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HISTOPATHOLOGICAL EVALUATION OF THE KIDNEY FOLLOWING WHEY PROTEIN SUPPLEMENTATION IN WISTAR ALBINO RATS

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ABSTRACT

Background: Whey protein is one of the most widely consumed dietary supplements among athletes, bodybuilders, and health-conscious individuals owing to its role in muscle growth and recovery. Concerns persist regarding the renal effects of prolonged protein supplementation, since excessive protein intake may increase renal workload, causing hyperfiltration and structural alterations in renal tissue. **Aim**: To assess the effect of whey protein supplementation in kidney of Wistar albino rats. **Materials and Methods**: Eighteen healthy adult Wistar albino rats (150–180 g) were divided into three groups of six. Group 1 (control) received normal diet and water for three months; Group 2 received whey protein for one month; Group 3 received for three months. Body weight, serum creatinine, kidney organ weight and renal histopathology (H&E staining) were assessed. **Results**: Serum creatinine and kidney weight rose in a duration-dependent manner (control < 1-month < 3-month group; one-way ANOVA $p < 0.001$ for both right and left kidney weight). Histopathology showed normal architecture in controls, mild tubular epithelial swelling and focal dilatation in the 1-month group, and marked tubular degeneration, glomerular congestion and interstitial inflammatory infiltration in the 3-month group. **Conclusion**: Whey protein supplementation produces progressive, duration-dependent functional and histological alterations in the kidney of Wistar albino rats. Long-term,

unsupervised use of protein supplements warrants cautious, medically regulated intake with periodic renal monitoring.

KEYWORDS: Whey protein; kidney; histopathology; Wistar albino rats; nephrotoxicity; kidney weight.

1. INTRODUCTION

Protein is an essential macronutrient required for growth, repair and maintenance of body tissues. The recommended daily protein intake for healthy adults is 0.8 g/kg body weight, ordinarily met through a balanced diet. With rising fitness awareness and expansion of the sports-nutrition industry, protein supplements particularly whey protein have become extremely popular among athletes, bodybuilders and health-conscious individuals.

Whey protein is a high-quality, complete milk-derived protein containing all essential amino acids and is rapidly absorbed, making it effective for muscle protein synthesis and post-exercise recovery. However, unsupervised and excessive whey protein consumption raises concerns about long-term renal safety. Metabolism of excess dietary protein increases nitrogenous waste (urea, ammonia) that must be filtered and excreted by the kidneys. Persistent high protein loads may raise renal blood flow and intraglomerular pressure, potentially leading to hyperfiltration, glomerular injury, proteinuria and structural renal change.

Given the widespread and often unmonitored use of whey protein, the present experimental study was undertaken to evaluate its histological effects on the kidney, with organ weight and microscopic architecture as key end-points.

AIM

To assess the effect of whey protein supplementation on the kidney of Wistar albino rats.

OBJECTIVE

To evaluate the histological changes in the kidney of Wistar albino rats following whey protein supplementation for one month and three months.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Eighteen healthy adult Wistar albino rats weighing 150–180 g were procured and housed in the institutional animal facility of Alva's ayurveda medical college, Moodubidire, under CPCSEA guidelines: polypropylene cages with stainless-steel grill tops, paddy-husk bedding changed daily, a 12-hour light/dark cycle, 50–70% relative humidity and 22 °C ambient temperature, with standard pelleted feed and water ad libitum.

2.2 Grouping and Dosage

- Group 1 (Control, n = 6): normal diet and water for 3 months.
- Group 2 (n = 6): whey protein 1 g/kg/day orally for 1 month (30 days), along with normal diet and water.
- Group 3 (n = 6): whey protein 1 g/kg/day orally for 3 months (90 days), along with normal diet and water.

The animal dose was derived from the standard human dose using the Paget and Barnes conversion factor (Animal dose = Human dose × 0.018 × 5/kg body weight). Whey protein was administered by oral gavage each morning between 11 a.m. and 12 noon.

