Preventative and treatment effects of *Blumea Lacera* extract on chronic renal failure (CRF) in a mouse model

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ABSTRACT: In Vietnam, there are many patients with chronic renal failure (CRF). They must undergo hemodialysis periodically, peritoneal dialysis, or a kidney transplant which is very expensive. Many patients die because they cannot afford treatment. Our study aimed at finding the scientific basis for developing an affordable, available drug from Vietnamese medicinal herbs to prevent and treat CRF in an experimental model. The aerial part of *Blumea lacera* was collected in Sa Pa, Lao Cai and dried and minced as raw materials for extraction with different polarizing solvents to qualitatively determine its chemical composition. The *B. lacera*'s ethanol extract was tested for both its preventive and treatment effects on a CRF model induced by adenine in mice. The results showed that *B. lacera* contains major chemical components including flavonoids, alkaloids, saponin, tannins, sterols, carotenoids, polysaccharides, organic acids, and reduced sugars. Additionally, the *B. lacera* ethanol extract has shown to have significant preventive as well as treatment effects on chronic renal failure models induced by adenine.

KEYWORDS: Blumea lacera; ethanol extract; adenine; chronic renal failure

Introduction. Chronic renal failure (CRF) is a global health burden with high economic costs to health systems. Global mean (95% CI) prevalence of stage 5 CRF accounts for 13.4% of cases.[7] According to preliminary statistics, in Vietnam 6.73% of the population has CRF. This is a burden for families and society.[10] On average, there are about 8,000 newly infected patients each year. At end-stage CRF, the main measures for treatment are cycle dialysis, peritoneal dialysis, or a kidney transplant, which is very expensive. Many patients cannot afford to pay for treatment, and so they have no option but to go home and wait to die.

Vietnam is a tropical country rich in herbs, many of which have traditionally been used to make medicines. This inspired the authors to find an available and affordable herb in Vietnam for the production of new drugs for the prevention and/or treatment of CRF. There have been previous studies analyzing the effect of herbs against CRF including Angelica sinensis, Ligusticum wallichii, Salvia miltiorrhiza, Rhizoma dioscoreae, Rhodiola crenilata, Astragalus membranaceus and Angelica sinensis,[9] and rhubarb in China.[12]

Blumea lacera is a popular herb in Vietnam which is used in some remedies for kidney disease.[2] However, there have been no studies on Vietnamese B. lacera's chemical composition or the effects of the herb against CRF. We wished to study *Blumea lacera* to test its usefulness in the development of medicine for patients with CRF. Wishing to have an affordable drug made from herbs to prevent and treat chronic renal failure, we conducted the project with following objectives:

- Determination of the chemical components of *Blumea lacera* aerial parts.
- Evaluation of preventive effects of *Blumea lacera* extracts against chronic renal failure in an experimental model.
- Evaluation of treatment effects of *Blumea lacera* extracts against chronic renal failure in an experimental model.

Results and Discussion: Determination of Blumea lacera's Chemical Composition. The qualitative investigation of the chemical composition of *B. lacera* helps to screen bioactive compounds for further study. Due to time limitations, we only have a preliminary survey of the chemical composition of the dry *Blumea lacera* without intensive research on specific substances. Table 1 shows that *Blumea lacera* contains 9 chemical components including: flavonoids, alkaloids, and saponin. These are just preliminary results; however, this is the first report in Vietnam about the chemical composition of *B.* lacera. Our research results are also consistent with previous studies outside Vietnam on the qualitative groups of organic compounds in *B. lacera*. [1, 8]

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Serial	Compounds	Qualitative reaction	Results
1	Alkaloid	Mayer's reagent	+
		Bouchardat reagent	+
		Dragendorff's reagent	+
2	Cardiac Glycoside	Liebermann-Burchard reaction	-
		Legal Reaction	-
		Baljet Reaction	-
		Keller – Kiliani Reaction	-
3	Anthranoid	Borntraeger Reaction	-
4	Flavonoid	Cyanidin Reaction	+
		Alkaline reaction	+
		Ferric chloride reaction	+
5	Coumarin	Ring-opening and ring-closing reactions of lactone	-
		Diazotization reaction	-
6	Saponin	Foaming Index	+
7	Tannin	Ferric chloride reaction	+
		Gelatin-Tannin Reaction	+
		Lead Acetate Test	+
8	Organic Acid	Na_2CO_3 Reaction	+
9	Reduced sugar	Fehling reagent	+
10	Polysaccharide	Lugol reagent	+
11	Fatty acid	Creation of blurring on paper	-
12	Sterol	Liebermann-Burchard reaction	+
13	Carotenoid	Reacts with concentrated H_2SO_4	+

Preventive effect of Blumea lacera extract against chronic renal failure. Figures 1A and 1B show that the concentration of urea and creatinine in the preventive and control groups were similar ($p>0.05$). Meanwhile, the levels of urea and creatinine in the non-preventive group were significantly higher than that in the other two groups ($p<0.05$ and $p<0.01$ respectively).

