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Full Length Research Paper

The effect of quince leaf decoction on renal injury induced by hypercholesterolemia in rabbits: A pilot study

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There has been no investigation on the efficacy of quince extracts (*Cydonia oblonga* Miller) against hyperlipidemia-induced renal injury. In the present study, this hypothesis was evaluated by a histological comparison of kidney tissues from New Zealand white rabbits fed on a cholesterol-rich diet with and without a quince leaf extract supplement. Mild glomerular injury and moderate tubular damage were apparent in all rabbits in disease group. Meanwhile, milder tubular injury was detected in all animals in treatment group. The results were consistent with the hypothesis and suggest that a more extended study would be justified.

Key words: Quince, kidney, cholesterol, herb, diet.

INTRODUCTION

Dyslipidemia and hypercholesterolemia have been linked to the pathogenesis of progressive renal injury (Attia et al., 2002; Mune et al., 1999). Studies have revealed that cholesterol-fed rats develop proteinuria, interstitial injury, glomerulosclerosis, and podocyte and mesangial cell damage (Eddy, 1996; Joles et al., 2000). Renal injuries induced by hyperlipidemia are in part attributable to enhanced oxidative stress and defective renal vascular functions (Attia et al., 2002; Joles et al., 2000; Feldstein et al., 1999). Interestingly, Kasiske et al. (1988) reported an improvement in glomerular injury following lipid-lowering therapy in hyperlipidemic rats.

Quince (*Cydonia oblonga* Miller), a tree belonging to the Rosaceae family, grows wild in the Caucasus and Northern Iran (Golgolab, 1961). Different parts of quince have been used as traditional remedies for cardio-vascular, respiratory, gastrointestinal and urinary tract symptoms (Oliveira et al., 2007; Fattouch et al., 2007). Recent experimental animal studies have revealed that the fruit and leaves of quince have cell-protecting properties owing to the abundance of antioxidants they contain, for example, phenolic acids and flavonoids (Oliveira et al., 2007; Fattouch et al., 2007; Hamauzu et al., 2006). Quince leaf has also been attested to possess anti-hemolytic (Costa et al., 2009), anti-diabetic and anti-lipoperoxidative (Aslan et al., 2010), and lipid-lowering characteristics (Osman et al., 2010).

Despite these medicinal features, there is currently no evidence that quince leaf has renoprotective effects. The aim of this study was to assess the effect of quince (*C. oblonga* Miller) leaf decoction on renal injury induced

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by hypercholesterolemia in rabbits.

MATERIALS AND METHODS

Eleven adult New Zealand white male rabbits were used in this study in accordance with the international and local institutional guidelines for use of animals in research. The animals were kept under constant laboratory conditions with respect to humidity, illumination and temperature for two weeks prior to the study. Their regular diet included drinking water and standard food in the form of compact granules containing all essential nutrients (Shakouri et al., 2010). The rabbits were randomly divided into three groups: Group 1 (hypercholesterolemia group) received a cholesterol-enriched diet. Group 2 (treatment group) received a cholesterol-enriched diet plus *C. oblonga* leaf decoction as drinking supplement. After almost six weeks of these dietary modifications, Groups 1 and 2 were switched to a normal diet for another six weeks. Group 3 (control group) was maintained throughout the whole period of study on a regular diet without cholesterol enhancement or quince leaf decoction.

To prepare the cholesterol-enriched diet, cholesterol (Merck, Germany) was dissolved in ethanol and the solution was sprayed on to the ground food in a 2% weight/weight ratio. The mixture was then prepared, dried and granulated and the dried granules were used for the study. The cholesterol enriched diet elevated the serum cholesterol levels in Groups 1 and 2 by at least 10 folds when compared to those in the normal diet fed rabbits. To prepare the quince leaf decoction, dried leaves (4.95 g) were added to 600 ml boiling water, and the mixture was kept in a 100°C water bath for two hours. The decoction was then cooled to room temperature and filtered through a membrane. The rabbits drank the decoction (100 ml/each rabbit in Group 2) and tolerated it well.

At the end of the study (12th week), the animals were sacrificed with an anesthetic overdose (ketamine plus acepromazine). Urine samples were drawn by direct catheterization of the bladder. The urine samples were stored at 5°C for three days until analyses were performed. Spot urine creatinine and protein concentrations were measured by standard techniques. The ratio of urine protein to creatinine was calculated and used to estimate 24 h urine protein (Price et al., 2005).

