

Effect of Ascorbic Acid, *Balanites aegyptiaca*, and Strawberry Supplementation on Glycemic Control and Renal Function Biomarkers

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Abstract: This study investigated the effects of ascorbic acid, *Balanites aegyptiaca*, and strawberry supplementation on glycemic control and renal function biomarkers over a six-week experimental period. Glycated hemoglobin (HbA1c) showed a non-significant reduction among treated groups ($P = 0.072$). The control group recorded $4.2 \pm 0.42\%$, compared with $3.8 \pm 0.22\%$ (ascorbic acid), $3.1 \pm 0.44\%$ (*Balanites aegyptiaca*), and $3.2 \pm 0.22\%$ (strawberry). In contrast, serum glucose levels were significantly decreased ($P = 0.000$). The control value was 114.03 ± 1.55 mg/dl, while levels declined to 107.75 ± 1.91 mg/dl with ascorbic acid, 100.0 ± 3.70 mg/dl with *Balanites aegyptiaca*, and 102.51 ± 2.08 mg/dl with strawberry, representing a maximal reduction of approximately 14 mg/dl in the *Balanites aegyptiaca* group. Serum urea levels differed significantly among groups ($P = 0.000$), decreasing from 41.81 ± 0.71 mg/dl in the control group to 35.36 ± 1.77 mg/dl (ascorbic acid), 39.11 ± 1.09 mg/dl (*Balanites aegyptiaca*), and 33.78 ± 0.48 mg/dl (strawberry), with the lowest value observed in the strawberry-treated group. Serum creatinine levels were also significantly reduced ($P = 0.002$), from 0.86 ± 0.10 mg/dl in controls to 0.60 ± 0.017 mg/dl (ascorbic acid), 0.65 ± 0.03 mg/dl (*Balanites aegyptiaca*), and 0.67 ± 0.08 mg/dl (strawberry). Weekly trend analysis demonstrated gradual and sustained reductions in glucose, urea, and creatinine across treated groups over the six-week period. Overall, *Balanites aegyptiaca* exhibited the strongest hypoglycemic effect, ascorbic acid produced the greatest reduction in creatinine, and strawberry supplementation showed the most pronounced decrease in urea levels. These findings support the beneficial biochemical effects of these natural interventions on glucose homeostasis and renal function.

Keywords: Ascorbic acid; *Balanites aegyptiaca*; Strawberry; Renal biomarkers; Glycemic regulation.

Introduction

Metabolic disorders, particularly hyperglycemia and impaired renal function, represent major health challenges worldwide, often leading to chronic complications if left unmanaged [1-5]. Oxidative stress and inflammation play a crucial role in the pathophysiology of these conditions, contributing to tissue damage and dysfunction in vital organs such as the liver and kidneys. In recent years, natural antioxidants have received significant attention due to their potential to modulate glucose metabolism, enhance renal function, and reduce oxidative damage [6]. Ascorbic acid (vitamin C) is a well-known water-soluble antioxidant that scavenges free radicals, enhances enzymatic antioxidant systems, and has been reported to improve glucose homeostasis and kidney function in experimental and clinical settings [7-9]. Similarly, *Balanites aegyptiaca*, a traditional medicinal plant, contains bioactive compounds such as saponins and flavonoids, which have demonstrated hypoglycemic and nephroprotective properties. Strawberry (*Fragaria × ananassa*) is rich in polyphenols and vitamin C, and previous studies have highlighted its capacity to improve glycemic control and reduce markers of oxidative stress in animal models [10-12]. Despite growing evidence of their beneficial effects, comparative studies investigating the simultaneous impact of ascorbic acid, *Balanites aegyptiaca*, and strawberry on glycemic control and kidney function remain limited [13-15]. Therefore, this study aimed to evaluate the effects of these natural interventions on serum glucose, glycated hemoglobin

(HbA1c), urea, and creatinine levels in a controlled experimental model, providing insights into their potential protective roles in maintaining metabolic and renal health.

