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Effect of *Moringa* Extract Against Renal Injury Caused by High Fat Diet-Induced Obesity in Male Rats

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Abstract:

This research endeavor delved deeply into the potential protective efficacy of moringa extract in ameliorating renal impairments instigated by a high-fat dietary regimen in male rat models. The subjects were judiciously classified into six discrete cohorts, each comprising six individuals, with the subsequent allocation: Group 1 serving as the non-intervention control; Group 2 receiving a daily dose of 300 mg/kg body weight moringa extract (ME) spanning 8 weeks; Group 3 subjected to a sustained high-fat diet (HFD) throughout an 8-week interval; Group 4 exposed to a dual-modality involving an HFD and daily administration of 300 mg/kg bw ME for the identical duration; Group 5 subjected to the combined impact of an HFD and a daily 40 mg/kg bw dose of simvastatin (SIM) across 8 weeks; lastly, Group 6 subjected to a concurrent treatment approach involving an HFD, 300 mg/kg bw ME, and 40 mg/kg bw SIM, daily, over an 8-week period. Through an intricately orchestrated sequence of experiments, we embarked upon an expedition to unearth the Reno protective potential of moringa extract against dietary-induced nephrological impairment. Our findings offer an all-encompassing outlook on the synergy between dietary interventions and the innovative agents under scrutiny. This inquiry not only advances our comprehension of potential remedies for diet-associated renal adversities but also accentuates the emergence of moringa extract as a formidable contender in this domain. Our revelations illuminated that the application of a high-fat diet ushered in a substantial surge in malondialdehyde (MDA) levels, emblematic of heightened oxidative stress. This was concomitant with a marked depletion in glutathione (GSH), aggregate antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-Rd) levels. Conversely, the administration of moringa extract adeptly mitigated these adverse repercussions induced by the high-fat dietary regimen.

Keywords: Moringa, High-fat diet, Renotoxicity.

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تأثير مستخلص المورينجا ضد الإصابة الكلوية التي تسببها السمنة الناتجة عن النظام الغذائي عالي الدهون في ذكور الجرذان

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الملخص

بحث هذا البحث بعمق في الفعالية الوقائية المحتملة لمستخلص المورينجا في تخفيف الضعف الكلوي الذي يحرضه نظام غذائي غني بالدهون في نماذج الفئران الذكور. تم تصنيف الموضوعات بحكمة إلى ستة مجموعات منفصلة ، كل منها يتألف من ستة أفراد ، مع التخصيص اللاحق: المجموعة 1 تعمل كعنصر تحكم في عدم التدخل ؛ المجموعة الثانية التي تتلقى جرعة يومية من 300 مجم / كجم من وزن الجسم من مستخلص المورينجا (ME) لمدة 8 أسابيع ؛ المجموعة 3 تخضع لنظام غذائي عالي الدهون (HFD) طوال فترة 8 أسابيع ؛ المجموعة 4 التي تعرضت لطريقة مزدوجة تتضمن HFD والإعطاء اليومي 300 ملغم / كغم من وزن الجسم لنفس المدة ؛ المجموعة 5 التي تعرضت للتأثير المشترك لـ HFD والجرعة اليومية 40 ملغم / كغم من وزن الجسم من سيمفاستاتين (SIM) على مدى 8 أسابيع ؛ وأخيراً ، خضعت المجموعة 6 لنهج معالجة متزامن يتضمن HFD، 300 ملغم / كغم من وزن الجسم ، و 40 ملغم / كغم من وزن الجسم يومياً على مدى 8 أسابيع. من خلال سلسلة تجارب منسقة بشكل معقد، شرعنا في رحلة استكشافية لاكتشاف الإمكانات الوقائية الكلوية لمستخلص المورينغا ضد ضعف الكلى الناجم عن النظام الغذائي. تقدم النتائج التي توصلنا إليها نظرة شاملة حول التآزر بين التدخلات الغذائية والعوامل المبتكرة قيد الفحص. لا يؤدي هذا الاستفسار إلى تعزيز فهمنا للعلاجات المحتملة للشدائد الكلوية المرتبطة بالنظام الغذائي فحسب، بل يبرز أيضًا ظهور مستخلص المورينغا كمنافس هائل في هذا المجال. أوضحت اكتشافاتنا أن تطبيق نظام غذائي غني بالدهون أدى إلى زيادة كبيرة في مستويات malondialdehyde (MDA) ، وهو رمز لزيادة الإجهاد التأكسدي. كان هذا متزامنًا مع استنفاد ملحوظ في الجلوتاثيون (GSH)، القدرة الكلية لمضادات الأكسدة (TAC)، ديسموتاز الفائق (SOD)، الكاتلاز (CAT)، الجلوتاثيون بيروكسيديز (GSH-Px)، ومستويات اختزال الجلوتاثيون (طريق GSH). على العكس من ذلك، خففت إدارة مستخلص المورينجا ببراعة من هذه الآثار السلبية الناجمة عن النظام الغذائي عالي الدهون.