The kidney clears toxins from the body including urea and creatinine. When kidney function is impaired the toxins cannot be eliminated and the levels of urea and creatinine increase causing dysfunction of the body. Hence, these are two important biomarkers to assess kidney function. [4, 9, 11] The results show that *B. lacera* has a preventive effect by reducing the concentrations of urea and creatinine in the preventive group.

Figures 1C and 1D show that the erythrocytes number and hemoglobin concentration in the preventive and control groups were similar ($p>0.05$). Meanwhile, those parameters in the non-preventive group were significantly lower than that in the other two groups ($p<0.05$ and $p<0.01$ respectively).

Healthy kidneys produce a hormone called erythropoietin (EPO), a substance that stimulates bone marrow to produce red blood cells. In CRF, the kidneys cannot produce EPO, leading to a in hemoglobin levels. The results show that *B. lacera* has a preventive effect on CRF by preventing anemia.

From the above analysis, we can conclude that *Blumea lacera* has significant preventive effects on adenine-induced CRF models.

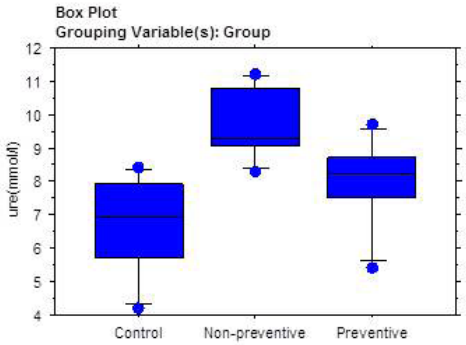

Figure 1A: Preventive effect on urea concentration.

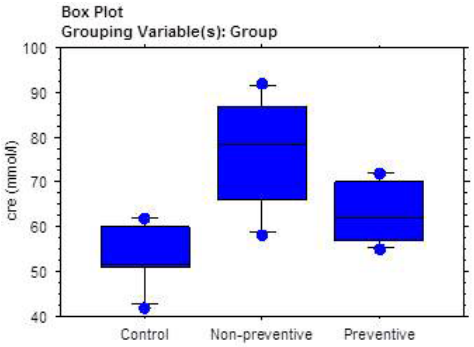

Figure 1B: Preventive effect on creatinine concentration.

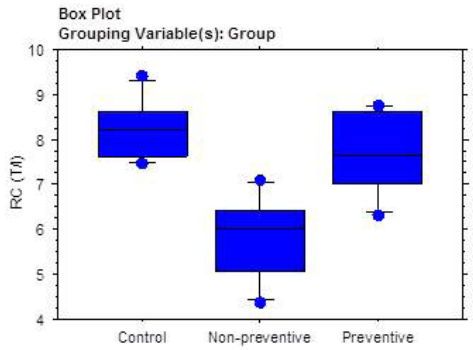

Figure 1C: Preventive effect on erythrocyte number

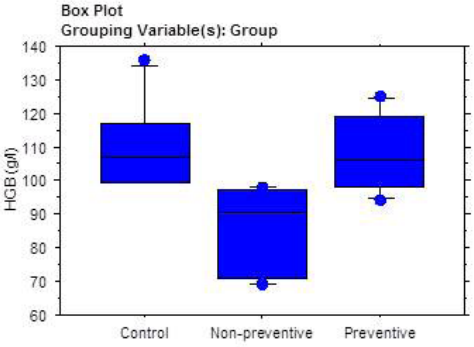

Figure 1D: Preventive effect on hemoglobin concentration

Treatment effect of Blumea lacera extract against chronic renal failure. Figures 2A and 2B show that the concentration of urea and creatinine in the post-treatment group was significantly lower than that in the pre-treatment group with $p<0.01$, and similar to the control group. Thus, the *B. lacera* extraction has a treatment effect in CRF, by showing a significant reduction in urea and creatinine levels compared to the pre-treatment group.

Figures 2C and 2D show that after treatment the number of erythrocytes increased significantly compared to before treatment with $p<0.05$. The concentration of hemoglobin is similar to the control group with $p>0.05$. Thus, after a 21-day treatment, the anemic status of mice improved. However, the treatment needs to be continued and followed up.