Materials and Methods

This experimental study was carried out in July 2025 at the Department of Chemistry, Faculty of Science, Omar Al-Mukhtar University. Ascorbic acid was obtained from a certified pharmaceutical supplier in Al-Bayda, Libya. Fresh strawberries were purchased from the local market. *Balanites aegyptiaca* plant materials were collected and prepared for experimental use. All materials were prepared in suitable concentrations for oral administration based on the body weight of the experimental animals. Twenty healthy adult male rabbits were obtained from verified local farms and used in this experiment. The animals were housed in stainless steel cages under controlled environmental conditions (temperature 22–26 °C, relative humidity 40–70%, and a 12-hour light/dark cycle). All rabbits were provided with a balanced diet and clean drinking water throughout the experimental period. Animal handling and care followed the guidelines of the US-EPA (2004) and the Libyan Ministry of Agriculture for laboratory animal welfare. The rabbits were randomly divided into four experimental groups ($n = 5$ per group) and treated for six weeks as follows: Group 1 (Ascorbic acid group): Rabbits received ascorbic acid at a dose of 100 mg/kg body weight orally on alternate days. Group 2 (Strawberry group): Rabbits received

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strawberry extract at a dose of 50 mg/kg body weight/day orally by gavage. Group 3 (*Balanites aegyptiaca* group): Rabbits received *Balanites aegyptiaca* extract at a dose of 200 mg/kg body weight/day orally by gavage. Group 4 (Control group): Rabbits received 8 ml of distilled water orally and served as the control. All treatments were administered orally each morning, and doses were adjusted according to the body weight of the animals. The experimental period lasted six weeks, during which body weight and physiological responses were monitored. Blood samples were collected weekly from the marginal ear vein of rabbits during the six-week experimental period to monitor biochemical changes. At the end of the experiment, blood samples were collected by cardiac puncture after animal sacrifice. The blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum, which was stored at $-20\text{ }^{\circ}\text{C}$ until biochemical analysis. Serum glucose levels were determined using standard enzymatic colorimetric methods. Glycated hemoglobin (HbA1c) was measured to evaluate long-term glycemic control. Kidney function markers including serum urea and creatinine were also determined using commercial diagnostic kits according to the manufacturer's instructions. All biochemical analyses were performed following established laboratory protocols. Statistical analysis was carried out using Minitab (version 17) and GraphPad Prism (version 8). Data were expressed as mean \pm standard error (SE). Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was considered at $P < 0.05$.

Results

The evaluated biochemical markers demonstrated significant improvements in glucose homeostasis and kidney function following treatment, while HbA1c showed a non-significant trend toward reduction. Glycated hemoglobin (HbA1c) percentage did not differ statistically among groups ($P = 0.072$). The control group recorded $4.2 \pm 0.42\%$, whereas values declined to $3.8 \pm 0.22\%$ with ascorbic acid, $3.1 \pm 0.44\%$ with *Balanites aegyptiaca*, and $3.2 \pm 0.22\%$ with strawberry. Although the reduction did not reach

statistical significance, the numerical decrease of approximately 1.1% in the *Balanites aegyptiaca* group compared to control suggests improved long-term glycemic regulation. In contrast, serum glucose levels showed a highly significant difference ($P = 0.000$). The control group exhibited 114.03 ± 1.55 mg/dl, while ascorbic acid reduced glucose to 107.75 ± 1.91 mg/dl. A more pronounced reduction was observed in the *Balanites aegyptiaca* group (100.0 ± 3.70 mg/dl) and strawberry group (102.51 ± 2.08 mg/dl). The decrease reached approximately 14 mg/dl in the *Balanites aegyptiaca* group compared with control, indicating a clear hypoglycemic effect. Kidney function markers were also significantly influenced. Serum urea levels differed markedly among groups ($P = 0.000$). The control value was 41.81 ± 0.71 mg/dl, whereas ascorbic acid reduced urea to 35.36 ± 1.77 mg/dl. The *Balanites aegyptiaca* group recorded 39.11 ± 1.09 mg/dl, and the strawberry group showed the lowest level at 33.78 ± 0.48 mg/dl, reflecting a reduction of approximately 8.03 mg/dl compared to control. Serum creatinine levels were significantly decreased in all treated groups ($P = 0.002$). The control group measured 0.86 ± 0.10 mg/dl, while ascorbic acid reduced creatinine to 0.60 ± 0.017 mg/dl. The *Balanites aegyptiaca* and strawberry groups recorded 0.65 ± 0.03 mg/dl and 0.67 ± 0.08 mg/dl, respectively. This represents a reduction of nearly 0.26 mg/dl in the ascorbic acid group relative to control, indicating improved renal filtration efficiency. Weekly trends illustrated in Figures 1–4 showed stable HbA1c values with gradual declines in glucose, urea, and creatinine in treated groups across the six-week period. The most consistent reductions were observed in the *Balanites aegyptiaca* and strawberry groups for glucose, and in the ascorbic acid group for creatinine. Overall, the numerical findings indicate that *Balanites aegyptiaca* exerted the strongest hypoglycemic effect, while ascorbic acid produced the most pronounced improvement in creatinine levels. Strawberry supplementation showed notable reductions in both glucose and urea. Importantly, all treatments improved biochemical parameters without evidence of metabolic deterioration, supporting their potential protective role in maintaining glycemic control and renal function.

