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Protection effect of intracellular melanin from *Lachnum* YM156 and Haikunshenxi capsule combination on adenine-induced chronic renal failure in mice†‡

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This study aimed to elucidate the therapeutic effects of oral administration of intracellular melanin from *Lachnum* YM156 (LIM) on chronic renal failure (CRF) in mice. The cytotoxicity of LIM was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. CRF was induced in mice by chronic dietary adenine intake. We have used this intervention to explore the effects of oral treatment with LIM (100 and 200 mg kg⁻¹) in CRF mice. The treatment with LIM alone and a combination of Haikunshenxi capsule (HC) add LIM increased the concentration levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX), and glutathione reductase (GSH), reduced malonaldehyde (MDA) in the nephridial tissues and also reduced the nephridial levels of tumor necrosis factor (TNF)- α , interleukin-1 β (IL-1 β), and interleukin (IL)-6, and the activities of inducible nitric oxide synthase (iNOS). Interestingly, the HC and LIM combination produced a higher therapeutic effect than HC alone. The mechanism of the reported salutary effects of LIM in adenine-induced CRF is associated with amelioration of the adenine induced inflammation and oxidative stress. The present findings recommend that LIM is a useful natural product which can be used to enhance the protection function of HC in CRF mice.

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Introduction

Chronic renal failure (CRF) is considered to be a worldwide public health problem, which represents a critical period in the evolution of chronic renal disease and is associated with complications and comorbidities that begin early in the course of the disease.¹ CRF is characterized by progressive loss of nephrons caused by increased intraglomerular pressure and hyperfiltration, and the loss of autoregulatory ability exposes the glomeruli to systemic blood pressure, leading to glomerular hypertrophy and sclerosis.² Many renal diseases in humans are progressive in nature and eventually result in renal failure. As a result, the number of patients with renal failure continues to increase worldwide.³ Consequently, more attention has been paid to the research and development of effective therapies and agents for CRF.

Haikunshenxi capsule (HC), of which the main ingredient is fucoidan, is a drug used for the clinical treatment of CRF in Chinese medicine. Fucoidan is a natural marine drug extracted from kelp, and it can improve renal blood flow, delay glomerular sclerosis, promote functional recovery, scavenge reactive oxygen free radicals and inhibit lipid peroxidation.⁴ Clinically, HC is beneficial to patients with CRF. However, long-term use of HC may result in hepatic injury, nausea or other adverse reactions.⁵ Therefore, useful natural products for the treatment of CRF or to enhance the protection function of high dose HC treatment would be of significant importance. Thus, it is meaningful to explore a useful natural product. Most animals and some plants produce melanin in their bodies, but the resources are limited.⁶ Some microbes, like *Bacillus thuringiensis*, also synthesize significant amounts of melanin.⁷ Melanin produced by microbial fermentation which is characterized by no seasonal restrictions, low cost and convenient operation.⁸ *Lachnum* is a class of saprophytic fungi. For many years, its biodiversity has been widely investigated.⁹ Melanin pigments are ubiquitous in nature, with a variety of biological functions, like anti-oxidant, anti-inflammatory, anti-tumor, anti-radiation, liver injury protection and anti-bacterial properties.^{10–12} It has been widely and conventionally used in different industrial fields

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including food, cosmetics, pharmacology, medicine and other fields.^{13,14} However, its biological activities are not yet fully investigated.

In the present study, the main objective is to investigate whether melanin could be useful in CRF treatment and to enhance the protection function of HC. We report on the possible anti-oxidant and anti-inflammatory activities of an intracellular melanin extract from YM156 (LIM), as evidenced by its effects on the nephridial levels of antioxidant enzymes and inflammatory mediators. We hypothesized that LIM alone and LIM combined with HC could attenuate adenine-induced inflammation and oxidation. LIM not only displays a markedly therapeutic effect on CRF mice, but can also enhance the protection function of HC in CRF mice.