الكلمات المفتاحية: المورينجا، النظام الغذائي الغني بالدهون، السمية الكلوية.

Introduction

Obesity has emerged as a pressing global health concern, giving rise to a range of health issues like dyslipidemia, diabetes, insulin resistance, hypertension, stroke, and arteriosclerosis [1]. According to the World Health Organization (WHO), there's an alarming statistic: over a billion adults are grappling with being overweight, while a staggering 300 million individuals are classified as obese on a worldwide scale [2].

The crux of obesity lies in the excessive accumulation of lipids within adipose tissue all across the body [3]. WHO defines obesity by a body mass index (BMI) of 22-29.9 for overweight and 30 kg/m² for outright obesity. BMI is a widely accepted metric for categorizing weight, derived from the ratio of one's weight (in kg) to the square of their height (in m²); it's often employed as an indirect indicator of total body fat [4].

The root cause of obesity development can be traced back to the disruption in the balance between energy intake and expenditure [5]. This imbalance is a complex interplay of various factors, encompassing genetics, metabolism, behaviors, and environmental influences [6].

Enter Moringa oleifera, commonly known as drumstick, a tree species belonging to the Moringaceae family and celebrated for its medicinal properties. Packed with an array of essential minerals, Moringa's distinctively long, stick-like appearance has earned it the nickname "drumstick." Revered as a miracle tree, it can reach impressive heights of up to 10 meters. Its petite flowers measure about 1.5 to 2 cm in length, while its leaves can extend up to 60 cm. This versatile plant thrives across the plains of India and has even established itself naturally in tropical regions. It's remarkably adaptive, growing splendidly in diverse soil types and particularly flourishing in the conditions found in both North and South India [8].

In an effort that leans towards the technical realm, a study was undertaken to probe the effects of moringa extract on renal disorders induced by high-fat diets in male rats. This research delves into the potential impacts of

harnessing the properties of moringa to combat the detrimental consequences of diets high in fats, aiming to shed light on novel interventions for managing the intricate relationship between dietary habits and renal health.

Material and methods

Chemicals

Zocor, scientifically known as Simvastatin, was sourced from the esteemed Global Napi Pharmaceuticals Egypt. The administration of Simvastatin was conducted through oral means, specifically at a dosage of 40 mg per kilogram of body weight. This particular dosage was selected in alignment with the parameters outlined in the referenced study [9].

The cholesterol utilized for the experimental procedures was procured from the reputable Sigma Chemical Company, located in St. Louis, MO, USA. This cholesterol component was incorporated into a high-fat diet, precisely at a concentration of 2%. This specific dosage of cholesterol was meticulously determined based on the specifications elucidated in the cited study [10].

In this manner, the experimental design ensured the utilization of Simvastatin and cholesterol doses that were both in accordance with the referenced studies, thereby maintaining scientific rigor and adherence to established protocols.

Moringa extract

I procured a supply of meticulously gathered, thoroughly cleansed, and impeccably dried leaves from the esteemed Moringa oleifera plant, sourced from the local marketplace in Egypt. A precisely measured quantity of approximately 40 grams of these botanical wonders was finely powdered. This verdant powder was then carefully immersed in a meticulously measured 250 millilitres of 70% ethanol solution, forming a harmonious union. [25].

Subsequently, this amalgam was bestowed a three-day sojourn within the chill embrace of a refrigerator, allowing for the intricate dance of extraction to unfold. Following this refrigerated interlude, the mixture was subjected to the refined embrace of filter paper of superior quality, designated as Whatman No. 1. This venerable medium facilitated the separation of the refined solution from any residual botanical remnants.

The refined filtrate was then ushered into the domain of a Rotary Evaporator, a marvel of scientific engineering that employed the alchemical principles of controlled evaporation to concentrate the essence within the solution. The culmination of this process led to the attainment of a concentrated extract, teeming with the essence of the revered moringa leaves. [24].

