EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 01
516-519
Year: 2017

DIUERETIC ACTIVITY OF PLANT EXTRACT OF GARDEN SPURGE (*EUPHORBIA HIRTA* L.) ON MALE WISTAR RATS

Siti Nurjanah[1], Samsuar[1], Nopiyansah[1], Martha Lulus Lande[2] and Mohammad Kanedi[2]*

[1]Department of Pharmacy, Faculty of Mathematics and Sciences, Tulang Bawang University, Lampung, Indonesia.
[2]Department of Biology, Faculty of Mathematics and Sciences, University of Lampung, Lampung, Indonesia.

***Corresponding Author Mohammad Kanedi**
Department of Biology, Faculty of Mathematics and Sciences, University of Lampung, Lampung, Indonesia.

Article Received on 18/11/2016 **Article Revised on 09/12/2016** **Article Accepted on 30/12/2016**

ABSTRACT
Because of its wide uses in many folk medicine system, the garden spurge *(Euphorbia hirta* L*)* is worth mentioning as a versatile herb plant. However, in relation to renal function, research results on the efficacy of these plants have not give a consistent result. This study, for that reason, aimed to reveal and verify the effect of ethanol extract of *E. hirta* against output of rat urine by using furosemide as a reference. Three different doses of whole plant extract of E. hirta (38.67, 77.35 and 154.7 mg/kg), furosemide (3.6 mg/kg) and distilled water (as negative control) were orally administered to the fasted healthy male rats. The urinary parameters assessed were the urine volume hourly for 6 hours, urine pH and urine color density. The results showed extract of *E hirta* at the dose of 77.35 mg/kg and 154.7 mg/kg significantly increase urine volume in comparison to the negative control but showed no difference with furosemide (α=0.05). However, all treatments showed no statistical difference in the urine pH and urine color scores. Thus, it can be conclude that the whole plant ethanolic extract of patikan kebo potentially for diuretic herb without make significant changes on pH and color of the urine output.

KEYWORDS: *Garden spurge, Patikan kebo, Euphorbia hirta, diuretic activity, urine pH, urine color.*

INTRODUCTION
Garden spurge (*Euphorbia hirta* L.), belongs to the family of Euphorbiaceae, which in Indonesia is called patikan kebo, deserved to be called as a versatile herb plant. In the Indonesian folk medicine system this plant used by households to increase lactation process, treat laryngitis, bronchitis, asthma, stomachache, diarrhea, dysentery, and some inflammatory illnesses.[1-2]

Based on some pharmacological studies, patikan kebo showed widepharmacological activities such as antifungal, antibacterial and larvicidal.[3] The antibacterial activity of this plant evident from the inhibitiion to growth of *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa*.These findings justify the traditional use of the plant in the treatment of sores, boils, wounds and control of dysentery and diarrhoea.[4]

Other studies reported that *E hirta* has diuretic activity. Whole plant methanolic extract of the plantsuggested to decrease urine output when compared to furosemide as a reference. On the contrary, extract of this plant has moderate and safe oral antidiuretic activity in comparison with vasopressin as reference.[5]

Different results were reported by Lingga et al.[6] By using ethanol as a solvent it was revealed that whole plant extract of *E. hirta* at the dose of 0,18 g/kg body weight showed a siginificant diuretic effects on male albino Wistar rats (Rattus norvegicus). Much earlier, it was reported that the aqueous and ethanolic extracts of *E hirta* could significantly induce diuresis in rats with a similar effect as acetazolamide.[7]

Given the results of the above studies have not give the consistent and exact description, due to the differences of types and levels of extract as well as the standard reference used, then the diueretic effects of *E. hirta* plant extract still need to be verified.

MATERIALS AND METHODS
Plant Material and Extraction
The fresh samples of whole plant of *Euphorbia hirta L.*) were collected from suburbs Bandar Lampung, and the botanical identification was done by plant taxonomist at the Laboratory of Botany, University of Lampung, Indonesia.