From the above analysis, we can conclude that *B. lacera* has significant treatment effects on adenine-induced CRF models.

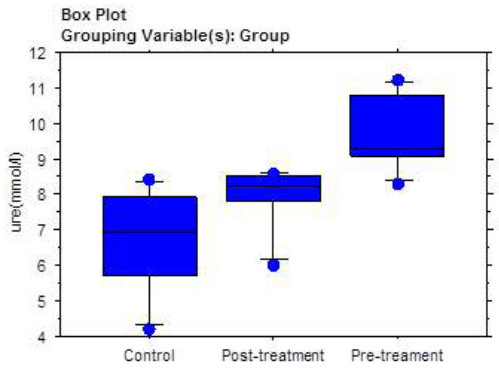

Figure 2A: Treatment effect on urea concentration.

Conclusion. The chemical compositions of *Blumea lacera* includes flavonoids, alkaloids, and saponin. *B. lacera* ethanol extract has preventive and treatment effects against chronic renal failure in mice induced by adenine in that it reduced urea and creatinine concentrations in blood as well as improved the anemic status in the preventive group and treatment group.

Methods. Herbs: the plant material was identified as *Blumea lacera* (Burm.f.) by the Institute of Ecology and Biological Resources. Chemicals included ethanol 98%, water (H_2O), n-hexane, diluted sulfuric acid (H_2SO_4), adenine (Sigma). Reagents: Dragendoff, Mayer, Bouchardat, ninhydrin, lugol, sulfuric acid, gelatin, FeCl3, Na2CO3, NaOH met the analytical criteria specified in the Vietnamese Pharmacopoeia V. Main equipment included: Reflux extraction system of 2-liter flask capacity, vacuum rotary storage system (Eyela, Japan) combining recirculating cooling unit (Daihan, Korea), balance analytical Mettler Toledo (Switzerland) accuracy of 0.1 mg, Glassware: external flask with capacity of 50-2000 ml, test tubes, precision pipettes, volumetric flasks, BTS350 semi-automatic biochemical analyzer from Spanish Biosystem to measure urea and creatine, and semi-automatic hematology analyzer. Animals included 18 white mice with total weight 20 ± 2g. Swiss mice were provided by the National Institute of Hygiene and Epidemiology.

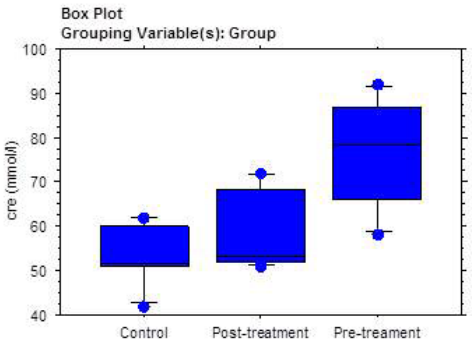

Figure 2B: Treatment effect on creatinine concentration

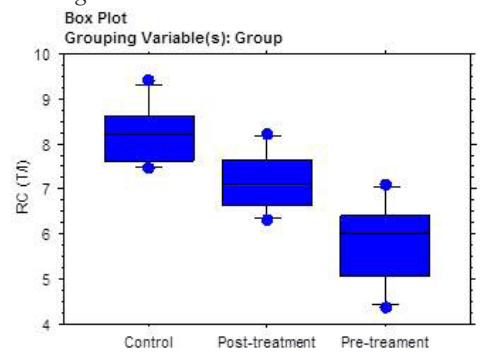

Figure 2C: Treatment effect on erythrocyte number.

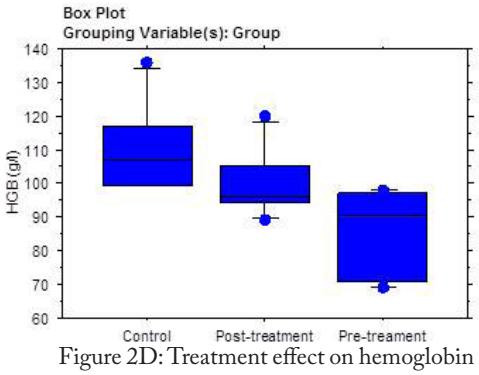

Figure 2D: Treatment effect on hemoglobin concentration.

Blumea lacera extractions for chemical composition determination. *Blumea lacera* was collected from Sa Pa town, Lao Cai province, Vietnam and was dried, minced and extracted using different polar solvents (ethanol, water, n-hexane, dilute H_2SO_4). 13 groups of organic substances in the extract were qualitatively determined by specific chemical reactions based on medicinal plant research methods.[6] Each experiment was repeated three times with the same results.