Results

Effects of LIM on HepG2 cell viability

As shown in Fig. 1, LIM had no cytotoxic effect on the HepG2 cell line in the tested concentrations. Compared with the control group, the experimental group cell viability was almost close to 100%. The percentage of cell viability (% of control) was calculated using the following formula: cell viability (% of control) = $B \times 100\% / A$, where A is the absorbance value of the control group and B is the absorbance value of LIM.

Treatment effects on serum levels of SCR, BUN, TP and ALB

As shown in Fig. 2, compared with the normal control group, the serum concentrations of SCR and BUN were significantly elevated in the model control group mice ($p < 0.01$), as expected, indicating that this was a successful experimental model as previously described.^{15,16} Compared with the model control group, SCR and BUN concentrations in LIM alone and LIM with HC groups decreased to different degrees ($p <$

0.05 or $p < 0.01$). In addition, SCR and BUN concentrations of mice in the HC plus LIM group were lower than those in the HC alone group at the same dosage.

TP and ALB levels were significantly lower in the model control groups than the normal control group. Compared with the model control group, TP and ALB concentrations of mice in LIM and LIM + HC groups increased at different degrees ($p < 0.05$ or $p < 0.01$). TP and ALB concentrations of mice in the HC plus LIM group were higher than those of the HC alone group at the same dosage, which were close to those of normal mice. All the results were obtained in a dose-dependent manner.

Treatment effects on mice status and movement

In our study, treatment effects on appearance and behaviour were examined. The results demonstrated that no significant effect was observed in all treatment groups except for the model control group. Mice could move about freely. Their fur looked smooth and normal, and their food intake and water consumption also appeared normal.

Treatment effects on weight growth rate and kidney index in CRF mice

Treatment effects on weight growth rate and kidney index are shown in Table 1. As expected, while the normal control mice had a 57.34% increase in weight, mice in the model control group had a 32.42% growth increase rate, which was the least compared with all other groups. This showed that the model control of CRF in mice was built successfully. This was followed by the HC alone group which could be due to the side effects of HC. However, the weight growth rate of mice in the other groups did not show obvious differences when compared with that in the normal control group. The weight growth rate of mice with LIM alone treatment markedly improved when compared with that of the model control group. In the two groups treated with LIM plus HC, the weight growth rates were almost the same as that of the normal control group, which could be due to the enhanced therapeutic effect of HC when it was used in combination with LIM.

Compared with the normal control group, the kidney index of the model control group was significantly increased ($p < 0.01$). Compared with the model control group, the kidney index values of the LIM alone and LIM with HC groups were significantly reduced, which confirms that LIM exerted a protective effect against CRF. Moreover, the kidney index of the mice increased in a dose-dependent manner. This showed that the kidney index of mice in the combination treatment group was lower than those of the HC alone group at the same dosage. These results demonstrated that the nutritional state and general well-being of the mice were the best in the combination treatment group and were the worst in the model control group.

Fig. 1 Effect of the different concentrations of LIM on HepG2 cell viability.

Fig. 2 Effects of LIM on SCR (A), BUN (B), TP (C) and ALB (D) in serum of CRF mice. ^a $p < 0.05$, significantly different from the model control group. ^b $p < 0.01$, highly significantly different from the model control group. HC: Haikunshenxi capsule, positive drug; L-LIM: low-dose LIM; H-LIM: high-dose LIM. There were ten mice in each group.