Table (5): Effect of the studied treatments on serum glucose, HbA1c, and kidney function markers in experimental groups.

Parameter	Experimental groups				P-value
	Control	Ascorbic acid	<i>Balanitesaegyptiaca</i>	Strawberry	
Glycated Hemoglobin HbA1c (%)	4.2 ± 0.42^a	3.8 ± 0.22^a	3.1 ± 0.44^a	3.2 ± 0.22^a	0.072
Glucose (mg/dl)	114.03 ± 1.55^a	107.75 ± 1.91^{ab}	100.0 ± 3.70^b	102.51 ± 2.08^b	0.000
Urea (mg/dl)	41.81 ± 0.71^a	35.36 ± 1.77^{bc}	39.11 ± 1.09^{ab}	33.78 ± 0.48^c	0.000
Creatinine (g/dl)	0.86 ± 0.10^a	0.60 ± 0.017^b	0.65 ± 0.03^b	0.67 ± 0.08^b	0.002

The means \pm SE for each treatment group are provided; n = 5. When mean values within a row did not share a common superscript letter (a, b, or c), significant differences ($p < 0.05$) were observed.

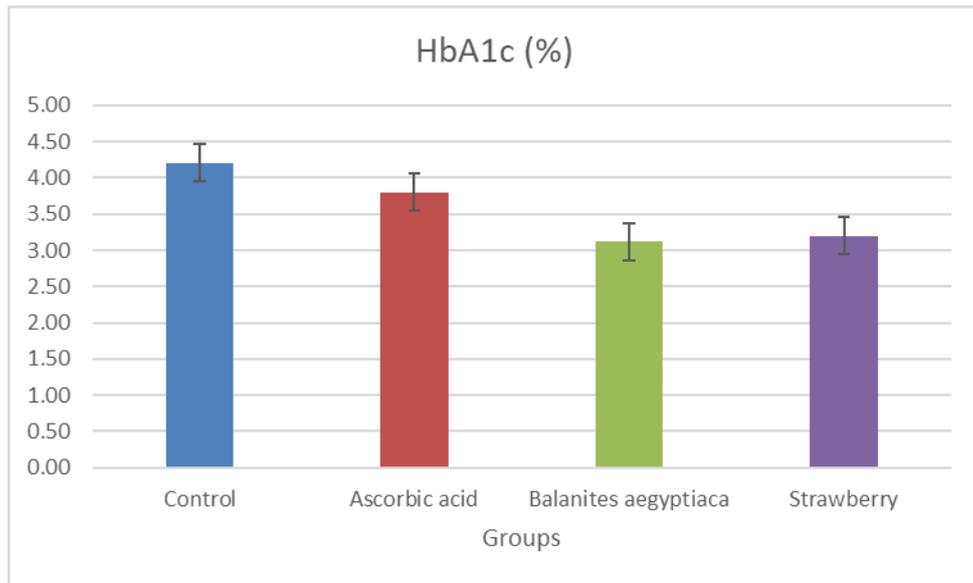


Figure 1: Comparison of Glycated Hemoglobin (HbA1c) percentage across control and treatment groups.