- Alcoholic extraction: Flavonoids were determined by cyanidine reaction; reacted with alkali then reacted with 5% $FeCl_3$; saponin was determined by a foaming reaction; cardiac glycosides were determined by Liebermann-Burchardt reactions, Legal reactions, Baljet reactions and Keller-Kiliani reactions; coumarin was determined using the lactone ring-closing reaction and diazotization reaction.[6]
- Water extraction: tannin was determined by reaction with 5% $FeCl_3$, 1% gelatin and 10% lead acetate; organic acids were determined by reaction with Na_2CO_3; polysaccharides were determined by reaction with Lugol reagent; reducing sugar was determined by reaction with Fehling reagent.[6]

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- N-hexane extraction: sterol was determined by Liebermann-Burchard reaction; fats were determined by reaction of blurring on filter paper; carotenoids were determined by reacting with large concentration of H_2SO4.[6]
- Diluted H_2SO_4 extraction: alkaloids were determined using Mayer, Dragendorff and Bouchardat reagents; anthranoids were determined by Borntraeger reaction. [6]

Samples and animal Preparation

- Preparation of extract: 1.1 kg dry powder of *Blumea lacera*; reflux was extracted three times with 96% EtOH, cooled, filtered and concentrated, then evaporated under reduced pressure to achieve 86.4 grams in EtOH.

Prevention and treatment studies: The EtOH residues were mixed with Dimethyl sulfoxide (DMSO) solution, then mixed with distilled water to reach a concentration of 628mg / 10ml and orally administrated at a dose of 0.1ml / 10grams (equivalent to 8 grams of dried medicinal herbs / kg / day).

- Adenine solution: 10mg of adenine was mixed in 1ml of vegetable oil and orally administrated at dose 0.1ml / 10gram, equivalent to a dose of 100mg / kg body weight/ day.

Animals were maintained under stable room temperatures (24±1oC) and humidity levels (50-60%) and a 12-hour light-dark cycle. Each polycarbonate animal cage contained six mice. All animals had access to food and water with body weights recorded daily throughout the course of all experiments. Five days before the study, mice were acclimatized to lab conditions and handled by participants for a few minutes every day to avoid fear by mice during experiments. When experiments ended, all animals were transferred to the Animal Care Unit without any deaths recorded.

Evaluating the effects of chronic renal failure preventing. 18 healthy white mice were randomly divided into three groups of six. The experiment was conducted within 35 days as follows:

- Control group (n = 6): mice are fed normal diets.
- Preventive group (n = 6): Adenine-induced CRF model according to Yokizawa and colleagues with some modifications [11]. Mice are orally administered adenine dissolved in vegetable oil at a dose of 100mg / kg every other day, alternating with herb extract with the corresponding dose of 8g of dried herbs / kg / day.
- Non-preventive group (n = 06): Mice are given adenine every other day as the above preventive group and were given distilled water on other days without the herb extract.

After 35 days, blood was taken via mouse tail vein to test urea, creatinine, erythrocytes, and hemoglobin using BTS350 of Biosystem Spain semi-automatic biochemical analyzer and HumaCount 30TS hematology analyzer respectively. The Mann-Whitney U test was used to compare differences in the median of the indexes between groups. A p-value < 0.05 was considered a significant difference.

Evaluating the effects of treatment of chronic renal failure. To minimize number of animals for research, they were re-used from the above preventive effect evaluation.

- The treated group included six mice with CRF taken from the non-preventive group were given the extract at the dose equivalent to 8g of dried medicinal herbs / kg / day alternating drinking adenine at dose of 100mg / kg / day every other day.
- Control group included six healthy mice, taken from the control group, fed normal diets.

After 21 days, blood was taken from the mouse tail vein to determine the concentration of urea, creatinine, hemoglobin and the number of erythrocytes using the same methods as the experiment for the preventive effect's evaluation.

The results obtained from the above control group were used as a control indexes to make comparison with pre-treatment and post-treatment groups.

Ethical issues in the study . The procedure involving animals was approved by the Ethical Committee in the Biomedical Research of Vietnam Military Medical University (1680B/QĐ-HVQY) and the National Ethics Committee of Ministry of Health (N 45/CN-HDDD) and the agreement of the use of mice for the study was from the Animal Husbandry Association of Vietnam (No. 18/KHCN-HCN), following the National and International Guidelines for Ethics in Biomedical Research.[3, 5]

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