Effects of Cysteine-stabilized Peptide Fraction of *Morinda lucida* Leaf on Selected Kidney Function Indices in Mice

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Objective: This study evaluated the effect of cysteine-stabilized peptide fraction (CSPF) of *Morinda lucida* leaf on selected kidney function indices in mice.
Methods: Sixty mice were assigned into six groups. Group A served as the control while groups B, C, D, E and F received 31.25, 61.5, 125, 250, and 500 mg/kg body weight of CSPF respectively for 7 or 28 days.
Results: Administration of CSPF for 7 and 28 days caused no significant ($p > 0.05$) alteration in kidney-body weight ratio, plasma concentrations of the selected electrolytes, urea and creatinine at all doses compared to controls. However, plasma uric acid concentration was significantly increased ($p < 0.05$) after administration of CSPF for 7 days at doses of 125 and 500 mg/kg body weight while it was significantly reduced ($p < 0.05$) after administration for 28 days at doses higher than 31.25 mg/Kg body weight compared to controls. The activities of Ca^{2+}, Mg^{2+}-ATPase and Na^{+}, K^{+}-ATPases in the kidney and the histology of the kidney remained unaltered ($p > 0.05$) throughout the experimental period compared to controls.
Conclusion: CSPF may adversely affect uric acid metabolism after prolonged administration.

Key words: *Morinda lucida*, cysteine-stabilised peptide, mice, renal function

INTRODUCTION

Natural products, especially plant extracts have been a major pillar for the survival of the human race for centuries [1]. It has been reported that about 65% of rural dwellers world-over (about 90% in Africa) depend on medicinal plants for their primary health care with plant-derived products also playing an indirect role in the health of the remaining 35% dwelling in developed countries [2]. The continued dependence of majority of the world population on phytochemicals has made evaluation of their safety or toxicity a continuous exercise.

Morinda lucida Benth (*Rubiaceae*) is a medicinal plant popularly known as Brimstone tree in English or 'Oruwo' in Yoruba (Western Nigeria). It is a tropical West Africa rainforest tree about 15 m tall with grey bark, short bent branches and shining green foliage [3]. Crude extracts of the leaf is used in folkloric medicine for the treatment of hypertension, malaria, ulcers, and gonorrhoea [4]. Our preliminary study showed that the leaf of *Morinda lucida* plant expresses cysteine-stabilised peptide and that this peptide fraction from the leaf of *Morinda lucida* exhibited antioxidant and antimalarial properties. However, the effect of the peptide fraction on kidney function has not been evaluated. Therefore, this study was carried out to evaluate the implications of consumption of cysteine stabilised peptide fraction of aqueous extract of *Morinda lucida* leaf on kidney function using selected kidney functional indices in mice as end points.

MATERIALS AND METHODS

Chemicals: Trifluoroacetic acid, acetonitrile and ouabain were purchased from Sigma Chemical Company, St. Louis, Mo, USA, C_{18} medium is a product of Phenomenex, Aschaffenburg Germany, while methanol and dichloromethane, sodium dihydrogen tetraoxophosphate (V) dihydrate, tetraoxosulphate (VI) acid, ammonium molybdate, ascorbic acid, Tris KCl, magnesium chloride hexahydrate, ethylene-glycol tetraacetic acid, adenosine triphosphate, sodium dodecyl phosphate, and sodium chloride were purchased from BDH Chemicals Limited, Poole England.
Plant material: *Morinda lucida* leaves were collected at the University of Ibadan campus, Ibadan, Oyo State, Nigeria. It was identified and authenticated at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria, where a voucher specimen was deposited (DPHUI : 1626).
Animals: Adult Swiss albino mice were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. The animals were housed in well-aerated plastic cages, fed with standard mouse chow (Grand Cereals Ltd., Jos Plateau State, Nigeria) and clean tap water *ad libitum*.
Ethical Approval: This study was approved by the University Ethical Review Committee of the University of Ilorin, Ilorin, Nigeria (with approval number UERC/ASN/2015/067), which complies with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH

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Table 1 Effects of *Morinda lucida* Leaf Peptide Extract on Kidney-Body Weight Ratios of Mice after 7 and 28 Days of Administration