The immediately excised kidneys were washed with 0.9% NaCl, cut into 5 × 5 mm pieces and fixed in 10% neutral buffered formaldehyde for light microscopic studies. Following graded dehydration through alcohol, the kidney tissues were embedded in paraffin and the blocks were retained until they were cut into sections for histochemical staining (hematoxylin and eosin (HE)), Periodic Acid-Schiff (PAS), and Masson's trichrome. On the basis of HE staining, the glomerular alterations were classified into no change (no lipoprotein thrombi, no foam cells in the glomeruli and no mesangial hyperplasia), mild (mesangial hyperplasia and dilatation of vascular lumen with no evidence of fat deposits), moderate (lipoprotein thrombi and foam cells in some glomeruli) and severe (lipoprotein thrombi and foam cells in many glomeruli) (Ishimura et al., 2009). Along with the glomerular changes, tubular damage was evaluated by categorization into no change, mild (cloudy swelling of tubular epithelium), moderate (desquamated and/or vacuolated tubular epithelial cells) and severe (necrosis, flattened epithelium and intraluminal casts). Basement membrane was assessed by PAS staining, and the presence of peritubular fibrosis was documented through Masson's trichrome staining.

RESULTS AND DISCUSSION

Light microscopy observations revealed mild glomerular injury in all rabbits in Group 1 (Figure 1A to C) and in two of the six rabbits in Group 2 (Figure 2B and D). The remaining animals had no glomerular changes in their HE- and PAS-stained sections (Figure 2A and C). While desquamated and vacuolated tubular epithelium (moderate tubular damage) was observed in kidney sections from Group 1 (Figure 1D), milder tubular injury was detected in all animals in Group 2. No glomerular or tubular alterations were seen in Group 3. Furthermore, no basement membrane thickening and fibrosis were discerned. The glomerular, tubular and basement membrane changes along with the urine analyses of the studied animals are listed in Table 1.

The results of this study showed that cholesterol-fed rabbits had both glomerular and tubular injuries, while the basement membrane was intact. Cholesterol-fed animals treated with the quince (*C. oblonga* Miller) leaf decoction supplement exhibited milder glomerular and tubular injuries. It is therefore plausible that quince leaf has a protective effect on the kidneys. To the best of our knowledge, this has not hitherto been reported.

Earlier investigations indicated glomerular macrophage inundation preceding glomerular mesangial matrix expansion, glomerular hypertrophy and tubulointerstitial changes in the early stages of hypercholesterolemia-induced renal injury (Eddy, 1996; Guijaro et al., 1995). A later study by Joles et al. (2000) highlighted podocyte injury as the primary lesion. On the other hand, Mazzolai et al. (2006) reported lipid accumulation initially in renal tubules and later in the glomeruli in high fat-fed mice. In our study, the renal tubules were more damaged than the glomeruli during the 6-week hypercholesterolemia. However, the core mechanism by which hypercholesterolemia induces renal injury is still unknown (Mune et al., 1999), though there has been particular focus on the role of reactive oxygen species (ROS) and increased oxidative stress (Trovato et al., 2010). Similarly, administration of antioxidants and lipid-lowering agents has been shown to protect the kidneys during hypercholesterolemia (Kasiske et al., 1988; Trovato et al., 2010).

Owing to their antioxidant components including phenolic acids and flavonoids, different parts of quince have been used as traditional remedies for numerous medical complaints (Osman et al., 2010; Silva et al., 2004). For instance, quince leaf decoction is used as a diuretic and for kidney stones in some regions of Turkey (Kültür, 2007). It is noteworthy that higher total concentrations of phenolics have been found in quince leaves than in pulps, peels and seeds (Oliveira et al., 2008). Because of its antioxidant properties, quince leaf can have anti-hemolytic, anti-diabetic and anti-lipidperoxidative effects (Costa et al., 2009; Aslan et al., 2010). Furthermore, Carvalho et al. (2010) and Osman et al. (2010) reported that quince leaf has anti-cancer and lipid-lowering characteristics. The probable protective effects of quince leaf on the hypercholesterolemia-induced renal