2.3 Parameters Assessed

- Weekly body weight.
- Daily behavioural and general clinical observation (activity, food/water intake, urine output, fur texture).
- Serum creatinine (Modified Jaffe's method) at 30 days (Groups 1 & 2) and 90 days (Groups 1 & 3).
- Kidney organ weight (right and left) recorded at sacrifice.
- Renal histopathology (H&E-stained sections) of both kidneys from every animal.

Animals of Group 2 were sacrificed on day 30 and animals of Groups 1 and 3 on day 90, under diethyl ether overdose anaesthesia. Kidneys were excised via mid-sagittal abdominal incision, weighed, and fixed in 10% formalin for histopathological processing.

2.4 Statistical Analysis

Normality was tested using the Shapiro–Wilk test and homogeneity of variance using Levene's test. Intergroup comparisons of body weight and serum creatinine were made with two-way mixed ANOVA, and kidney weight with one-way ANOVA, followed by Tukey's / Bonferroni post-hoc tests. $p < 0.05$ was considered statistically significant.

3. OBSERVATIONS AND RESULTS

3.1 Body Weight and General Observations

Body weight increased progressively over time in all three groups, with the greatest cumulative gain in Group 3, consistent with the anabolic action of whey protein. Two-way mixed ANOVA showed significant effects of time, group and time × group interaction on body weight ($p < 0.05$). Whey protein-treated animals additionally showed mildly increased activity, food intake, water intake and urine output compared to controls, though no mortality or overt behavioural toxicity occurred in any group throughout the study.

3.2 Serum Creatinine

Serum creatinine remained within the normal range in the control group throughout the study (≈0.60 mg/dL). Group 2 animals (1-month supplementation) showed a mild but consistent rise (≈0.69–0.74 mg/dL), while Group 3 animals (3-month supplementation) showed a marked, statistically significant elevation (≈0.84–0.93 mg/dL). Mixed ANOVA confirmed significant effects of time, group and their interaction, indicating a duration-dependent rise in serum creatinine with prolonged whey protein intake.

3.3 Kidney Weight

Both right and left kidney weights increased with whey protein supplementation in a duration-dependent manner. Kidneys remained grossly normal in shape and colour on gross examination, with only a mild increase in size and weight noted in the supplemented groups.

Table 1: Mean kidney weight (right and left) across the three experimental groups.

Group	Duration	Mean Right Kidney Wt (g)	Mean Left Kidney Wt (g)
Group 1 (Control)	3 months	0.835	0.815
Group 2 (Whey protein)	1 month	0.905	0.882
Group 3 (Whey protein)	3 months	0.993	0.965

One-way ANOVA revealed a highly significant difference between groups for both right kidney weight ($F = 68.00$, $df = 2,15$, $p < 0.001$) and left kidney weight ($F = 66.00$, $df = 2,15$, $p < 0.001$). Tukey's post-hoc analysis showed significantly higher kidney weight in Group 2 versus Control (right: mean difference −0.07 g, $p = 0.002$; left: −0.06 g, $p = 0.004$), in Group 3 versus Control (right: −0.15 g, $p < 0.001$; left: −0.15 g, $p < 0.001$), and in Group 3 versus Group 2 (right: −0.08 g, $p = 0.001$; left: −0.09 g, $p < 0.001$). These findings confirm a clear, duration-dependent increase in renal organ weight with whey protein supplementation.

3.4 Histopathological Findings

Light photomicrographs (H&E stain) of representative kidney sections from each group are shown in Figures 1–3. Detailed microscopic descriptions are summarised below.

