

Figure 3 presents the first measurement of glucose

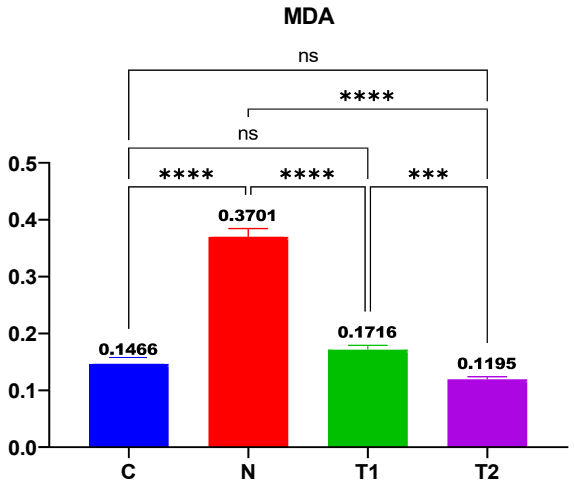

Fig.1. Levels of Malondialdehyde (MDA) in Different Experimental Groups

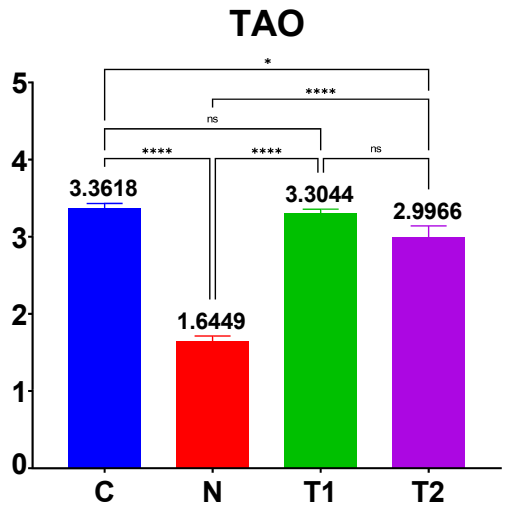

Fig. 2. Total Antioxidant Capacity (TAC) Levels in Different Experimental Groups

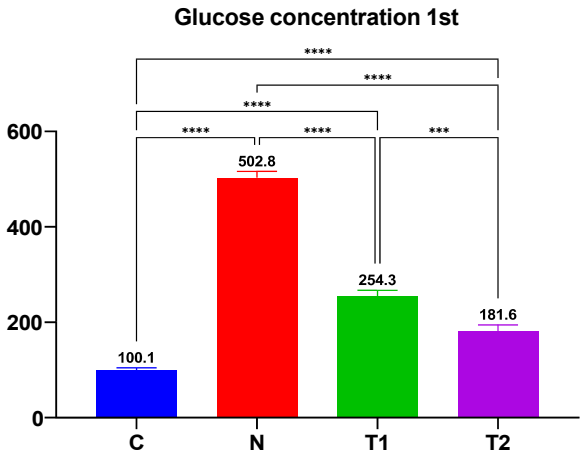

Fig. 3. First Measurement of Glucose Concentration in Experimental Groups

concentration across different experimental groups. The N group, showed the highest glucose level (502.8 mg/dL), confirming severe hyperglycemia induced by diabetes. In contrast, the C group maintained a significantly lower glucose concentration (100.1 mg/dL), reflecting normal glycemic regulation in healthy rats. The treatment groups, T1 and T2, demonstrated a substantial reduction in glucose levels compared to the negative control. T1 exhibited a glucose concentration of 254.3 mg/dL, while T2 showed an even lower level of 181.6 mg/dL, suggesting a dose-dependent hypoglycemic effect of Moringa pterigosperma.

Figure 4 presents the second measurement of glucose concentration across different experimental groups in the second week. The N group, which received alloxan without treatment, exhibited the highest glucose level (509.1 mg/dL), indicating persistent severe hyperglycemia in diabetic rats. In contrast, the C group maintained a significantly lower glucose concentration (114.9 mg/dL), reflecting normal glycemic regulation. The treatment groups, T1 and T2, showed a substantial decrease in glucose levels compared to the negative control. T1 exhibited a glucose concentration of 171.8 mg/dL, while T2 had a slightly lower level of 165.9 mg/dL. Statistical analysis revealed highly significant differences (**p < 0.0001, ******) between the negative control (N) and all other groups, reinforcing the effectiveness of Moringa pterigosperma in reducing hyperglycemia. However, the difference between T1 and T2 was not statistically significant (ns), suggesting that both doses provided a similar hypoglycemic effect after prolonged administration.