Treatment	Day 7 Body Weight (g)	Day 7 Kidney Weight (g)	Day 7 Kidney-Body Weight Ratio (%)	Day 28 Body Weight (g)	Day 28 Kidney Weight (g)	Day 28 Kidney-Body Weight Ratio (%)
Control	16.08 ± 0.82[a]	0.17 ± 0.02[a]	1.07 ± 0.06[a]	21.90 ± 0.78[a]	0.22 ± 0.00[a]	1.00 ± 0.03[a]
31.25mg/Kg b.w	14.70 ± 0.29[a]	0.18 ± 0.01[a]	1.24 ± 0.09[a]	17.24 ± 0.88[a]	0.16 ± 0.01[b]	0.95 ± 0.08[a]
62.5 mg/Kg b.w	16.91 ± 1.54[a]	0.18 ± 0.01[a]	1.10 ± 0.12[a]	18.82 ± 0.68[a]	0.18 ± 0.01[a]	0.96 ± 0.05[a]
125 mg/Kg b.w	15.47 ± 2.17[a]	0.21 ± 0.00[a]	1.42 ± 0.16[a]	19.92 ± 1.39[a]	0.18 ± 0.01[a]	0.92 ± 0.05[a]
250 mg/Kg b.w	14.08 ± 0.81[a]	0.19 ± 0.03[a]	1.35 ± 0.13[a]	17.65 ± 4.52[a]	0.20 ± 0.01[a]	0.92 ± 0.06[a]
500 mg/Kg b.w	12.94 ± 0.50[a]	0.16 ± 0.01[a]	1.25 ± 0.11[a]	18.22 ± 1.06[a]	0.21 ± 0.02[a]	1.11 ± 0.08[a]

Values are means of 5 replicates ± SEM. Means in the same column with different superscripts are significantly different ($p < 0.05$).

publication No. 85–23, revised 1996).

METHODS

Plant Material: Cysteine-stabilised peptide fraction was prepared as described by Hellinger *et al.* [5]. Briefly, air-dried leaves were pulverised and percolated in a mixture of dichloromethane/methanol (50 : 50 v/v) and left for 18 h with continuous agitation. The mixture was filtered using muslin cloth. The filtrate was transferred into a separating funnel where equal volume of double distilled water was added, the upper aqueous layer was separated, concentrated at 40 °C in a rotary evaporator and then freeze-dried. The freeze-dried aqueous extract was reconstituted in a trifluoroacetic acid- distilled water acid (0.05 : 100, v/v) and thereafter passed through C_{18} column that has been preconditioned with methanol and activated with acetonitrile-distilled water mixture (9 : 1, v/v). The cysteine-stabilised peptide fraction was obtained from the C_{18} column by eluting with increasing concentrations (20%, 80%, and 100%) of acetonitrile-distilled water mixture. The eluent from 80% acetonitrile-distilled water mixture was concentrated using a rotary evaporator, freeze-dried and kept at 4 °C for subsequent use. The percentage yield was 0.5%.

Experimental Design: Sixty adult Swiss mice were randomly divided into six groups (A–F) with ten animals in each group. Mice in groups B, C, D, E and F were daily administered 0.2 ml of 31.25, 62.5, 125, 250 and 500 mg/kg body weight of the peptide fraction respectively through the oral route while control animals (group A) were administered 0.2 ml of 5% DMSO (the vehicle in which the peptide fraction was dissolved).

Sample Collection and Preparation

Half of the mice in each group were sacrificed after 7 days of peptide fraction administration by diethyl ether anaesthesia, while the remaining five mice in each group were sacrificed after 28 days of peptide fraction administration using the same procedure. Blood was collected into heparin bottles and centrifuged at 1000 rpm for 10 min and the plasma was separated into plain sample bottles and kept frozen at -20 °C for biochemical analysis. The mice were carefully dissected; the kidneys were excised, freed of fat, cleaned of blood and weighed. Parts of the weighed kidneys were transferred into specimen bottles containing 10% formalin for histopathological examination while the others were transferred into specimen bottles containing ice cold 0.25 M sucrose solution. The organs in ice cold sucrose solution (1 : 5, v/v) were homogenised, the homogenate was centrifuged at 10,000 g for 4 min in a refrigerated centrifuge (eppendorf 5804 F) and the supernatant separated into new sample bottles and kept at -20 °C for biochemical analysis.