A 600g sample of fresh plant of *Euphorbia hirta* L. were extracted by maceration in 95% ethanol for 72 h and the solvent was replaced every 24 h. The final residue removed by filtration and the filtrate was concentrated to be a viscous extract using a rotary evaporator. The viscous extract then freez-dried to be a powder form. To

make the powdered extract is in effect then the ethanolic extract of *E hirta* was suspended in distilled water contains 0.5% CMC (Carboxy Methyl Cellulose).

Experimental Animals and Treatment

Twenty-five albino male Wistar rats (*Rattus norvegicus*) weighing 200-250g, aged 3-4months, obtained from Lampung Veteriary Office, Indonesia. Before treatment animals were allowed to acclimatize for a week, during which the rats placed in cages (one animal per cage) at room temperature, 12/12 hour light/dark cycle, and given water and food *ad libitum*. All the animal treatment procedure in accordance with the Ethical Research Committee, Faculty of Medicine, University of Lampung, Indonesia.

By using a completely randomized design, the animals were divided into five groups consisted of 5 rats. Group 1 received 1ml distilled water as a negative control; group 2 given 3.16 mg/kg body weight of furosemide as a positive control; group 3 treated with 38.67 mg/kg body weight of extract of *Euphorbia hirta*; group 4 fed with 77.35mg/kgof the extract, group 5 treated with 154.7mg/kg of the extract. The substances wereorally administered by gavage using a Sonde feeding needle. All rats treated twice during the day of experiment, one in the morning and one in the afternoon.

Study Parameters and Statistical Analysis

Immediately after the respective treatment the animals placed in hand made metabolic cages and urine was collected in a measuring cylinder containing mineral oil after 1st, 2nd, 3rd, 4th, 5th, and 6th hour. After 6 hours the total urine of each rat was assessed for volume, colors, and pH. The scale of urine colors were assessed qualitatively based on the color density as follows. Scores 1 for dark yellow; 2 for yellow; 3 for light yellow; and 4 for clear urine.

Both parametric and nonparametric statistic were used for data analysis. Parametric statistic, one way ANOVA and LSD test applied for dependent variables, volume and pH of the urine. While nonparametric statistics, Mann-Whitney Test, was applied for qualitative parameters of urine colors.

RESULTS AND DISCUSSION

The mean values of cumulative volume and pH of urine of experimental rats after 6 hours treatment along with the results of ANOVA and its post hoc test (LSD) are presented in Table 1. Based on the post hoc test results (LSD test) against the urine output tabulated in Table 1 it can be assumed that whole plant ethanolic extract of E hirta at the dose of 77.35 mg/kg and 154.7 mg/kg was significantly increase the volume of urine on the same level of furosemide (α=0.05). However, there is none of the treatment given showed a different effect on the urine pH.

Tabel 1 Cummulative volume and pH of urine of rats given distilled water, furosemide and whole plant extract of *Euphorbia hirta* L (EH) after 6 hours treatment.

Treatment	Volume (ml) (mean ± SD)	pH (mean ± SD)
Distilled Water	1.52 ± 0.550^{ab}	7.45 ± 0.268^{a}
Furosemide	3.38 ± 0.444^{cd}	7.41 ± 0.191^{a}
EH 38.67 mg/kg	1.34 ± 0.321^{a}	7.53 ± 0.311^{a}
EH 77.35 mg/kg	2.12 ± 0.349^{b}	7.59 ± 0.889^{a}
EH 154.7 mg/kg	2.98 ± 0.277^{c}	7.43 ± 0.965^{a}
F-value	24.8601	0.0712
P-value	0.0000	0.9900
F-crit	2.8661	2.8661
Values in the same column followed by the same superscript are not significantly different at α=0.05		

The urine color scales along with the result of nonparametric statistical analysis are presented in Tables 2.

Table 2 Urine color slaces and the Mann-Whitney Test results of rats given distilled water, furosemide and whole plant extract of *Euphorbia hirta* L (EH) after 6 hours treatment.

