Journal of Ayurvedic and Herbal Medicine 2016; 2(3): 78-81

Research Article

J. Ayu. Herb. Med.
2016; 2(3): 78-81
May- June

www. ayurvedjournal.com

Blood chemistry and electrolyte analysis in *Mus musculus* treated with *Euphorbia hirta* Linn extracts

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ABSTRACT

Euphorbia hirta's effects on the blood chemistry in albino mice (*Mus musculus*) had been investigated in this study. There were 15 mice per gender (10 experimental, 5 control). 0.5 mL of the 1:10 g:mL decoction was administered after 40 days of acclimatization. Approximately 2 hours after the oral administration of the extract, 0.5 mL of blood was obtained through intracardiac extraction. Laboratory tests showed that in males, only K showed significant decrease and none in females (p=0.001). Analyses on the degree variation between male and female, significant changes were observed to have occurred in their levels of creatinine, blood urea nitrogen, and uric acid (p=0.048, 0.035, 0.006, respectively). Highest electrolyte decrease was male's K (26.5%), and female's UA for blood chemistry (30.1%), while highest increase in males' Na and females' BUN was observed (7.9% and 25.5%, respectively). It is then hypothesized that hormones may play in the blood chemistry and not much in the electrolytes. Further, it could also be that the extracted molecules do not react on individual molecules, rather than more complicated molecules such as those in the blood chemistry. Using other extracting method and longer periods of extract administration can be considered for further investigation.

Keywords: Blood chemistry, Electrolytes, *Euphorbia hirta*.

INTRODUCTION

The use of herbal plants can be considered as an indispensable practice of society. This ancient practice of the pharmaceutical science has led to major biomolecular discoveries that helped shaped the medical world. Yet, though ancient it may seem, herbal medicine had a difficult time finding its way to mainstream medicine since many claimed that the traditional preparation of plant extracts contained high levels of toxins. Such is the case of the herbal plant *Euphorbia hirta.*

Originally, *E. hirta* is native to Central America and has since spread throughout the tropical and subtropical countries such as China, Australia, Malaysia, and the Philippines [1]. A member of the Euphorbiaceae family, the herb is thought to possess several pharmacological attributes traditionally used against various infections, diseases in respiration and even tumors [2]. Its active components includes flavonoids, triterpenes, alkanes, phytosterols, polyphenols, and tannins.

The plant's extracts, usually prepared as decoction, is the most common practice among the locals in treating some infections and diseases. One such infection is dengue. In the Philippines, dengue is fast becoming one of the primary infections since it affects thousands and mortality is by the dozens each year. Since dengue's first epidemic in the 1920s [3], indigenous knowledge to combat such infection considered *E. hirta*'s extracts to increase platelet level in dengue patients.

Though the plant has long been considered a medicinal herb, there are traces of toxins that can be found in its extracts. Furthermore, studies on its side effects of the extracts have been poorly studied [4]. Few of the side effects of the extracts include reduction in the tubular diameter of the seminiferous tubules in rats, potent molluscicidal, alteration of total protein, amino acids and nucleic acid levels (DNA and RNA) [5,6].

How the plant's biocomponents alters the blood chemistry in albino mice is poorly studied. Its efficiency in increasing platelets is hypothesized to also affect the levels of chemical components of blood such as blood urea nitrogen (BUN), creatinine, serum glutamic pyruvic transmitase (SGPT), uric acid and electrolytes. Further, this study investigates possible hormonal influences on the said tests as it compares results between male and female mice.

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MATERIALS AND METHODS

Plant and Plant Collection

Taxonomic verification of the plant used a summarized description by Kumar *et al* [2]. The plant is under the family *Euphorbiaceae.* It can grow as high as 40 cm with leaves oppositely arranged in its slender stem. Leaf shape may vary from elliptic-oblong or oblong-lanceolate and its edges are toothed. It has yellow fruits, and its hairy, three-celled and keeled capsules (diameter of 1-2 mm), which contained three wrinkled seeds that are angular and four-sided. Figure 1 shows a digital image of the collected plant.