Table 1 Effects of LIM on weight growth rate and kidney index in CRF mice

Groups	Weight growth rate (%)	Kidney index (mg g ⁻¹)
Normal control	57.34 ± 1.52 ^b	1.12 ± 0.12 ^b
Model control	32.42 ± 1.43	1.94 ± 0.03
Positive control (HC)	42.68 ± 2.35 ^a	1.20 ± 0.07 ^b
Low dose LIM	50.12 ± 1.21 ^b	1.72 ± 0.09 ^a
High dose LIM	52.68 ± 1.27 ^b	1.35 ± 0.04 ^b
Low dose LIM + HC	54.34 ± 1.28 ^b	1.18 ± 0.08 ^b
High dose LIM + HC	56.64 ± 2.18 ^b	1.13 ± 0.05 ^b

^a $p < 0.05$, significantly different from model control group. ^b $p < 0.01$, highly significantly different from model control group. The mice in each group were ten.

Treatment effects on kidney tissue homogenate oxidative stress in CRF mice

The effects of LIM treatment with or without HC on kidney tissue oxidative stress in mice are presented in Table 2. The

kidney homogenate solution of SOD, CAT, GSH-Px and GSH levels in the CRF model control group mice were significantly decreased ($p < 0.01$), and the MDA concentration was significantly increased ($p < 0.01$). A significant change in these

Table 2 Nephridial SOD (U mg⁻¹ protein), CAT (U mg⁻¹ protein), GSH-Px (U L⁻¹ protein) and GSH (mg GSH g⁻¹ protein) levels and nephridial MDA (nmol mL⁻¹) concentrations

Groups	SOD (U mg ⁻¹)	CAT (U mg ⁻¹)	GSH-PX (U L ⁻¹)	GSH (mg g ⁻¹)	MDA (nmol mL ⁻¹)
Normal control	49.46 ± 0.18 ^b	78.22 ± 0.13 ^b	892.44 ± 16.32 ^b	5.42 ± 0.24 ^b	2.84 ± 0.21 ^b
Model control	30.45 ± 1.12	44.13 ± 1.25	688.45 ± 12.67	3.05 ± 0.11	5.05 ± 0.15
Positive control (HC)	45.61 ± 0.75 ^b	70.52 ± 0.46 ^b	842.21 ± 13.38 ^b	4.98 ± 0.62 ^b	3.07 ± 0.68 ^b
Low dose LIM	32.32 ± 4.02 ^a	60.53 ± 0.14 ^b	743.09 ± 11.42 ^b	4.19 ± 0.26 ^b	3.95 ± 0.38 ^b
High dose LIM	38.25 ± 1.24 ^b	64.45 ± 0.98 ^b	790.43 ± 13.68 ^b	4.84 ± 0.26 ^b	3.74 ± 0.45 ^b
Low dose LIM + HC	46.52 ± 5.96 ^b	74.25 ± 1.12 ^b	850.44 ± 12.32 ^b	5.23 ± 0.34 ^b	3.05 ± 0.11 ^b
High dose LIM + HC	48.58 ± 2.50 ^b	76.47 ± 1.34 ^b	878.91 ± 12.43 ^b	5.38 ± 0.27 ^b	2.88 ± 0.35 ^b

^a $p < 0.05$, significantly different from model control group. ^b $p < 0.01$, highly significantly different from model control group. The mice in each group were ten.

parameters was found in the model control group as compared to the normal control group ($p < 0.01$), which indicated that superoxide formation was significantly higher in the kidneys of the model control group. The antioxidant enzyme levels of the mice in the LIM alone and LIM with HC groups were significantly higher than those of the model control group mice ($p < 0.01$ or $p < 0.05$), and the MDA concentration was significantly lower ($p < 0.01$). The data also showed that the effect of HC plus LIM treatment is slightly better than that of HC treatment alone in increasing the antioxidant enzyme levels and decreasing the MDA production, suggesting that the effect of HC in CRF mice may be enhanced by LIM. All the results were obtained in a dose-dependent manner.

Inflammatory stress in CRF mice kidney tissue

To examine the potential anti-inflammatory effects of LIM treatment with or without HC, the treatment effects are presented in Table 3. It can be seen that compared with the normal control group, the nephridial levels of TNF- α , IL-1 β , IL-6 and activities of iNOS in the model control group mice were significantly increased ($p < 0.01$). The levels of TNF- α , IL-1 β , IL-6 and activities of iNOS in the LIM and LIM with HC groups were significantly lower than those in the model control group, and the treatment effect in the high dose LIM plus HC combination group was better than that in the HC alone group ($p < 0.01$). All the results were obtained in a dose-dependent manner.