This essence, a testament to the profound botanical artistry at play, was skilfully reconstituted by dissolution in distilled water. It is noteworthy that the dose of this resplendent moringa extract, administered for the purpose of the study, adhered to the parameters elucidated in study [11-28], where a dosage of 300 milligrams of extract per kilogram of body weight was employed. This intricate and methodical endeavour represents a humble contribution to the realm of scientific exploration into the manifold potentialities of the moringa plant [23].

Animals

A cohort of white male albino rats (Rattus norvegicus), aged 8 weeks, was selected as the subjects for this experimental study. These rats were specifically sourced from the reputable Egyptian Institute for Serological and Vaccine Production, located in Helwan, Egypt. Once acquired, they were comfortably housed within the animal facility of the esteemed Department of Zoology, situated within the Faculty of Sciences at Mansoura University.

Each of the rats was accommodated within individual cages, thoughtfully furnished with wood-chip bedding that was meticulously replaced on a daily basis. These enclosures were meticulously maintained in a climate-controlled environment, where a precisely regulated 12-hour light-dark cycle was established to mimic natural conditions. Prior to the commencement of the experimental procedures, a week-long acclimatization period was observed for all the rats, allowing them to gradually adapt to their new surroundings.

Throughout the course of the study, the rats were provided with unrestricted access to water, ensuring their hydration needs were met. Their diets encompassed a combination of standard nourishment and a high-fat diet, both integral to the experimental design. It is worth noting that all protocols and procedures undertaken were executed in strict alignment with the guidelines set forth by the National Research Council for the Care and Use of Laboratory Animals. Additionally, ethical considerations were diligently observed, as evidenced by the official approval received from the local experimental animal ethics committee affiliated with the Department of Zoology at Mansoura University.

In summary, this meticulously orchestrated study aimed to explore the effects of differing diets on white male albino rats, offering valuable insights into their physiological responses. The attention to detail in sourcing, housing, and ethical adherence underscores the scientific rigor that underpinned this research endeavor.

Animal grouping

After an initial acclimatization period of one week, the rats were divided randomly into six distinct groups, each consisting of six male rats. The groups were structured as follows:

1. Control Group: This group of rats did not receive any specific treatment or intervention.
2. Moringa Extract (ME) Treated Group: Rats in this group were administered Moringa Extract orally, at a dosage of 300mg per kilogram of their body weight, on a daily basis for a duration of 8 weeks.
3. High Fat Diet (HFD) Treated Group: Rats assigned to this group were fed a High Fat Diet on a daily basis for a total period of 8 weeks.
4. High Fat Diet and Moringa Extract (HFD+ME) Treated Group: Rats in this particular group were provided with the High Fat Diet concurrently with the Moringa Extract (at a dosage of 300 mg/kg bw) on a daily basis for a span of 8 weeks.
5. High Fat Diet and Simvastatin (HFD+SIM) Treated Group: Rats in this group were exposed to the High Fat Diet in combination with Simvastatin (at a dosage of 40 mg/kg bw) daily for a duration of 8 weeks.
6. High Fat Diet, Moringa Extract, and Simvastatin (HFD+ME+SIM) Treated Group: Rats belonging to this group were subjected to the High Fat Diet concurrently with both Moringa Extract (at a dosage of 300 mg/kg bw) and Simvastatin (at a dosage of 40 mg/kg bw) on a daily basis for a period of 8 weeks.

Sample collection

After a span of eight weeks, the rats in the fasting group were humanely euthanized a day after the final treatment. At this point, the rat kidneys were carefully removed and their weights were recorded individually. To prepare the kidney tissue for further analysis, a 10% w/v solution of the tissue in distilled water was created through homogenization. This resulting kidney homogenate was then preserved at a temperature of -20°C in properly labeled Eppendorf tubes, awaiting subsequent biochemical assessments.

In addition to these biochemical investigations, portions of the kidney tissue were also dedicated to histological examinations. To ensure the structural integrity of the tissue, these samples were meticulously placed in a solution of 10% neutral formalin. This step aimed to fix the tissue and prevent any degradation, preserving the tissue's cellular architecture for subsequent histological studies.