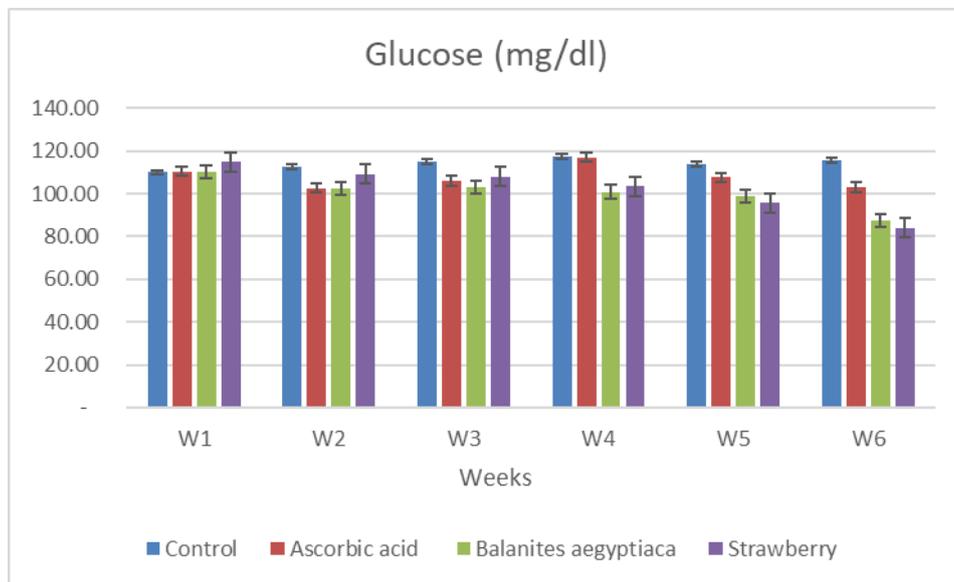


Figure 2: Weekly serum Glucose levels in control and treatment groups over a six-week period.

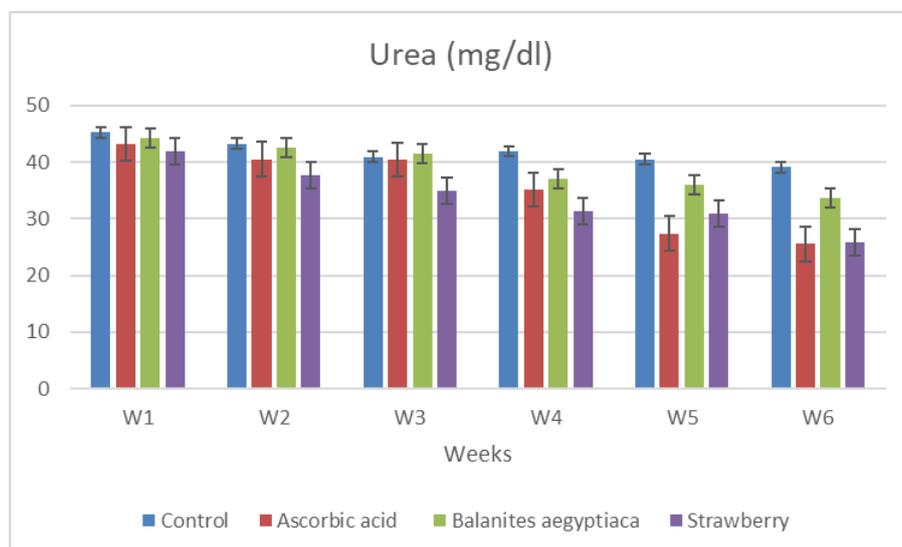


Figure 3: Weekly changes in serum Urea levels across control and treatment groups over a six-week period.

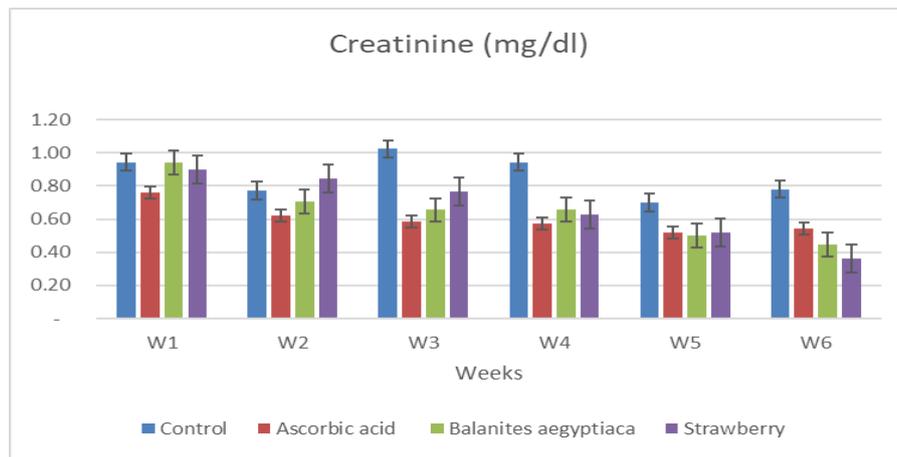


Figure 4: Weekly serum Creatinine levels for control and treatment groups over a six-week duration.