Treatment effects on kidney tissue pathological changes

As shown in Fig. 3, no obvious histological alteration was observed in the kidneys of mice from the normal control group or HC, high dose LIM and LIM plus HC combination treatment groups. The kidneys of the model control group were pale, and adenine crystals were seen mainly in the cortex area. There was marked deposition of crystalline bodies in the renal tubules and interstitium. However, the glomeruli were observed sparsely. Renal tubular atrophy and severe interstitial fibrosis were also found. These findings are compatible with characteristics of CRF.¹⁷ In the low dosage LIM group, the mice had some complete glomeruli and kidney tubules in the kidney tissue. The groups that were treated with high dose LIM and LIM plus HC combination had a reduced number of adenine crystals, renal tubular atrophy and interstitial fibrosis.

Discussion

HC is a commonly used drug for CRF and its high doses are known to cause severe hepatic injury, nausea, emesis or other adverse reactions.⁵ *Lachnum* sp. is a class of saprophytic fungi, which can produce a large amount of melanin under submerged culture conditions. *Lachnum* sp. melanin has many biological activities, such as anti-oxidant, anti-aging and anti-radiation.^{14,18,19} In this study, LIM showed no cytotoxic effect and it has a therapeutic effect in CRF mice. We observed the anti-oxidant and anti-inflammatory properties

Table 3 Nephridial levels of TNF- α (pg mg⁻¹ protein), IL-1 β (pg mg⁻¹ protein) and IL-6 (pg mg⁻¹ protein) and nephridial iNOS (U mg⁻¹ protein) activities

Groups	TNF- α (pg mL ⁻¹)	IL-1 β (pg mL ⁻¹)	IL-6 (pg mL ⁻¹)	iNOS (U mg ⁻¹)
Normal control	59.74 ± 1.18 ^b	12.35 ± 0.13 ^b	8.44 ± 0.32 ^b	0.48 ± 0.10 ^b
Model control	250.15 ± 8.10	18.34 ± 0.25	20.85 ± 0.11	1.98 ± 0.12
Positive control (HC)	105.32 ± 8.32 ^b	14.81 ± 0.37 ^b	12.28 ± 0.42 ^b	0.81 ± 0.02 ^b
Low dose LIM	218.12 ± 7.22 ^a	17.66 ± 0.24 ^a	18.27 ± 0.72 ^a	1.79 ± 0.26 ^a
High dose LIM	168.32 ± 6.54 ^b	16.03 ± 0.17 ^a	14.16 ± 0.09 ^b	1.64 ± 0.26 ^a
Low dose LIM + HC	98.13 ± 6.78 ^b	14.51 ± 0.34 ^b	12.07 ± 0.07 ^b	0.79 ± 0.34 ^b
High dose LIM + HC	84.35 ± 5.70 ^b	13.81 ± 0.24 ^b	10.74 ± 0.03 ^b	0.68 ± 0.27 ^b

^a $p < 0.05$, significantly different from model control group. ^b $p < 0.01$, highly significantly different from model control group. The mice in each group were ten.

Fig. 3 Effects of LIM on nephridial histological in CRF mice; magnification: 400×.

of LIM and explored its treatment benefit effects that enhanced the protection function of HC in CRF mice. It was observed that compared with the model control group, LIM and the combined use of LIM and HC showed a therapeutic effect *via* improving the activity of anti-oxidant enzymes, reducing the kidney levels of cytokines related to its activation (TNF- α , IL-1 β and IL-6) and activities of iNOS. The protection function of HC was enhanced by LIM dose dependently and LIM has further beneficial effects on CRF, which might be due to its anti-inflammatory and anti-oxidative effects as shown in this study.