Concentrations of plasma sodium and potassium ions were determined by flame photometry (JENWAY PFP7). The concentration of plasma calcium ion was determined by the method of Moorehead and Biggs [6] while the method described by Burtis *et al.* [7] was used to determine the concentrations of plasma chloride and phosphate ions. The concentrations of total protein, urea, creatinine and uric acid in the plasma were determined by the methods of Gornall *et al.* [8], Weatherburn [9], Bartels and Bohmer [10], and Fossati and Prencipe [11] respectively. The activities of alkaline phosphatase (ALP), Gamma-Glutamyltransferase (GGT) were also determined using the methods of Wright *et al.* [12] and Szasz [13] respectively. Mg^{2+}-ATPase activity was determined using the method of Ronner *et al.* [14] as modified by Fleschner and Kraus-Friedmann [15], while Ca^{2+}, Mg^{2+}-ATPase and Na^{+}, K^{+}-ATPase activities were determined by the method of Ronner *et al.* [14]. Histopathological study was done as described by Krause [16].

Statistical analysis

The data are presented as Means ± standard error of mean. Data were analysed using one-way ANOVA and Dunnett's test using SPSS 16.0 computer software package (SPSS Inc. Chicago, IL, USA). Differences at $p < 0.05$ were considered significant. Graphs were generated using GraphPad Prism 6 by GraphPad Software, California, USA.

RESULTS

Kidney-body weight ratio

There was no significant ($p > 0.05$) alteration in the kidney-body weight ratio at all doses after 7 and 28 days of administration compared to controls (Table 1).

Plasma Electrolytes and Biomolecules

Administration of peptide fraction for 7 and 28 days had no significant effect ($p > 0.05$) on the plasma concentrations of sodium ion (Na^{+}), potassium ion

Table 2 Effects of cysteine-stabilised peptide fraction of aqueous extract of *Morinda lucida* Leaf on Kidney Function Indices of Mice after 7 Days of Administration

Treatment	Na^+concentration (mmol/L)	K^+concentration (mmol/L)	Ca^{2+}concentration (mg/dL)	Cl^- concentration (mg/dL)	HCO_3^- concentration (mmol/L)	PO_4^{3-} concentration (mg/dL)	Urea concentration (mmol/L)	Creatinine concentration (µmol/L)	Uric acid concentration (mmol/L)
Control	135.75 ± 3.14[a]	3.50 ± 0.14[a]	2.37 ± 0.25[a]	103.25 ± 4.40[a]	20.00 ± 1.35[a]	1.15 ± 0.15[a]	6.49 ± 0.09[a]	77.14 ± 9.09[a]	0.45 ± 0.04[a]
31.25 mg/Kg b.wt	141.75 ± 3.57[a]	3.68 ± 0.08[a]	2.29 ± 0.12[a]	110.50 ± 1.55[a]	21.25 ± 1.93[a]	1.03 ± 0.21[a]	7.33 ± 0.06[a]	79.71 ± 10.76[a]	0.47 ± 0.08[a]
62.5 mg/Kg b.wt	142.00 ± 0.58[a]	4.03 ± 0.35[a]	2.28 ± 0.14[a]	106.50 ± 2.06[a]	17.75 ± 1.03[a]	1.40 ± 0.37[a]	7.03 ± 0.06[a]	82.29 ± 7.04[a]	0.51 ± 0.05[a]
125 mg/Kg b.wt	135.25 ± 1.89[a]	4.00 ± 0.04[a]	2.25 ± 0.10[a]	101.00 ± 3.37[a]	20.00 ± 1.47[a]	1.25 ± 0.14[a]	7.10 ± 0.10[a]	92.57 ± 10.76[a]	0.59 ± 0.07[b]
250 mg/Kg b.wt	138 .75 ± 1.70[a]	4.03 ± 0.41[a]	2.13 ± 0.01[a]	112.75 ± 9.72[a]	21.50 ± 0.65[a]	1.40 ± 0.20[a]	7.56 ± 0.10[a]	90.00 ± 12.86[a]	0.54 ± 0.05[a]
500 mg/Kg b.wt	138.50 ± 1.47[a]	4.15 ± 0.17[a]	2.25 ± 0.07[a]	97.25 ± 0.95[a]	19.96 ± 0.25[a]	1.45 ± 0.21[a]	6.80 ± 0.04[a]	92.57 ± 14.08[a]	0.56 ± 0.07[b]

Values are means of 5 replicates ± SEM. Means in the same columns with different superscripts are significantly different ($p < 0.05$).