Treatment	N					Mean Rank
	1	2	3	4	5	
Distilled water	2	3	3	3	4	15.40^{a}
Furosemide	4	2	3	3	3	17.10^{a}
EH 38.67 mg/kg	2	2	2	2	3	9.20^{a}
EH 77.35 mg/kg	3	2	2	2	3	10.70^{a}
EH154.7 mg/kg	3	1	4	4	3	15.90^{a}
Color scales: 4=clear; 3=light yellow; 2=yellow; 1=dark yellow. Mean rank values that shared the same superscript not significantly different at α=0.05						

The actual depiction of the urine color scale presented in Table 2 above is based on the color densities urinated by experimental rats collected in the test tubes as shown in Fig.1.

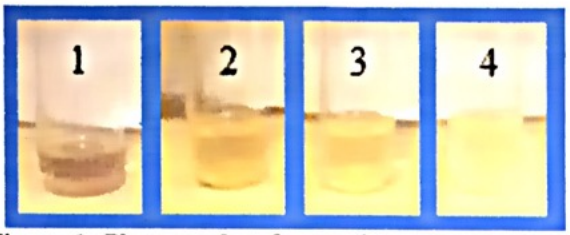

Figure 1. Photographs of rat urine scales depicting the difference of urine color densities collected in the test tubes (4=clear; 3=light yellow; 2=yellow; 1=dark yellow).

As a whole the findings of this study signifies that the whole plant extract of E. hirta is diuretic which by definition mean able to increase urine volume.[8] Herb plants that have proven to have exhibited a significant increase in indices of diuresis including urine volume, urination frequency, diuretic action, natriuretic and saliuretic indices and renal fractional excretion of electrolytes and metabolic acidosis generaly positive for containing saponins, tannins, flavonoids, phenols, anthraquinones, alkaloids, and deoxysugar.[9]

Another previous study done using *Flueggea leucopyrus* (Phyllanthaceae) has also indicated that aqueous extract of the plant increased urine output. Phytochemical screening showed that this plant extract contains alkaloids, triterpenoids, sterols, anthocyanins, tannins of pyrogallol type, and cyanogenic glycoside.[10]

Garden purge, in fact, phytochemically contains flavonoids such as euphorbianin, leucocyanidol, camphol, quercitrin and quercitol; polyphenols such as gallic acid, myricitrin, 3,4-di-O-galloylquinic acid,2,4,6-tri-O-galloyl-Dglucose; tannins such as euphorbins A, B, C, D, E; triterpenes and phytosterols such as β-amyrin, 24-methylenecycloartenol, and β-sitosterol; and alkanes which include heptacosane, n-nonacosane.[11]

The findings of this study showed no significant difference in the pH of urine between treated rats, however all of the data showed that the pH of the urine of the animals tend to be alkaline (pH>7). There were factors known to affectthe level of urine pH of a subject such as alcohol consumption[12] and acid-base load of the diet.[13] Perhaps, the active ingredient contained in the extract of garden purge has properties associated with the urine pH.

With regard to urine color, this study found that the plant extract of patikan kebo of all levels showed no significant effect on urine color scale (Table 2) either in comparison to negative control (distilled water) or the positive control (furosemide). Actually, the relationship between diet intake and urinary pigment has been reported long ago in 1927. Urine color, according to the report, is determined by some factors including fasting, acid and base treatment, calorigenic stimulation, and diuresis. Urinary pigment increase up to of 46.4% by fasting treatment, 34.6% by the administration of hydrochloric. Diueresis—theincrease of urine volume, almost invariably occurred simultaneously with increased pigment output.[14]

CONCLUSSION

In conclussion the whole plant ethanolic extract of patikan kebo (*Euphorbia hirta* L.) potentially for diuretic herb without make significant changes on pH and color of the urine output.

ACKNOWLEDGEMENT

The authors are grateful profusely to Vicky Yugasworo for his support in laboratory work for this project.