Figure 5 presents the levels of Aspartate Aminotransferase (AST) in different experimental groups, indicating significant variations among them. The N group, which received alloxan without treatment, exhibited the highest AST level (175.4 IU/g), suggesting severe liver dysfunction and hepatocellular damage in diabetic rats. In contrast, the C group showed a significantly lower AST level (55.3 IU/g), reflecting normal liver function in healthy rats. The treatment groups, T1 and T2, demonstrated a reduction in AST levels compared to the negative control. T1 had an AST level of 118.7 IU/g, while T2 showed a much lower level of 58.8 IU/g, which was close to the control group. Statistical analysis revealed highly significant differences (**p < 0.0001, ******) between the N and all other groups, confirming the protective effect of Moringa pterigosperma on liver function. Furthermore, no significant difference was observed between the C and T2, indicating that a higher dose of Moringa pterigosperma (200 mg/kg) effectively restored AST levels to near-normal values.

Figure 6 shows significant differences in the Alanine Aminotransferase (ALT) levels across different

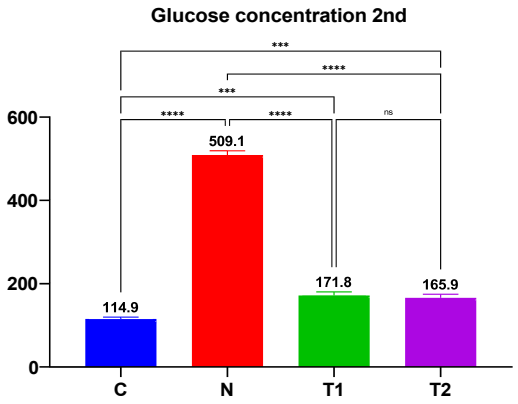

Fig. 4. Second Measurement of Glucose Concentration in Experimental Groups (Second Week)

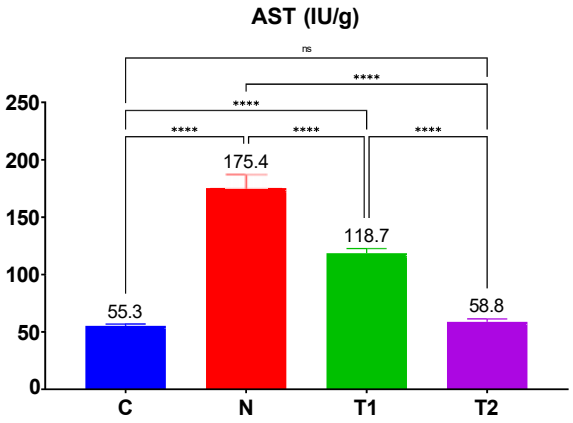

Fig. 5. Levels of Aspartate Aminotransferase (AST) in Different Experimental Groups

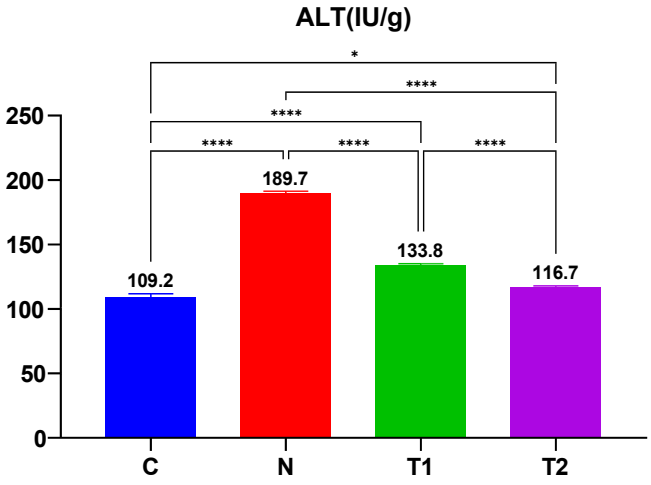

Fig. 6. Levels of Alanine Aminotransferase (ALT) in Different Experimental Groups

experimental groups. The N group, which received alloxan without treatment, exhibited the highest ALT level (189.7 IU/g), indicating severe liver dysfunction and metabolic stress associated with diabetes. In contrast, the C group displayed a significantly lower ALT level (109.2 IU/g), reflecting normal liver enzyme activity. The treatment groups, T1 and T2, showed notable reductions in ALT levels compared to the negative control. T1 exhibited an ALT level of 133.8 IU/g, while T2 had a lower level of 116.7 IU/g, which was closer to the control group.

The Figure 7 shows significant differences in the TNF-a levels across the different experimental groups. The N group, which received alloxan without treatment, exhibited the highest TNF-a level (14.2738 pg/mL), reflecting increased systemic inflammation due to diabetes-induced oxidative stress. In contrast, the C group had a significantly lower TNF-a level (11.0882 pg/mL), demonstrating normal inflammatory status in healthy rats. The treatment groups, T1 (100 mg/kg Moringa extract) and T2 (200 mg/kg Moringa extract), showed a decreased in TNF-a levels compared to the N group on other hand, the T1 exhibited a TNF-a level of 12.6910 pg/mL, while T2 showed a slightly lower level of 12.1987 pg/mL.