Parameters assayed

Kidney homogenates were employed in the assessment of malondialdehyde (MDA) levels, following the methodology outlined by Ohkawa et al. [12-29]. The determination of glutathione (GSH) content was executed in accordance with the protocol established by Beutler [13], while the evaluation of total antioxidant capacity (TAC) was conducted using the method previously detailed by Koracevic et al. [14-30].

The enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-Rd) were assayed in kidney homogenates, drawing upon the procedures described in earlier works [15], [16], [17], [18].

Statistical analysis

Statistical analyses were conducted using GraphPad Prism 5.0 software, with the presentation of data as the mean accompanied by the standard error (SE) based on a sample size of n=6. The evaluation of statistical significance involved the utilization of a one-way analysis of variance (ANOVA) with a significance threshold set at $P \leq 0.05$, as reported by reference [19-31].

Results

In Table 1, the data clearly demonstrate a notable rise in the MDA (malondialdehyde) content, accompanied by a reduction in the levels of GSH (glutathione), TAC (total antioxidant capacity), SOD (superoxide dismutase), CAT (catalase), GSH-Px (glutathione peroxidase), and GSH-Rd (glutathione reductase) in rats that were fed a high-fat diet (HFD). Interestingly, the administration of a medicinal extract (ME) to the rats that were on the HFD exhibited a significant improvement in the MDA levels, coupled with an augmentation in the levels of kidney antioxidants. This suggests a potential beneficial effect of the ME treatment on the oxidative stress induced by the HFD, as evidenced by the restoration of antioxidant levels and the attenuation of lipid peroxidation, as reflected by the decrease in MDA content. These results underscore the potential of ME as a therapeutic intervention to counteract the adverse impact of a high-fat diet on oxidative balance and cellular health.

Table 1: Effect of Moringa extract on oxidative stress and antioxidants in kidney.

Parameters	Control	ME	HFD	HFD+ME	HFD+SIM	HFD+ME+SIM
MDA (nmol/g)	409.9^{a} ±22.39	402.0^{a} ±11.68	1478^{b} ±59.46	560.2^{c} ±25.31	470.0^{a} ±15.28	420.2^{a} ±15.44
GSH (mmol/g)	238.7^{a} ±4.301	241.8^{a} ±4.891	96.30^{b} ±2.703	150.2^{c} ±6.343	176.4^{d} ±7.281	236.6^{a} ±4.302
TAC (mM/g)	1.206^{a} ±0.01083	1.194^{a} ±0.01016	0.9755^{b} ±0.08263	1.173^{a} ±0.002750	1.180^{a} ±0.004425	1.191^{a} ±0.005625
SOD (U/g)	739.6^{a} ±46.14	827.0^{a} ±22.26	181.3^{b} ±7.149	698.0^{a} ±29.80	704.3^{a} ±26.93	712.7^{a} ±30.82
CAT (U/g)	180.6^{a} ±6.042	180.3^{a} ±6.67	43.5^{b} ±4.089	126.3^{c} ±8.545	133.8^{c} ±4.697	188^{a} ±5.422
GSH-Px (U/g)	1219^{a} ±10.86	1232^{a} ±10.14	762.3^{b} ±17.44	1126^{c} ±13.42	1176^{a} ±14.97	1192^{a} ±13.85
GSH-Rd (U/g)	648.6^{a} ±7.400	679.4^{a} ±15.01	440.5^{b} ±17.64	576.2^{a} ±18.47	595.1^{a} ±29.56	615.6^{a} ±21.48

Results were presented as means ± SE n=6.
Different letters (significant), and similar letters (non-significant) change at $p \leq 0.05$
C: Control ME: Moringa extract, HFD: High-fat diet, SIM: Simvastatin.

Plate 1 Figures A-F
Histopathological kidney change
Figure 1 illustrates kidney sections from various experimental groups, each subjected to different treatments. Let's delve into the details of these microscopic images:

A. In the control group, the kidney section exhibits typical and healthy glomeruli (G) alongside normal renal tubules (R).
B. Moving on to the normal animal treated with the ME extract, the kidney section retains its regular appearance with intact glomeruli (G) and renal tubules (R).
C. Conversely, the kidney section from the HFD-treated animal portrays evident degenerative alterations within the epithelium lining of the renal tubules (arrow), underscoring the impact of the high-fat diet.
D. Shifting our focus to the kidney section of the diseased animal treated with the ME extract, we observe a slight degree of degeneration in the renal tubular epithelium (arrow), suggesting a potential ameliorative effect of the extract on the degenerative process.
E. The kidney section of the diseased animal treated with SIM presents a similar scenario, with a mild level of degeneration visible in the renal tubular epithelium (arrow), indicating the possible impact of the SIM treatment.
F. Finally, the kidney section from the diseased animal subjected to a combination of ME and SIM treatment displays a comparable mild degeneration of the renal tubular epithelium (arrow), hinting at the combined effect of these treatments on the degenerative changes.