Table 3 Effects of cysteine-stabilised peptide fraction of aqueous extract of *Morinda lucida* Leaf on Kidney Function Indices of Mice after 28 Days of Administration

Treatment	Na^+concentration (mmol/L)	K^+concentration (mmol/L)	Ca^{2+}concentration (mg/dL)	Cl^- concentration (mg/dL)	HCO_3^- concentration (mmol/L)	PO_4^{3-} concentration (mg/dL)	Urea concentration (mmol/L)	Creatinine concentration (µmol/L)	Uric acid concentration (mmol/L)
Control	132.75 ± 1.49[a]	3.93 ± 0.14[a]	2.44 ± 0.11[a]	91.00 ± 1.47[a]	17.75 ± 0.85[a]	0.98 ± 0.23[a]	7.54 ± 0.63[a]	126.17 ± 17.51[a]	0.58 ± 0.06[a]
31.25 mg/Kg b.wt	133.00 ± 1.00[a]	3.85 ± 0.18[a]	2.35 ± 0.06[a]	93.75 ± 2.21[a]	19.50 ± 1.55[a]	1.20 ± 0.18[a]	7.35 ± 0.95[a]	105.14 ± 14.36[a]	0.57 ± 0.00[a]
62.5 mg/Kg b.wt	132.00 ± 1.58[a]	3.75 ± 0.26[a]	2.29 ± 0.05[a]	95.50 ± 2.53[a]	13.75 ± 3.97[a]	1.08 ± 0.11[a]	7.83 ± 0.33[a]	142.99 ± 16.11[a]	0.55 ± 0.01[b]
125 mg/Kg b.wt	136.00 ± 3.16[a]	4.00 ± 0.07[a]	2.42 ± 0.12[a]	95.75 ± 3.71[a]	19.75 ± 1.11[a]	1.00 ± 0.20[a]	7.16 ± 0.87[a]	100.93 ± 0.00[a]	0.55 ± 0.00[b]
250 mg/Kg b.wt	134.00 ± 1.83[a]	3.90 ± 0.47[a]	2.13 ± 0.05[a]	96.25 ± 2.56[a]	20.50 ± 1.66[a]	1.50 ± 0.18[a]	8.30 ± 0.82[a]	109.35 ± 16.11[a]	0.53 ± 0.01[b]
500 mg/Kg b.wt	133.50 ± 2.10[a]	3.95 ± 0.58[a]	2.13 ± 0.06[a]	94.50 ± 1.94[a]	17.75 ± 1.49[a]	1.63 ± 0.15[a]	8.97 ± 0.25[a]	71.50 ± 17.34[a]	0.54 ± 0.00[b]

Values are means of 5 replicates ± S.D. Means in the same columns with different superscripts are significantly different ($p < 0.05$).

(K^+), calcium ion (Ca^{2+}), chloride ion (Cl^-), bicarbonate ion (HCO_3^-), phosphate ion (PO_4^{3-}), urea and creatinine at all doses compared to controls (Tables 2 and 3). However, plasma uric acid concentration was significantly increased ($p < 0.05$) at doses of 125 and 500 mg/kg body weight after 7 days of administration, whereas it was significantly reduced ($p < 0.05$) at doses higher than 31.25 mg/kg body weight after 28 days of administration compared to controls (Tables 2 and 3).

Cellular Enzymes

From the results, there was no significant change ($p > 0.05$) in ALP activity in the kidney at all doses of the peptide fraction administered after 7 and 28 days compared to controls (Fig. 1). After 7 and 28 days of administration of peptide fraction, there was no significant alteration ($p > 0.05$) in the activity of γ-GT at all doses in the kidney and plasma (Fig. 2).

The results revealed that after 7 days of peptide fraction administration, Mg^{2+}-ATPases activity was significantly reduced in the kidney at 500 mg/kg body weight with no significant alteration ($p > 0.05$) at other doses compared to control (Fig. 3). The result obtained after 28 days of administration of the fraction showed that there was no significant alteration ($p > 0.05$) in Mg^{2+}-ATPase activity at all doses compared to control (Fig. 3).