The present study illustrated and provide the the Moringa pterigosperma extraction improved the metabolic, inflammatory, and oxidative stress aginest the alloxan-induced diabetic in male rats [8]. The study result illustrated that Moringa extraction improved the glucose metabolism, reduced the oxidative stress, and protected the liver cell function, which its indicated as a natural antidiabetic. The previous studies showed the Moringa pterigosperma have a bioactive phytochemicals, including flavonoids, polyphenols, and alkaloids, which have been reported to enhance insulin secretion, improve insulin sensitivity, and reduce hepatic glucose production[9]. The researcher provide the Moringa play an important role in glucose transporters (GLUT4) and insulin receptor signaling pathways, leading to improved glucose uptake and utilization[10]. Furthermore, the last researcher illustrated the anti-inflammatory role effect of Moringa extract, which causes decreased the level of the TNF-α levels, which indicator as key inflammatory cytokine since plays a critical role in insulin resistance, furthermore the lead to chronic inflammation due to pancreatic cell dysfunction, and cause impaired insulin secretion. The present study confirms that Moringa significantly reduced the TNF-a levels compared to induced group, that explain its protect the pancreatic function and preventing the metabolic disturbances. The present study agreement with previous research that shows the drug extract from the plant source causes modulate inflammatory pathways, reducing insulin resistance in diabetic

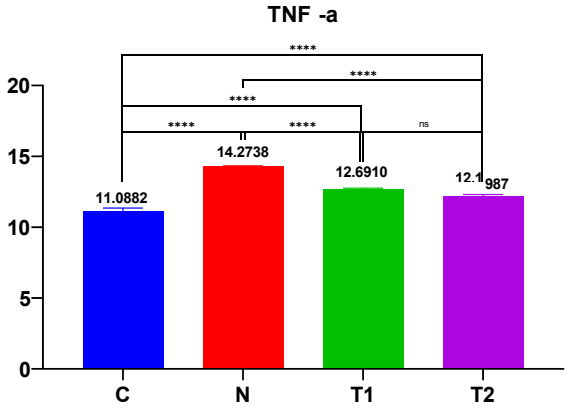

Fig. 7. Levels of TNF-α in Different Experimental Groups

models[9]. Furthermore, the significant reduction in malondialdehyde (MDA) levels and the increase in total antioxidant capacity (TAC) illustrated the strong antioxidant properties of Moringa. Moreover, oxidative stress is a major contributor to diabetes complications, as persistent hyperglycemia generates reactive oxygen species (ROS), leading to lipid peroxidation and cellular damage. These findings are consistent with earlier reports that Moringa supplementation enhances cellular antioxidant defense mechanisms[10]. Different studies reported that Moringa extract reduces fasting blood glucose levels, enhances antioxidant defense, and protects against hepatic damage in diabetic models due to its polyphenols and flavonoids which are act as free radical scavengers, neutralizing ROS and preventing oxidative stress-related damage. The present findings [illegible] levels, which provide its improved liver function on other hand the induction group showed hyperglycemia and increased in oxidative stress then lead to increase liver enzyme activity, indicating hepatocellular injury. The reduction in these enzymes in treatment groups provide the protective role against liver damage, through antioxidant and anti- inflammatory pathways which Moringa pterigosperma extract enhancing TAC and reducing oxidative stress markers.

The current study's findings on TNF-α suppression and improved insulin sensitivity suggest that Moringa may act through similar pathways, supporting its role in modulating key inflammatory signaling cascades. In contrast, Ishizawa *et al*[8] highlighted the role of TRPV1 activation in diabetic neuropathy and metabolic stress. While their study focused on the neurogenic aspects of diabetes, the reduction of oxidative stress markers (MDA) and inflammatory cytokines (TNF-α) in our study suggests that Moringa may indirectly protect against diabetic neuropathy by reducing systemic inflammation and oxidative stress.

The results of the present study showed TNF-α suppression and enhanced insulin sensitivity indicate that Moringa causes moderating in inflammatory signaling cascades, on the other hand as showed by Ishizawa *et al*[8].

CONCLUSION

The present study indicates that Moringa pteridosperms extract has significant anti- inflammatory properties against alloxan-induced diabetes in rats through reduced TNF-α levels in the treatment groups (T1 and T2) compared to the negative control group, which showed that Moringa supplementation may mitigate inflammation linked to diabetes which both doses (100 mg/kg and 200 mg/kg) decreased TNF-a levels. The findings indicate that Moringa's bioactive compounds, including flavonoids and polyphenols, contribute to its anti-inflammatory properties.

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