Throughout the images, 'G' represents glomeruli, and 'R' designates renal tubules. The staining utilized for visualization is Hematoxylin and Eosin (H&E), with a magnification of 40 µm.
It's important to note that these findings provide a visual insight into the effects of different treatments on kidney tissue morphology, shedding light on potential avenues for further investigation and understanding.

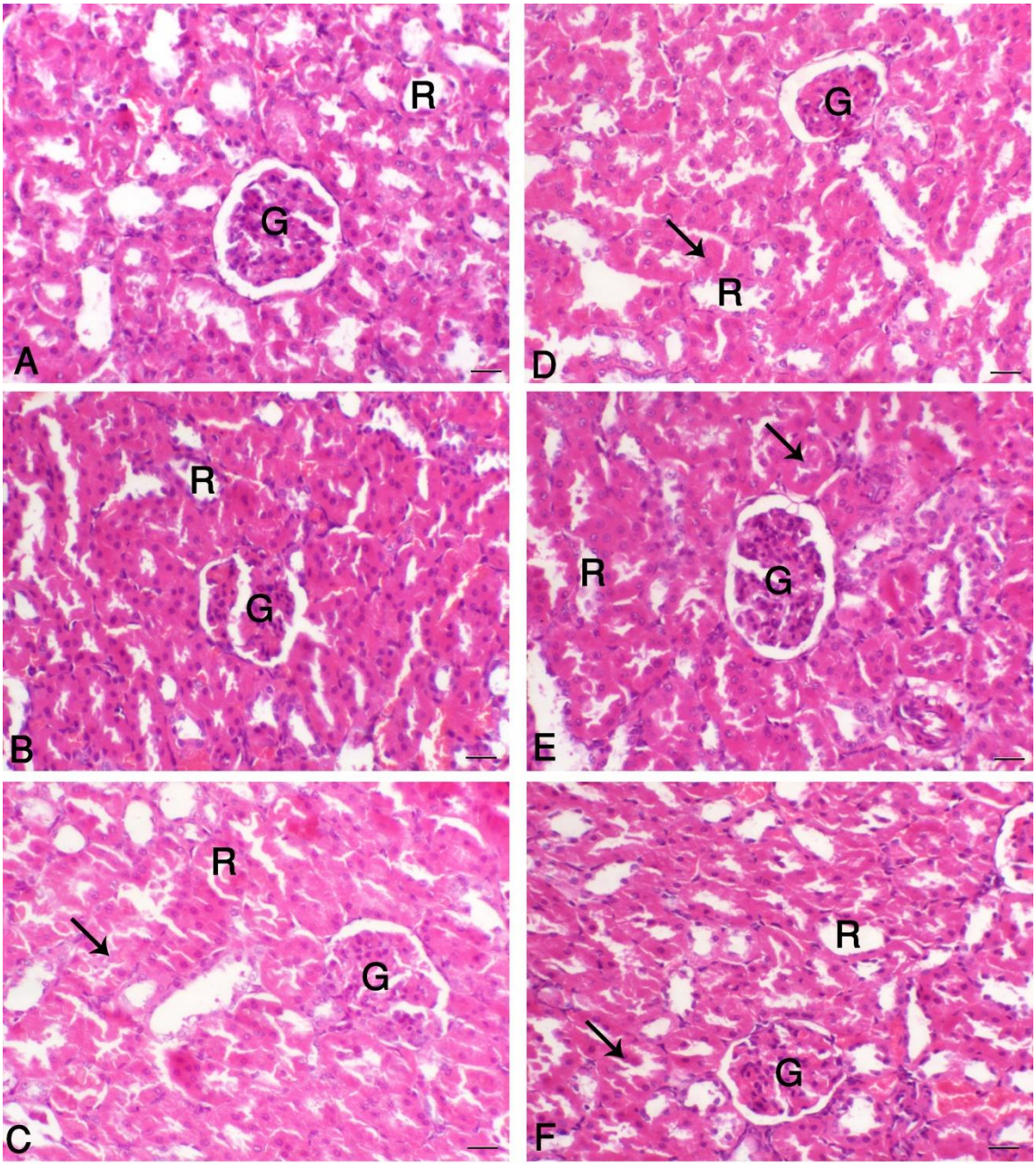

Figure 1 Kidney sections from various experimental groups.