REFERENCES

1. Mihardja L., Adimunca C., Widowati L., Raflizar, Pujiastuti', Winarno, Wahjoedi B. 2001. Manfaat Ekstrak Etanol Patikan Kebo *(Euphorbu Hirta* L.) sebagai Laktagogum pada Tikus Putih yang Menyusui (Lactogogum Effect of the Ethanolzc Extract of Patikan Kebo (*Euphorbia Hirta* L.) on Lactation Process of Mother Rats).Bul. Penelit. Kesehat, 2001; 29(3): 118-125.
2. Nafisah M, Tukiran, Suyatno, dan Hidayati N.2014. Uji Skrining Fitokimia Pada Ekstrak Heksan, Kloroform danMetanol dari Tanaman Patikan Kebo (*Euphorbiae hirta*). (Phytochemical Screening Test on Hexane, Chloroform And Methanol Extracts of Patikan Kebo (Euphorbiae Hirtae). Prosiding Seminar Nasional Kimia, ISBN : 978-602-0951-00-3 Jurusan Kimia FMIPA Universitas Negeri Surabaya, 20 September 2014.
3. Gayathri A.and Ramesh K.V., 2013. Antifungal activity of Euphorbia hirta L. inflorescence extract against*Aspergillus flavus* A mode of action study.Int.J.Curr.Microbiol.App.Sci., 2013; 2(4): 31-37.
4. Ogbulie J. N., Ogueke C. C., Okoli I. C. and Anyanwu B. N. 2007. Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*. African Journal of Biotechnology., 2001; (13): 1544-1548.
5. Vijayabhaskar K.,, Prasad A.S., Uddin M.T., Vamshi M., Gopal G.R. and Ravi B. Evaluation of Diuretic Activity on Whole Plant Methanolic Extract of *Euphorbia Hirta* in Rats with Comparison of Furosemide, Vasopressin (Antidiuretic Hormone). World Journal of Pharmacy and Pharmaceutical Sciences, 2016; 5(5): 1337-1346.
6. Lingga I.S., Citraningtyas G. dan Lolo W.S.2014. Uji Efek Ekstrak Etanol Patikan Kebo (*Euphorbia Hirta* Linn.) Sebagai Diuretik pada Tikus Putih Jantan Galur Wistar (*Rattus norvegicus* sp.) PHARMACONJurnal Ilmiah Farmasi – UNSRAT, Agustus, 2014; 3(3): 287-293.

7. Huang L., Chen S. and Yang M. *Euphorbia hirta* (Feiyangcao): A review on its ethnopharmacology, phytochemistry and pharmacology. Journal of Medicinal Plants Research., 2012; 6(39): 5176-5185, 10 October, 2012.
8. Johnson PB, Abdurahman EM, Tiam EA, Abdu-Aguye I, Hussaini IM. Euphorbia hirta leaf extracts increase urine output and electrolytes in rats. Journal of Ethnopharmacology, 1999; 65: 63-69.
9. Ekpenyong C., Daniel N. and Akpan E. 2014. Phytoconstituents and diuretic activity of Cymbopogon citratus leaf infusions in humans. Journal of Coastal Life Medicine, 2014; 2(9): 704-713.
10. Ellepola N.U., Deraniyagala S.A., Ratnasooriya, W.D. and Perera K. 2015. Aqueous Extract of *Flueggea leucopyrus* Increases UrineOutput in Rats. Tropical Journal of Pharmaceutical Research January, 2015; 14(1): 95-101.
11. Patil S.B., Naikwade N.S. and Magdum C.S. Review on Phytochemistry and Pharmacological Aspects of Euphorbia Hirta Linn. JPRHC, July 2009; 1(1): 113-133.
12. Eggleton M.G.1946. Urine Acidity in Alcohol Diuresis in Man. J. Physiol, I946; I04: 3I2-320.
13. Welch A.A. 2008. Dipstick Measurements of Urinary pH have Potential for Monitoring Individual and Population Dietary BehaviorsThe Open Nutrition Journal, 2008; 2: 63-67.
14. Drabkin D.L. 1927. The Normal Pigment Of The Urine. I. The Relationship Of Urinary Pigment Output To Diet And Metabolism. J. Biol. Chem., 1927; 75: 443-479. http://www.jbc.org/content/75/2/443.full.pdf.