Results after 7 days and 28 days of peptide fraction administration showed that there was no significant alteration ($p > 0.05$) in Ca^{2+}, Mg^{2+}-ATPase activity in the kidney of treated animals compared to controls (Fig. 4). Administration of the fraction for 7 and 28 days caused no significant change ($p > 0.05$) in Na^+, K^+-ATPase activity at all doses considered compared to controls (Fig. 5).

Results of Histopathological studies

The photomicrographs of the kidney after 7 days of peptide fraction administration are shown in Fig. 6, while Fig. 7 shows the photomicrographs of the kidney after 28 days of fraction administration. The figures revealed that there were no detectable differences between the structural features of the kidneys of the experimental groups compared to controls.

DISCUSSION

Cysteine-stabilised peptide fraction administration for 7 and 28 days caused no change in kidney-body weight ratios at all doses, suggesting that the fraction exerted no adverse effect on the size of this organ. The results of histopathological studies showed that there were no observable differences in structural features of the kidney after 7 and 28 days of peptide fraction administration compared to controls. This suggests that the peptide fraction, did not adversely affect the structural architecture of the kidney.

ALP is a marker enzyme for the integrity of the plasma membrane and endoplasmic reticulum [17]. However, ALP activity was not significantly affected after 7 and 28 days of administration at all doses of the fraction. This suggests that the plasma membrane integrity of the cells of the heart has not been compromised. This also suggests that the role of ALP in producing inorganic phosphate for cellular use has not been compromised.

Although increased plasma GGT is primarily used as a marker for hepatobiliary disease, it was of recent also implicated as an early indicator of kidney disease [18]. Serum GGT was proposed to be a sensitive and dependable indicator of oxidative stress which has been reported to play an active role in kidney damage [19]. This makes serum GGT to have a strong association with chronic kidney disease [18]. From the results obtained in this study, administration of the fraction for both 7 and 28 days did not significantly affect kidney GGT activities at all doses, suggesting that consumption of the peptide fraction may not predispose subjects to kidney disease.

The reduction in Mg^{2+}-ATPase activity in the kidney at the dose of 500 mg/kg body weight after 7 days of peptide fraction administration suggests that its role in maintaining membrane fluidity and permeability may be adversely affected at this dose. However, the kidney was able to offset this anomaly after prolonged administration. The fact that the fraction did not adversely affect Na^+ K^+ ATPase and Ca^{2+}, Mg^{2+}-ATPase in the kidney after 7 and 28 days of administration suggests that it may not adversely affect active transport of Na^+ and Ca^{2+} ions in the kidney, especially as it relates to their reabsorption.

The results also revealed that the peptide fraction caused no alteration in all plasma electrolyte concentrations after 7 and 28 days of administration compared to controls. This suggests that the osmoregulatory role of the kidney was not adversely affected after 7 and 28 days of administration. These results suggest that prolonged consumption of the peptide fraction may not lead to electrolyte imbalance in subjects, even at higher doses.

Plasma urea, creatinine and uric acid concentrations are indicators of kidney function. Elevated levels of plasma urea, creatinine and uric acid are red flags of renal dysfunction [20]. Also, increase in plasma urea concentration may be caused by increased protein catabolism [21], while impairment of the urea cycle leading to reduced production of urea may lead to decreased plasma urea concentration [22]. The plasma creatinine concentration is independent of protein and water intake, rate of urine production and exercise. Since its rate of production is constant, increase in plasma creatinine concentration indicates impaired excretion, suggesting kidney impairment. The absence of significant change in plasma urea and creatinine levels after 7 and 28 days of treatment with the fraction suggests that the urea cycle, protein catabolism and renal function (especially glomerular filtration) were not adversely affected by administration of the fraction at these doses. Thus, the increase in plasma uric acid concentration at higher doses after 7 days of administration suggests an increase in nucleic acid catabolism yielding uric acid at a higher concentration than the kidney can handle rather than impaired glomerular filtration. Meanwhile, uric acid is also known to exhibit beneficial antioxidant potentials [23]. Uric acid scavenges highly reactive and toxic oxygen species such as singlet oxygen, hydroxyl radicals and superoxide anion [18]. Thus, the increased uric acid concentration may enhance the antioxidant status of the blood. However, the observed reduction in plasma