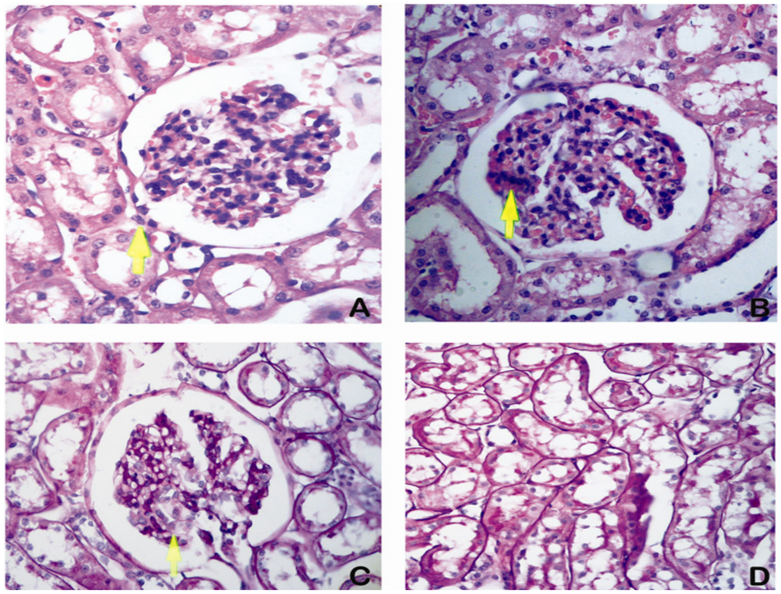

Figure 1. Histopathological findings in the kidneys of rabbits in Group 1 (disease group) showed mesangial hyperplasia and dilatation of vascular lumen with no evidence of fat deposits (mild glomerular change). Moderate tubular damage, that is, desquamated and/or vacuolated tubular epithelial cells, was noted as well. Paraffin-embedded kidney sections from rabbit 1 (A) and rabbit 2 (B), revealing glomerular and tubular alterations, were stained with HE. (C) Glomerular and tubular changes in PAS-stained kidney section from rabbit 1. (D) Tubular changes in rabbit 2 (PAS). Arrows indicate the glomerulus.

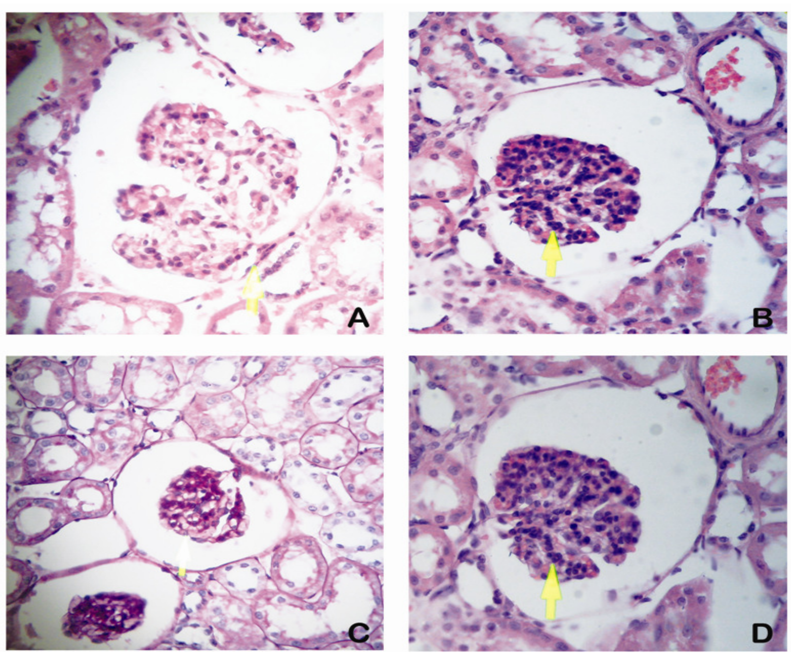

Figure 2. Microscopic examination of glomeruli in renal specimens from rabbits in Group 2 (treatment group). HE- (B) and PAS- (D) stained kidney sections from rabbit 6 showed mild glomerular and tubular change. Cloudy swelling of tubular epithelium is seen in B and D. No glomerular and tubular changes are apparent in HE-stained kidney sections from rabbit 1 (A) or in a PAS-stained specimen from rabbit 5 (C). Arrows indicate the glomerulus.

Table 1. The glomerular, tubular and basement membrane changes and the urine analyses of the studied rabbits.

Parameter		Glomerular injury	Tubular injury	Basement membrane changes	Fibrosis	Urine protein (mg/d)	Urine creatinine (mg/d)	Urine protein/ creatinine ratio
Group 1 (disease)	Rabbit 1	Mild	Moderate	No change	None	33.75	134	0.25
	Rabbit 2	Mild	Moderate	No change	None	82.50	126	0.65
	Rabbit 3	Mild	Moderate	No change	None	95.63	86	1.11
Group 2 (treatment)	Rabbit 1	No change	Mild	No change	None	18.75	92	0.20
	Rabbit 2	No change	Mild	No change	None	46.88	110	0.43
	Rabbit 3	No change	Mild	No change	None	15	78	0.19
	Rabbit 4	Mild	Mild	No change	None	56.25	124	0.45
	Rabbit 5	No change	Mild	No change	None	37.50	210	0.18
	Rabbit 6	Mild	Mild	No change	None	26.25	86	0.31
Group 3 (control)	Rabbit 1	No change	No change	No change	None	18.75	160	0.12
	Rabbit 2	No change	No change	No change	None	95.63	212	0.45

injury observed in the present study might be attributed to both its antioxidants and its lipid-lowering characteristics.

In conclusion, our results indicate that quince leaf decoction (*C. oblonga* Miller) partially protects rabbit kidneys from glomerular and tubular injuries during hypercholesterolemia. To the best of our knowledge, this is the first report concerning this effect of quince leaf.

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