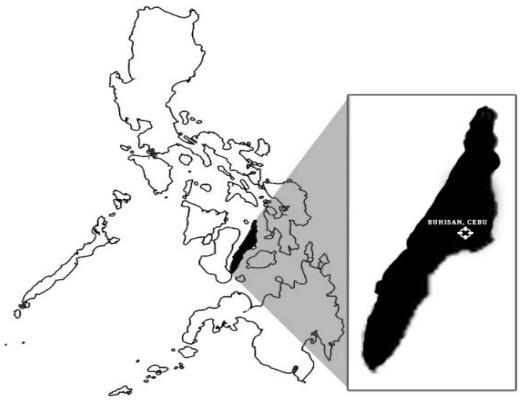

Figure 1: Geographic location of the sampling site

Buhisan, a mountainous part of Cebu City, Philippines, was the site where approximately 200 grams of *E. hirta* was collected (coordinates 10° 19' 18" North, 123° 50' 18" East) (Fig. 1). Using surgical gloves, the plants were hand-picked and then were placed in a tightly-sealed plastic container and were transported to the laboratory where it was thoroughly rinsed with water.

Test Organism

30 adult white mice (*Mus musculus)* served as the test organisms and were divided into groups. The experimental group consisted of 10 males and 10 females, while the control group consisted of 5 males and 5 females. Every mice group were housed in the same containers with ventilation. Mixed feeds and tap water were provided equally among the groups. Constant monitoring had been observed. The mice were acclimatized for 40 days before blood extraction.

Extract Preparation and Administration

100 g of the collected plant was thoroughly rinsed with triple distilled water. The decoction was prepared with a ratio of 100 g:1000 ml plant:triple distilled water. It was then boiled for about 15 minutes. The extract was then transferred to a dark-colored container.

Using gavage, 0.5 mL of the extract was administered in every mouse of the experimental groups. For the control group, 0.5 mL of triple distilled water had been administered.

Blood Extraction

The entire blood extraction process is in accordance with the recommendation of the Institutional Animal Care and Use Committee (IACUC). Diethyl ether was used to sedate the mice where approximately 2-3 drops of the said solvent was placed in a cotton ball. Using BD fine 1mL syringe, blood was directly extracted from heart. 0.5 mL of the extracted blood has been immediately transferred to a vial and were placed in a sealed container with ice.

Analyses

Blood samples were brought to a veterinary clinical laboratory for blood chemistry analyses. Using Arkray Automated analyzer for clinical chemistry (SPOTCHEM EZ SP-4430), levels of different blood chemistry contents such as blood urea nitrogen (BUN), creatinine, serum glutamic pyruvic transmitase (SGPT), uric acid (UA) and blood electrolytes (K, Na, Cl) were analyzed.

Laboratory results were statistically treated using the T-test.

RESULTS

In males, decrease in values were observed in levels of potassium (K), chlorine (Cl), serum glutamic pyruvic transmitase (SGPT), blood urea nitrogen (BUN) and uric acid (UA). However, an increase in sodium (Na) and no level changes for creatinine (Crea). In the other hand, only K, Cl and SGPT showed decrease in values while increasing values in Na, creatinine, BUN and UA has been observed.

Though varying contents were observed among the groups, some of the changes are minimal such as the case of the blood creatinine in both male (same) and female (increase of 0.07 mg/dl), and the male's BUN (decrease of 0.2 mg/dl).

Highest increase (in percentage) in the UA levels in male (30.1) followed by female's BUN (25.5). On the other hand, highest decrease has been observed in males' K level and females' SGPT (26.5 and 20.2, respectively). Table 1 shows the full average values of blood electrolytes and chemistry variables.