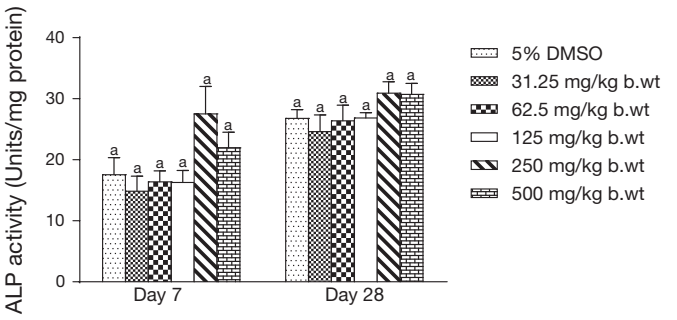

Fig. 1 Effects of peptide extract of *Morinda lucida* leaf on the activities of alkaline phosphatase in kidney of mice after 7 and 28 days of administration.
Values are means ± SEM of 5 replicates. Bars for each day with the same alphabet are not significantly different ($p > 0.05$).

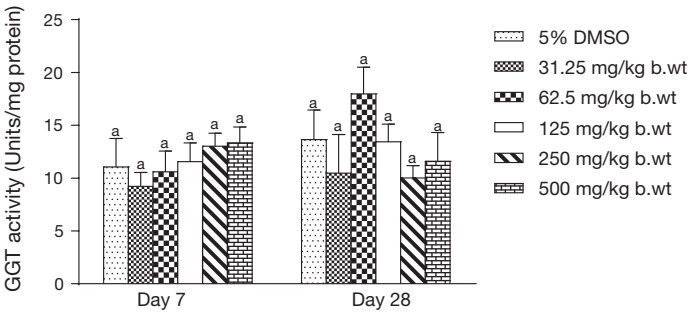

Fig. 2 Effects of peptide extract of *Morinda lucida* leaf on the activities of gamma glutamyl transferase in kidney of mice after 7 and 28 days of administration.
Values are means ± SEM of 5 replicates. Bars for each day with the same alphabet are not significantly different ($p > 0.05$).

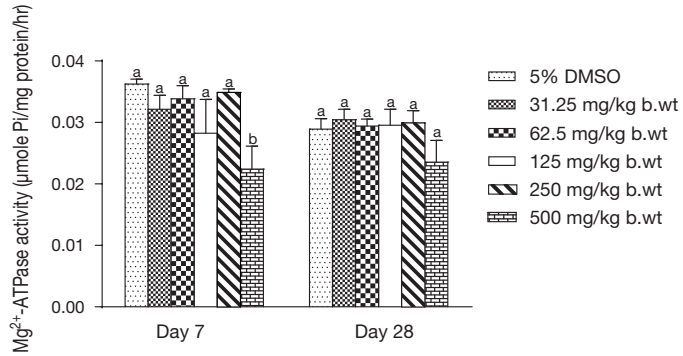

Fig. 3 Effects of cysteine-stabilised peptide fraction of aqueous extract of *Morinda lucida* leaf on the activities of Mg^{2+}-ATPase in the kidney of mice after 7 and 28 days of administration.
Values are means ± SEM of 5 replicates. Bars for each day with different alphabets are significantly different ($p < 0.05$).

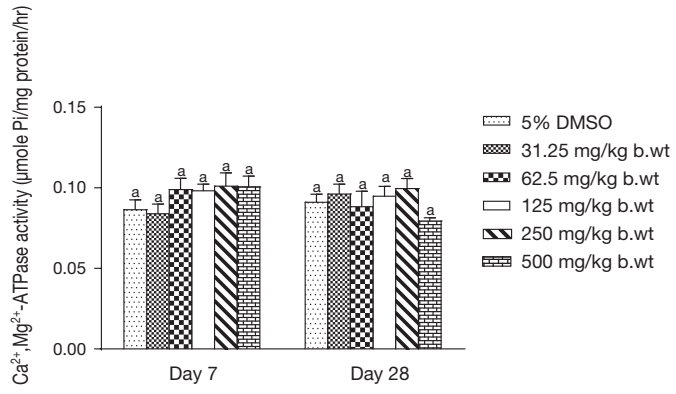

Fig. 4 Effects of cysteine-stabilised peptide fraction of aqueous extract of *Morinda lucida* leaf on the activities of Ca^{2+}, Mg^{2+}-ATPase in the kidney of mice after 7 and 28 days of administration.
Values are means ± SEM of 5 replicates. Bars for each day with the same alphabet are not significantly different ($p > 0.05$).