CRF is confirmed by SCR and BUN level tests. It has been shown to be increased in serum which indicates a lower glomerular filtration rate and, as a result, a decreased ability of the kidneys to excrete waste products.^{16,20} He, L. and Shen, P. *et al.* reported that kangqianling decoction showed a positive effect on CRF mice, which led to a significant decrease in levels of SCR and BUN.²⁰ Our data demonstrated that the levels of SCR and BUN in the combined LIM and HC group and LIM alone group decreased compared with that of the model control group, which is in concordance with the results of He's studies. CRF always led to a significant decrease

in TP and ALB levels.²¹ It has been reported that the levels of TP and ALB significantly in rhubarb-treated mice.²² In this study, similar results were found. Both LIM alone and HC plus LIM groups increased the levels of TP and ALB ($p < 0.01$) compared with the model control group. Furthermore, the combination of LIM and HC treatment showed significant effects. The results indicated that LIM had effective restorative action, returning the SCR, BUN, TP and ALB levels in the serum to normal values.

Feng *et al.* reported that after renal failure was induced by adenine, kidney enlargement resulted in the increase of the kidney index which was critical to evaluate the health state of mice kidneys.²³ In the present study, we found that the weight growth rate of mice in the LIM alone and LIM plus HC groups were significantly higher than those in the model group. The weight growth rate of the high dosage LIM plus HC group was close to that of normal mice. Compared with the model group, the kidney index of mice in the HC plus LIM group decreased significantly and showed a dosage-effect relationship. These observations implied that LIM could enhance the HC protection function of adenine-induced CRF in mice.

It has been reported that increased oxidative stress, indicating an imbalance between the intracellular production of free radicals and the cellular defence mechanisms, has been recognized to play an important role in CRF treatment-induced adverse effects.^{24,25} To confirm the anti-oxidative action mechanism of LIM in inhibiting CRF and enhancing HC protection function, the current study has investigated the effects of LIM with/without HC treatment related to oxidative stress, namely SOD, CAT, GSH-Px, GSH and MDA. SOD, CAT, GSH-Px and GSH are important antioxidant enzymes in organisms, which play an important role in the regulation of a variety of cell functions and in the protection of cells against oxidative injury.^{26–28} MDA is one secondary product of lipid peroxidation and has been extensively studied as a potential biomarker for oxidative stress.²⁹ Here, our results showed that adenine can significantly induce oxidative stress in mice as it causes a reduction of antioxidant enzyme activities in kidney tissues but increases in MDA concentrations. And the effect of LIM plus HC is slightly better than that of HC alone treatment in increasing the antioxidant enzyme levels and decreasing the MDA production. LIM can increase the decreased levels of these antioxidant enzymes induced by adenine; all these findings were in accordance with the findings of previous studies that melanin can significantly improve the levels of SOD, CAT and GSH-Px,^{14,18} suggesting that the effect of HC in CRF may be potentially enhanced by LIM.

In the present study, the levels of TNF- α , IL-6, IL-1 β and iNOS activities were measured to determine the inflammation status of the CRF mice and evaluate the protection effect of LIM. According to previous studies, apart from hematological abnormalities and oxidative stress, CRF can also lead to kidney damage and immune function decline. NO has been implicated in a multitude of biological functions in inflammatory processes, and the role of NO in both cell death and survival is concentration dependent.³⁰ However, NO arising from iNOS is believed to play a significant detrimental role in a variety of allotransplanted cells or tissues, including the lung, kidney, pancreatic islets, cornea and aorta.³¹ Thus, iNOS may play different roles in the various cells that can produce NO during immune-mediated renal injury. A decrease in its production in adult patients with CRF may contribute to their pronounced susceptibility to infections. The production of iNOS in the CRF mice was significantly lower than that of normal mice, indicating an impaired cell-mediated immunity. TNF- α , IL-1 β and IL-6 are potent pro-inflammatory cytokines which are produced by many cell types including monocytes/macrophages and renal mesangial and epithelial cells. These cytokines exert pleiotropic biological effects on a wide range of target cells and, due to their pro-inflammatory and immunoregulatory properties, play a crucial role in inflammation, immune response and haematopoiesis.^{20,32} Some reports have demonstrated the increase of the levels of pro-inflammatory cytokines including IL-1 β , IL-6 and TNF- α in both CRF patients and CRF animals.^{25,33,34} In this process, similar results had been observed in mice. TNF- α , IL-6, and IL-1 β levels and iNOS activi-