Group 1 — Control

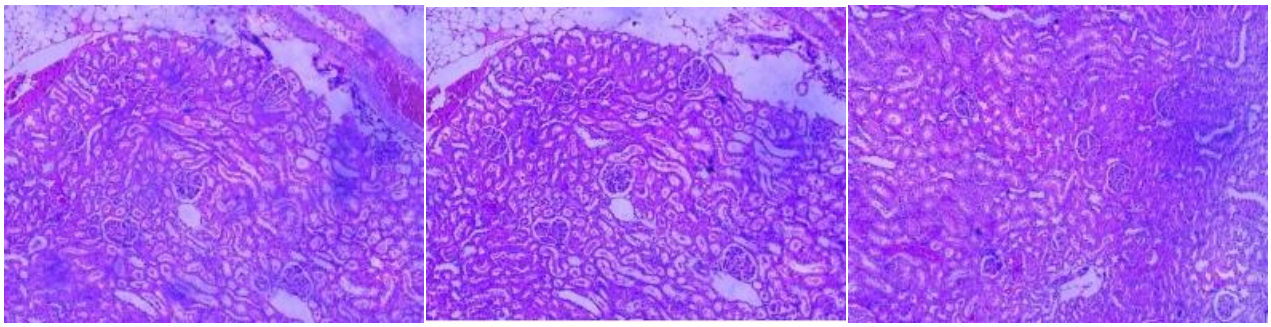


Figure 1: Kidney sections of control-group rats (H&E) showing normal cortical architecture: intact glomeruli, preserved Bowman's space, well-maintained proximal tubular brush border, and unremarkable interstitium.

Sections from both kidneys showed well-preserved cortical and medullary architecture. Glomeruli were normal in size, shape and cellularity with intact Bowman's capsule and space, and capillary tufts were delicate without congestion, sclerosis or mesangial proliferation. Proximal and distal tubules and collecting ducts showed intact epithelium, preserved brush border, and no dilatation, vacuolation, necrosis or cast formation. The interstitium and blood vessels were unremarkable, with no significant histopathological abnormality identified in either kidney.

Group 2 — Whey Protein, 1 Month

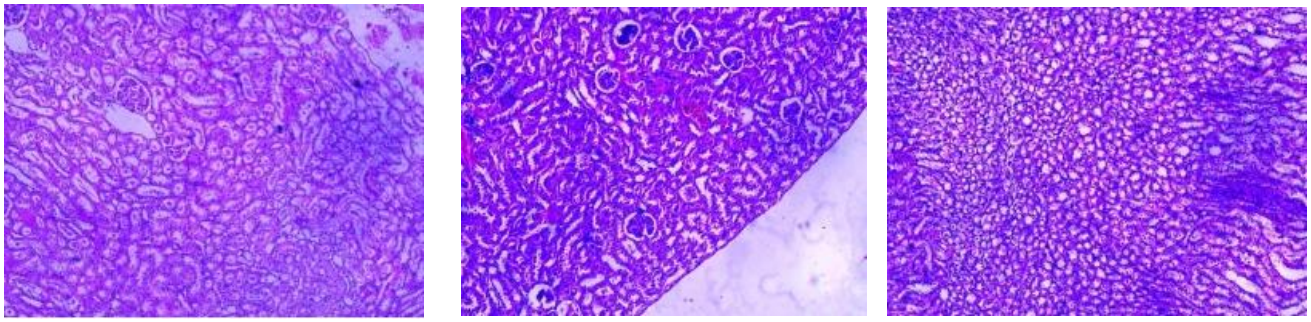


Figure 2: Kidney sections of Group 2 rats (H&E) showing mild early alterations: glomerular congestion, focal tubular dilatation, and mild epithelial vacuolation.

Sections showed mild alteration of renal architecture compared to controls. Glomeruli were mildly congested with slight prominence of capillary loops and mild focal reduction of Bowman's space, without sclerosis or severe mesangial proliferation. Proximal tubules

showed mild epithelial swelling, focal dilatation and occasional cytoplasmic vacuolation, though the brush border remained largely preserved. Mild focal interstitial inflammatory infiltration and mild vascular congestion were noted, without fibrosis or haemorrhage. Overall architecture remained largely preserved, with only mild early changes suggestive of increased renal workload.