Table 1: Comparative average electrolytes and blood chemistry contents between male and female albino mice

	Electrolytes (mmol/L)			Blood Chemistry			
	Na	K	Cl	SGPT (IU/L)	Crea (mg/dl)	BUN (mg/dl)	UA (mg/dl)
Experimental Male	153	10.03	131.8	57.9	0.54	17.6	4.22
Control Male	141.8	13.64	129	58.94	0.54	17.8	6.04
Impression	Increase	Decrease	Increase	Decrease	Same	Decrease	Decrease
Percentage	7.9	26.5	2.2	1.8	-	1.1	30.1
Experimental Female	147.6	11.28	124.8	71.3	0.77	15.56	7.62
Control Female	144.2	12.6	139.6	89.4	0.7	12.4	6.72
Impression	Increase	Decrease	Decrease	Decrease	Increase	Increase	Increase
Percentage	2.4	10.5	10.6	20.2	10	25.5	13.4

Table 2 presents the statistical difference between the experimental and control mice groups in terms of the effects of the extract on its electrolytes and factors in blood chemistry. Statistically, there is no significant difference among the levels of SGPT, creatinine, blood urea nitrogen and uric acid. However, in the blood chemistry, uric acid showed the most activity since it is close to the acceptable level of significance (p=0.058)

In the levels of the electrolytes, potassium levels vary significantly (p=0.001) between the experimental and the control groups, while other electrolytes, sodium and chlorine, do not exhibit statistical significance between the two groups.

Table 2: T-Test Results on the Differences of the Chemical Constituents and electrolytes between Experimental Male and Control Male

Variables	Experimental Male (n=10)	Control Male (n=5)	t-value (df)	p-value
	Mean ± SD	*Mean ± SD*		
Electrolytes				
Na	153.0±13.7	141.8±4.1	1.76^{ns}(13)	0.102
K	10.0±1.7	13.6±1.5	-4.03***(13)	0.001
Cl	131.8±18.7	129.0±7.1	0.32^{ns}(13)	0.755
Chemical Constituents				
SGPT	57.9±14.7	58.9±33.6	-0.09^{ns}(13)	0.933
Crea	0.54±0.26	0.54±0.18	0.00^{ns}(13)	1.000
BUN	17.6±3.6	17.8±4.1	-0.10^{ns}(13)	0.924
UA	4.2±0.9	6.0±2.5	-2.08^{ns}(13)	0.058

*Note: ns-not significant (P>0.05) ***-significant (P≤0.001)*

In the female group, there have been no significant difference both in its electrolytes and its blood chemistry factors (Table 3).

In Table 4, the results between the female and male groups have been statistically analyzed for a possible gender difference in its reaction to the extracts. Here it is shown that there is no difference in the electrolytes level between the male and female. However, creatinine, BUN and UA all showed statistical difference (p=0.048, 0.035, 0.006, respectively). Meanwhile, there is no significant difference in its SGPT (p=0.188).

Table 3: T-Test Results on the Differences of the Chemical Constituents and electrolytes between Experimental Female and Control Female

Variables	Experimental Female (n=10)	Control Female (n=5)	t-value (df)	p-value
	Mean ± SD	*Mean ± SD*		
Electrolytes				
Na	147.6±4.8	144.2±3.8	1.36^{ns}(13)	0.196
K	11.3±1.3	12.6±2.0	-1.54^{ns}(13)	0.148
Cl	124.8±4.5	139.6±33.5	-1.43^{ns}(13)	0.178
Chemical Constituents				
SGPT	71.3±41.1	89.4±69.5	-0.64^{ns}(13)	0.533
Crea	0.77±0.3	0.70±0.3	0.40^{ns}(13)	0.697
BUN	15.6±4.8	12.4±0.5	1.43^{ns}(13)	0.175
UA	7.6±2.8	6.7±2.7	0.60^{ns}(13)	0.561

Note: ns-not significant (P>0.05)