ties within the kidneys were significantly decreased in the LIM and HC plus LIM treatment groups compared with the model control group. It showed obvious dose-dependent anti-inflammatory activities which may be due to the anti-inflammatory function of LIM. In addition, LIM and HC combination treatment showed a trend of having a higher ability in decreasing the TNF- α , IL-6, IL-1 β levels and iNOS activities than HC treatment alone. In conclusion, data from our present study suggest that LIM can reduce the development of renal inflammation and tissue injury, indicating that it may have therapeutic utility in kidney diseases and/or associated conditions.

Conclusions

The therapeutic effects of intracellular melanin from *Lachnum* YM156 (LIM) on chronic renal failure (CRF) were evaluated in an acute adenine-induced kidney injury model. We demonstrated that LIM displays a markedly therapeutic effect on CRF mice, and the combined use of LIM and Haikunshenxi capsule (HC) shows a better therapeutic effect than HC treatment alone. Treatments with LIM alone or a LIM and HC combination markedly induced significant decreases in the concentrations of the measured inflammatory mediators. Further, LIM mitigated oxidative stress *via* improving the activity of anti-oxidant enzymes. This study provided experimental evidence that LIM displays a markedly therapeutic effect on CRF mice and can enhance the protection function of HC in CRF mice, suggesting a potential role for LIM in the protection against renal failure progression.

Laboratory animals

One hundred Kunming mice (male), weighing 20 ± 5 g, were purchased from the Experimental Animal Center of Anhui Medical University (certificate number: no. 1 license of the Medical Laboratory Animal of Anhui) and were kept under standard conditions, which include a 12 h light/dark cycle, temperature of 18–20 °C and a standard diet. This project was reviewed and approved by the Committee for Protection of Animal Care Committee at the Hefei University of Technology.

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References

- 1 M. T. James and B. M. Hemmelgarn, *Lancet*, 2010, 375, 1296–1309.
- 2 C. Yang, *Indian J. Pharm. Sci.*, 2015, 77, 103–107.
- 3 Y. Wu, Y. Ming, Z. Du and L. Jing, *Carbohydr. Polym.*, 2014, 114, 190–195.
- 4 Y. Chen, Y. Q. Zhang and H. U. Hong, *Zhongguo Xinyao Zazhi*, 2015, (14), 1628–1630.