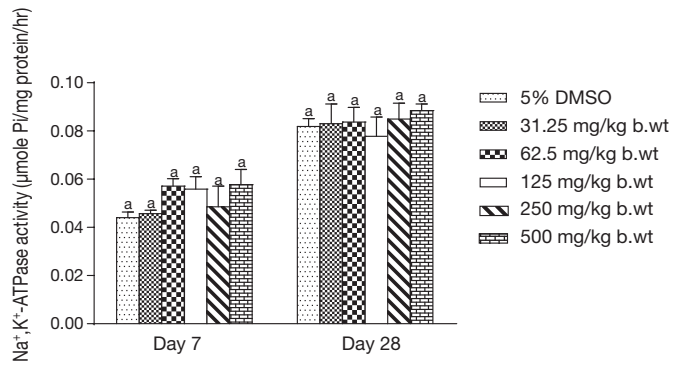

Fig. 5 Effects of cysteine-stabilised peptide fraction of aqueous extract of *Morinda lucida* leaf on the activities of Na^{+}, K^{+}-ATPase in the kidney of mice after 7 and 28 days of administration.
Values are means ± SEM of 5 replicates. Bars for each day with the same alphabet are not significantly different ($p > 0.05$).

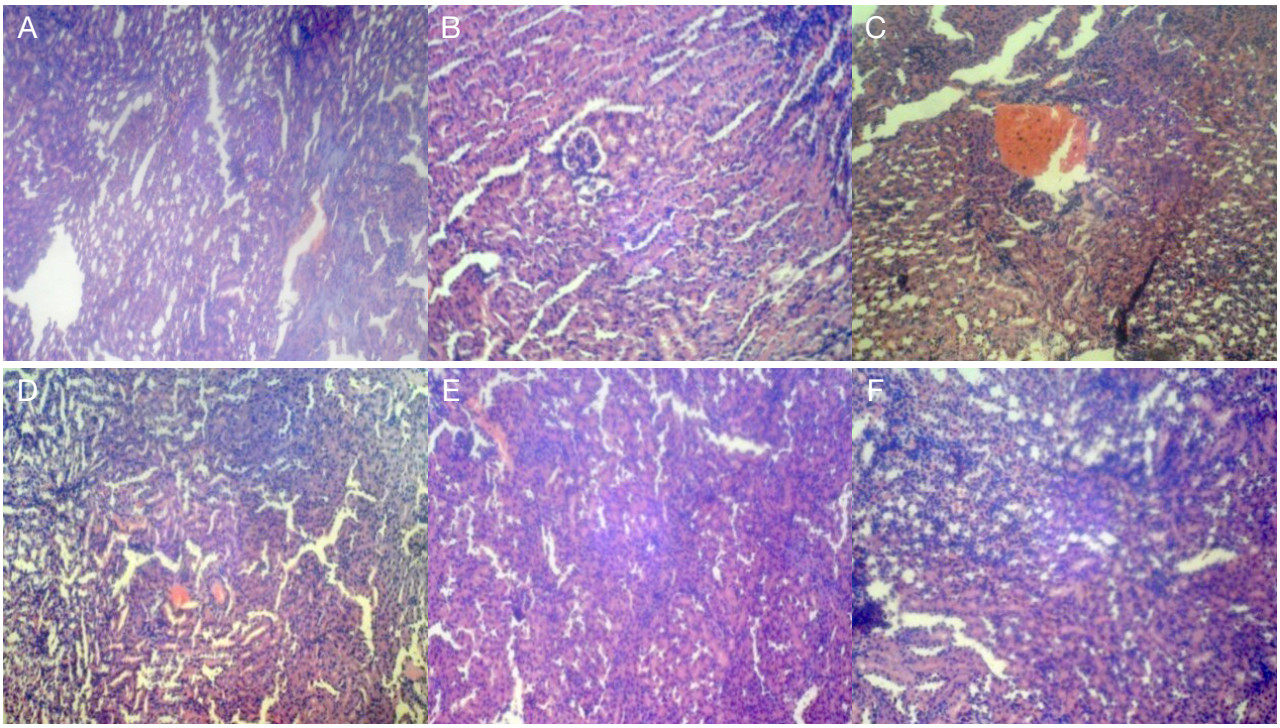

Fig. 6 Cross-section of mouse kidney after 7 days of administration of cysteine-stabilized peptide fraction of aqueous extract of *Morinda lucida* leaf (× 400; H & E).
A: 5% DMSO; B: 31.25 mg/kg body weight; C: 62.5 mg/kg body weight; D: 125 mg/kg body weight; E: 250 mg/kg body weight; F: 500 mg/kg body weight.