Table 4: T-Test Results on the Differences of the Chemical Constituents and electrolytes between Male and Female Mice

Variables	Male (n=15)	Female (n=15)	t-value (df)	p-value
	Mean ± SD	*Mean ± SD*		
Electrolytes				
Na	149.3±12.5	146.5±4.7	0.81^{ns}(28)	0.423
K	11.2±2.4	11.7±1.6	-0.66^{ns}(28)	0.518
Cl	130.9±15.5	129.7±19.6	0.18^{ns}(28)	0.862
Chemical Constituents				
SGPT	58.2±21.5	77.3±50.4	-1.35^{ns}(28)	0.188
Crea	0.54±0.23	0.75±0.31	-2.07*(28)	0.048
BUN	17.7±3.6	14.5±4.2	2.22*(28)	0.035
UA	4.8±1.8	7.3±2.7	-2.99**(28)	0.006

*Note: ns-not significant (P>0.05) *-significant (P≤0.05) **-significant (P≤0.01)*

DISCUSSION

The varying effects of the extracts on the levels of electrolytes and blood chemistry contents has been observed in this study. Some of the possible factors that influence this variations can be traced to its molecular-physiological response. It can be that the extracts affect differently in the individual molecules (say, polarity) that causes these levels to increase, decrease or maintain its normal range. Synergistic activities are also plausible.

In males, potassium showed the most significant activity (p=0.001), where in this case a decrease has been observed. On the other hand, no significant activities were detected in females. Due to the insignificant effects of the extracts to the electrolytes (except for K in males), it can then be that individual molecules may not interact with the extracted water-soluble compounds of the plant.

Another is hormonal, where the extracts is hypothesized to alter the hormone levels that is responsible for the activities of the levels of the said chemical constituents. Though electrolytes level between the male

and female groups is statistically similar, significant differences among the factors in its blood chemistry has been observed, specifically creatinine, BUN and UA. This result strongly suggest that hormones play a vital role in the production, formation and decrease of the said chemicals as per observed by several studies [7-11]. Table 1 further suggest that more increase in males' electrolyte levels than females' could be hormonally-influenced, so with an opposing trend in the mice's blood chemistry levels. The studies of Bharti *et al* [12], Kjeld [13], Luckhoff and Horster [14], and Owiredu *et al* [15] all suggested that electrolytes are strongly influenced by hormones.

With the current trend of pharmaceutical productions shifting from synthetic medicine to medicine sourced from nature, it is important that every aspect of its potential therapeutic effects shall be evaluated, especially its toxicity and other biochemical effects. As mentioned earlier, the decoction of *E. hirta* is thought to be a cure of the dengue diseases (dengue fever, dengue hemorrhagic fever and dengue shock syndrome) as it increases the platelet count of the patients [16].

Modern techniques of medicine production already considered specific drug designs tailored for individual needs. If pharmaceutical industries will consider this herb in its product line, it is best that toxicity and other physiological effects be rigorously considered.

CONCLUSION

The study concluded that levels of potassium in males are affected by the extracted water-soluble compounds of *E. hirta* and none in females. Blood chemistry analyses also showed that both gender do not exhibit significant increase in its levels of creatinine, blood urea nitrogen, serum glutamic pyruvic transmitase and uric acid. However, if these results are compared between male and female, significant changes of levels of creatinine, SGPT and UA were observed suggesting that hormonal influences may cause this difference.

Recommendations

Using other methods of compound extractions (methanolic, ethanolic, super critical fluid extractions, etc) are strongly recommended by this study to evaluate whether different solvents affect differently on the mice's levels of blood chemistry and electrolytes. Furthermore, longer exposure to the extracts is also suggested for deeper investigation.

Acknowledgement

The researcher would like to thank the Cebu Normal University Research Council for the research grant and to Dr. Jasmin Burgos and Edgar Tumapa from the Institutional Animal Care and Use Committee of Cebu Doctor's University for the guidelines and supervision of the experiment.