- 5 J. Wang, Q. Zhang, W. Jin, X. Niu and H. Zhang, *Carbohydr. Polym.*, 2011, **84**, 417–423.
- 6 F. H. E. Brindle and E. H. Froeliger, *EP Pat.*, 0635577, 1995.
- 7 A. E. Aghajanyan, A. A. Hambardzumyan, A. S. Hovsepyan, R. A. Asaturian, A. A. Vardanyan and A. A. Saghiyan, *Pigm. Cell Res.*, 2005, **18**, 130–135.
- 8 L. F. Gibson and A. M. George, *FEMS Microbiol. Lett.*, 2006, **169**, 261–268.
- 9 C. Li, Y. Hong, Y. X. Tan and J. H. Ai, *Clinical Proteomics*, Humana Press, 2008, pp. 193–208.
- 10 N. Avramidis, A. Kourounakis, L. Hadjipetrou and V. Senchuk, *Arzneim. Forsch.*, 1998, **48**, 764–771.
- 11 A. El-Obeid, S. Al-Harbi, N. Al-Jomah and A. Hassib, *Phytomedicine*, 2006, **13**, 324–333.
- 12 V. M. Sava, Y. C. Hung, V. A. Blagodarsky and M. Y. Hong, *Food Res. Int.*, 2003, **36**, 505–511.
- 13 A. Takahashi, K. Tsuchiya, T. Yamanome and M. Amano, *Peptides*, 2004, **25**, 1613–1622.
- 14 M. Ye, Y. Wang, G. Y. Guo and Y. L. He, *Food Chem.*, 2012, **135**, 2490–2497.
- 15 Y. Tong, B. Han, H. Guo and Y. Liu, *Afr. J. Tradit. Complementary Altern. Med.*, 2010, **7**, 331–338.
- 16 Y. Wang, H. Yin, X. Lv and Y. Wang, *Fitoterapia*, 2010, **81**, 397–402.
- 17 K. Ataka, H. Maruyama, T. Neichi and J. Miyazaki, *Am. J. Nephrol.*, 2003, **23**, 315–323.
- 18 Y. Lu, M. Ye, S. Song and L. Li, *Appl. Biochem. Biotechnol.*, 2014, **174**, 762–771.
- 19 M. Ye, G. Y. Guo, Y. Lu, S. Song, H. Y. Wang and L. Yang, *Int. J. Biol. Macromol.*, 2014, **63**, 170–176.
- 20 L. He, P. Shen, Q. Fu and J. Li, *J. Ethnopharmacol.*, 2009, **122**, 367–373.
- 21 R. Chen, L. Cheng and H. F. Du, *J. Comput. Sci. Technol.*, 2005, **20**, 728–734.
- 22 J. Wang, Y. Zhao, X. Xiao and H. Li, *J. Ethnopharmacol.*, 2009, **124**, 18–25.
- 23 X. Feng, X. M. Qiu, Y. L. Huang and X. Z. Liu, *Food Sci.*, 2010, **9**, 064.
- 24 I. Kucukkurt, S. Ince, I. Aytekin and Y. O. Birdane, *Kocatepe Vet. J.*, 2010, **3**, 13–17.
- 25 B. H. Ali, I. Alhusseni, S. Beegam and A. Nemmar, *PLoS One*, 2013, **8**, 181–182.
- 26 Z. L. Liu and B. Zhou, *Pure Appl. Chem.*, 2005, **77**, 1887–1903.
- 27 G. Siegel, A. Schmidt, P. Schäfer and M. Malmsten, *Eng. Life Sci.*, 2009, **9**, 363–375.
- 28 T. Yangui, S. Sayadi, H. Chakroun and A. Dhib, *Eng. Life Sci.*, 2011, **11**, 511–516.
- 29 F. Nielsen, B. B. Mikkelsen, J. B. Nielsen and H. R. Andersen, *Clin. Biochem.*, 1997, **43**, 1209–1214.
- 30 K. K. Dietrich, K. Fehsel, C. Suschek and K. Bachofen, *Int. Immunopharmacol.*, 2001, **1**, 1407–1420.
- 31 M. F. Usta, M. Tuncer, A. Baykal and M. A. Çiftçioglu, *Urology*, 2002, **59**, 953–957.
- 32 E. Kontny, M. ZiÓŁKowska, A. Ryżewska and W. Maśliński, *Cytokines*, 1999, **11**, 839–848.
- 33 D. M. Tatjana, M. Siniša, G. Ljiljana and P. Lada, *Eur. J. Intern. Med.*, 2014, **25**, e88.
- 34 M. I. Muniz-Junqueira, C. B. Lopes, C. C. Schleicher and J. P. R. Veiga, *Life Sci.*, 2005, **77**, 3141–3155.