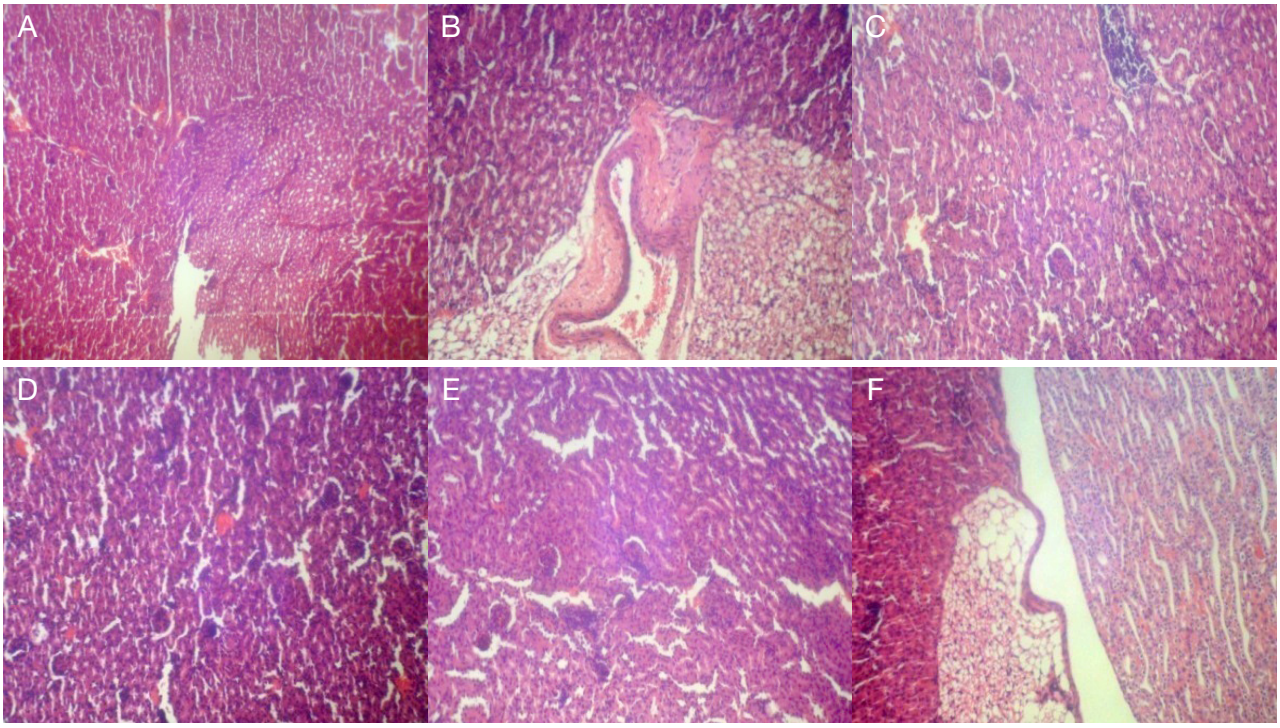

Fig. 7 Cross-section of mouse kidney after 28 days of administration of cysteine-stabilized peptide fraction of aqueous extract of *Morinda lucida* leaf (× 400; H & E).
A: 5% DMSO; B: 31.25 mg/kg body weight; C: 62.5 mg/kg body weight; D: 125 mg/kg body weight; E: 250 mg/kg body weight; F: 500 mg/kg body weight.

uric acid concentration at doses higher than 31.25 mg/kg body weight after 28 days of peptide fraction administration suggests a compensatory mechanism by the system to reduce nucleic acid catabolism which was increased at higher doses of the peptide fraction after 7 days of administration.

CONCLUSION

Cysteine-stabilised peptide fraction may adversely affect uric acid metabolism in subjects after prolonged administration.

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