REFERENCES

1. Lanhers MC, Fleurentin J, Cabalion P. Behavioral effects of *Euphorbia hirta* L.: sedative and anxiolytic properties. J. Ethnopharmacol. 1990. 29:189-198.
2. Kumar, S, Malhotra R and Kumar D. Euphorbia hirta: its chemistry, traditional and medicinal uses and pharmacological activities. Pharmacogn Rev. 2010 Jan-Jun; 4(7): 58–61.
3. Ooi, EE, Gubler DJ. Dengue in Southeast Asia: epidemiological characteristics and strategic challenges in disease prevention. Cad Saude Publica. 2009; 25 Suppl 1():S115-24.
4. Huang, L, Chen S, Yang M. *Euphorbia hirta* (Feiyangcao): A review on its ethnopharmacology, phytochemistry and pharmacology. Journal of Medicinal Plants Research. 2012. Vol 6(39):5176-5185.
5. Adedapo, AA, Abatan MO, Akinloye AK, Idowu SO, Olorunsogo OO. Morphaometric and histopathological studies on the effects of some chromatographic fractions of *phyllanthus amarus* and *Euphorbia hirta* on the male reproductive organs of rats. J. Vet. Sci. 2003. 4(2):181-185.
6. Sunil KS, Ram P, Yadav ST, Ajay S. Toxic effect of stem bark and leaf of *Euphorbia hirta* plant against freshwater vector snail Lymnaea acuminate. Chemosphere. 2005. 59:263-270.
7. Ranabir, S, Reetu K. Stress and hormones. Indian Journal of Endocrinology and metabolism. 2011. Jan-Mar; 15(1):18-22.
8. Rahimi, R, Qaderi M, Faraji H, Boroujerdi SS. Effects of very short rest periods on hormonal responses to resistance exercise in men. J Strength Cond Res. 2010. 24(7):1851-9.
9. Afzali, S, Taheri SK, Torabian S, Jamilian M. Changes in female sex hormones in patients with intentional drug and chemical poisoning. Iranian Journal of Toxicology. 2013. Vol 7, No. 20.
10. Reckellhoff, JF. Gender differences in the regulation of blood pressure. Hypertension. 2001. 37:1199-1208.
11. Iddah, MA, Macharia BN, Ng'wena AG, Keter A, Ofulla AVO. Thyroid hormones and hematological indices levels in thyroid disorders patients at Moi Teaching and Referral Hospital, Western Kenya. ISRN Endocrinology. 2013. Volume 13.
12. Bharti, A, Shrestha S, Rai R, Singh MK. Assessment of serum minerals and electrolytes in thyroid patients. International Journal of Advances in Scientific Research. 2015. 1(06):259-263.
13. Kjeld M. Concentrations of electrolytes, hormones, and other constituents in fresh postmortem blood and urine of fin whales (Balaenoptera physalus). Canadian Journal of Zoology. 2001. 79(3):438-446.
14. Kjeld M. Concentrations of electrolytes, hormones, and other constituents in fresh postmortem blood and urine of fin whales (Balaenoptera physalus). Canadian Journal of Zoology. 2001. 79(3):438-446.
15. Owiredu RKD Ephraim, Eghan BA Jnr, Amidu N, Laing EF. Relationship between parathyroid hormone and electrolytes in chronic kidney disease. Journal of Medical Research. 2012. Vol 1(8). Pp.0103-0111.
16. Arollado, EC, Pena IG, Dahilig VRA. Platelet augmentation activity of selected Philippine Plants. International Journal of Pharmaceutical and Phytopharmacological Research. 2013. 3(2):121-123.

HOW TO CITE THIS ARTICLE

Opena ELL. Blood chemistry and electrolyte analysis in *Mus musculus* treated with *Euphorbia hirta* Linn extracts. J Ayu Herb Med 2016;2(3):78-81.