Group 3 — Whey Protein, 3 Months

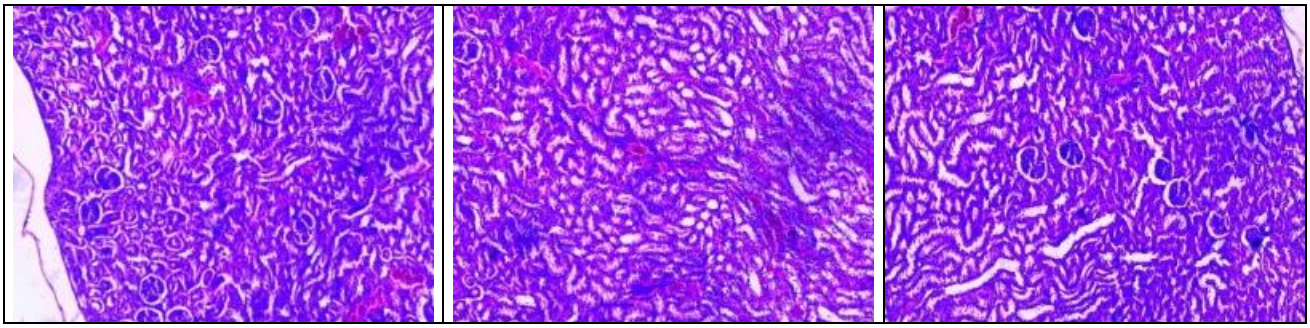


Figure 3: Kidney sections of Group 3 rats (H&E) showing marked tubular dilatation, epithelial degeneration with loss of brush border, glomerular congestion, and interstitial inflammatory infiltration.

Sections revealed moderate-to-marked distortion of renal architecture with significant tubular and glomerular alterations. Numerous tubules showed marked dilatation with widened, irregular lumina lined by flattened, attenuated epithelium. Tubular epithelial degeneration was evident, with cytoplasmic vacuolation, loss of epithelial polarity, nuclear pyknosis and focal desquamation, with partial loss of the proximal brush border in several profiles. Glomeruli exhibited marked congestion with engorged capillary loops, increased vascularity, focal reduction of Bowman's space, and mild mesangial prominence. Focal interstitial inflammatory infiltration, mild interstitial oedema and moderate vascular congestion were also present. Changes were bilaterally symmetrical, consistent with systemic renal involvement.

4. DISCUSSION

The present study demonstrates a clear, progressive, duration-dependent pattern of renal biochemical and structural change with whey protein supplementation. Control animals maintained stable serum creatinine and normal renal histology throughout, confirming that housing and diet alone did not induce renal stress. In contrast, Group 2 animals showed mild biochemical and histological change after one month, while Group 3 animals supplemented for three months showed significant elevation of serum creatinine together with marked

histopathological alterations, including tubular degeneration, brush-border loss, glomerular congestion and interstitial inflammation.

These findings parallel the concurrent rise in kidney organ weight, which increased significantly and progressively across groups (one-way ANOVA, $p < 0.001$ for both kidneys), most likely reflecting glomerular hyperfiltration, tubular hypertrophy and interstitial change secondary to the increased nitrogenous load imposed by high protein intake. The parallel occurrence of greater anabolic benefit (body-weight gain) and more severe renal alteration in Group 3 highlights a mechanistic link between sustained protein catabolism, nitrogenous waste generation and renal excretory burden the very efficacy of whey protein as an anabolic supplement appears accompanied by a proportional rise in renal metabolic load.

Notably, no mortality or overt behavioural toxicity was observed in any group, indicating that these renal changes occurred silently and sub-clinically a finding of considerable clinical relevance, since it suggests that supplement-induced renal stress may not be detectable through clinical observation alone. From an Ayurvedic perspective, this progressive alteration resonates with the classical principle of *Satmya* that even a nutritionally beneficial substance becomes harmful when consumed in excess or beyond the body's adaptive capacity.

These results are consistent with existing literature suggesting that chronic high-protein intake can produce hyperfiltration-mediated renal injury, particularly at higher doses or over prolonged durations, and argue for regulated, dose- and duration-conscious use of whey protein supplements, together with periodic monitoring of renal function in individuals consuming them chronically.

5. CONCLUSION

Whey protein supplementation produced measurable, duration-dependent functional and histological alterations in the kidney of Wistar albino rats. Short-term (1-month) administration produced comparatively mild changes, whereas long-term (3-month) supplementation produced significantly greater elevation in serum creatinine, kidney organ weight, and more pronounced histopathological injury including tubular degeneration and glomerular congestion. The null hypotheses are therefore rejected: whey protein supplementation causes significant histological change in the kidney at both one month and three months of administration. These findings underscore the importance of cautious, medically supervised use of protein supplements, particularly with prolonged consumption.

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