Discussion

The present findings demonstrate that supplementation with ascorbic acid, *Balanites aegyptiaca*, and strawberry exerted favorable effects on glucose regulation and renal function, while glycated hemoglobin showed only a modest, non-significant decline. From a biochemical standpoint, fasting glucose reflects short-term glycemic control, whereas HbA1c represents cumulative glycemic exposure over several weeks. The absence of statistical significance in HbA1c despite improvement in glucose levels may be attributed to the relatively short experimental duration and the fact that baseline glycemic status was within physiological limits. HbA1c typically requires a longer period to exhibit marked variation, particularly in non-diabetic models [16-20]. The significant reduction in serum glucose, particularly in the *Balanites aegyptiaca* group, suggests a hypoglycemic effect mediated by its bioactive constituents. *Balanites* fruits and seeds are rich in saponins, flavonoids, and steroidal compounds, which have been reported to enhance insulin sensitivity, stimulate peripheral glucose uptake, and inhibit intestinal glucose absorption [21-30]. Experimental studies have demonstrated that *Balanites aegyptiaca* extracts improve glucose tolerance and reduce hyperglycemia in diabetic animal models [31]. The underlying mechanism may involve modulation of pancreatic β -cell function and attenuation of oxidative stress, which plays a central role in insulin resistance [32]. However, some investigations have reported variable hypoglycemic responses depending on extraction method, dosage, and metabolic status of the subjects [33]. Such discrepancies likely reflect differences in phytochemical concentration and bioavailability, as well as variations in experimental design. Ascorbic acid also contributed to lowering serum glucose, which aligns with its known role in improving insulin sensitivity and reducing oxidative stress-induced β -cell dysfunction. Vitamin C competes with glucose for cellular uptake due to structural similarity, and its antioxidant action mitigates glucotoxicity-mediated oxidative damage [34]. Clinical and experimental studies have shown that vitamin C supplementation can reduce fasting glucose in individuals with impaired glycemic control [35]. Nevertheless, other studies in normoglycemic subjects reported minimal effects, suggesting that the glucose-lowering impact is more pronounced under hyperglycemic conditions [36]. Strawberry supplementation demonstrated a significant glucose-lowering effect as well. Strawberries are rich in anthocyanins and ellagic acid, which modulate carbohydrate metabolism by

inhibiting α -glucosidase and improving insulin signaling pathways [37]. Polyphenols in berries have been shown to activate AMP-activated protein kinase (AMPK), enhancing glucose uptake in peripheral tissues [38]. Some human trials reported improved postprandial glucose responses after berry consumption, while others observed only modest effects, possibly due to differences in dosage and baseline metabolic condition [39]. Renal function markers showed significant improvement in treated groups, as evidenced by reductions in urea and creatinine. Elevated serum urea and creatinine are indicators of impaired glomerular filtration and renal oxidative stress. The observed decline suggests improved renal filtration efficiency and reduced nephron stress. Oxidative damage is a major contributor to renal dysfunction, particularly under metabolic imbalance [40]. Ascorbic acid, through its antioxidant capacity, protects renal tubular cells from oxidative injury and enhances nitric oxide bioavailability, thereby improving renal hemodynamics [41]. Similarly, phytochemicals present in *Balanites aegyptiaca* and strawberry possess anti-inflammatory and antioxidant activities that mitigate renal lipid peroxidation and preserve glomerular integrity. Previous studies reported nephroprotective effects of plant-derived antioxidants through suppression of ROS production and modulation of pro-inflammatory cytokines [42]. Nevertheless, some reports found limited renal improvement in healthy animals, suggesting that antioxidant supplementation yields more prominent benefits under oxidative or metabolic stress conditions. The improvements observed in this study, despite physiological baseline values, may indicate enhanced metabolic efficiency rather than correction of overt dysfunction [43-45].

In conclusion, Supplementation with ascorbic acid, *Balanites aegyptiaca*, and strawberry improved glycemic control and renal function biomarkers in experimental rabbits. *Balanites aegyptiaca* showed the strongest hypoglycemic effect, while ascorbic acid produced the greatest reduction in serum creatinine. Overall, these natural supplements demonstrated potential protective effects on glucose regulation and kidney function.

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