Discussion

In the context of this investigation, the introduction of a High Fat Diet (HFD) led to a discernible reduction in kidney levels of vital antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH), Total Antioxidant Capacity (TAC), Glutathione Reductase (GSH-Rd), and Glutathione Peroxidase (GSH-Px) in rats subjected to the HFD regimen. This reduction in antioxidant defenses was accompanied by an escalation in oxidative stress, which in turn triggered conspicuous degenerative alterations in the epithelial lining of the renal tubules in these HFD-exposed rats, as opposed to the rats on a normal diet. The underlying mechanisms contributing to the decline in antioxidant enzyme levels in obese rats appear multifaceted. Heightened lipid peroxidation resulting from the HFD appeared to incapacitate these enzymes, as evidenced by their interaction with Malondialdehyde (MDA). This interaction had the consequence of not only stifling their function but also augmenting the buildup of harmful reactive oxygen species, including superoxide, hydrogen peroxide, and hydroxyl radicals, thereby fueling the oxidative cascade. Furthermore, the exhaustion of antioxidant enzyme reservoirs due to the augmented demand for neutralizing free radicals during the course of obesity development could also play a role in their depletion.

Conversely, rats administered with a supplementary dose of moringa extract alongside the HFD exhibited notable mitigation of oxidative stress, coupled with a substantial augmentation in key antioxidants including GSH, TAC,

SOD, CAT, GSH-Rd, and GSH-Px. This amelioration was largely attributed to the rich antioxidant content present in moringa extract, primarily composed of phenolic compounds and tannins. Of particular significance were the polyphenols, flavonoids, β-sitosterol, kaempferol, and quercetin found within the extract, all of which feature hydroxyl groups that possess remarkable resonance properties enabling them to efficiently donate electrons to quench free radicals, thereby nullifying their damaging effects. Moreover, the hydroxyl groups foster intermolecular hydrogen bonding with non-protein thiols and enzymes, thereby orchestrating the rejuvenation of the compromised antioxidant system in the face of oxidative stress.

Notably, the exceptional phenolic constituents in moringa extract have the potential to stimulate the production of endogenous antioxidant enzymes. The presence of β-Carotene, a significant component derived from moringa leaves, showcased considerable potency as it facilitated the conversion into vitamin A, bolstering the antioxidant arsenal. Remarkably, histological examination of kidney sections from rats exposed to the HFD along with moringa extract unveiled a restoration of the normal cellular arrangement. This rejuvenation was attributed to the combined effect of fat reduction and the regenerative prowess of moringa extract's antioxidant defense system within the kidney cells. This intricate mechanism ultimately culminated in the recovery of cellular integrity and normalcy.

In summation, the experimentation uncovered the intricate interplay between HFD-induced oxidative stress, antioxidant enzyme modulation, and the ameliorative potential of moringa extract. The findings underscored the extract's capacity to effectively counterbalance the harmful effects of oxidative stress by virtue of its diverse antioxidant constituents and their orchestrated interaction with the antioxidant defense system.

Conclusion

The data unequivocally supports the noteworthy efficacy of ME supplementation as a potent renal protective agent against the deleterious impacts of kidney histopathology and oxidative stress induced by a high-fat diet (HFD). The empirical evidence underscores the pivotal role of ME supplementation in mitigating renal disturbances at a histopathological level, while concurrently exerting antioxidative effects.

The utilization of ME supplementation emerges as a judicious intervention strategy, offering a multifaceted defense mechanism against the intricate interplay of histological perturbations and oxidative imbalances triggered by an HFD. These findings herald ME supplementation as a promising avenue for safeguarding renal integrity and function amidst the challenges posed by dietary choices.

In light of these empirical substantiations, the integration of ME supplementation into therapeutic protocols warrants serious consideration, as it stands as a testament to the potential synergy between pharmacology and nutrition. This insight not only deepens our comprehension of renal pathophysiology but also accentuates the practical utility of ME supplementation in upholding renal well-being amid the modern landscape of dietary proclivities.

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