新引進藥用植物VUKA (*Mondia whitei* L.)對小鼠餵飼之安全性評估[1]

郭肇凱[2]、張隆仁[2]、陳榮五[2]、廖俊旺[3]

摘　要

爲探討VUKA (*Mondia whitei* L.)使用之安全性，以水萃凍乾樣品對小鼠(ICR品系)進行口服急毒性試驗，測試劑量爲15 g/kg body weight，投予後連續觀察14天。試驗期間全部鼠隻均無死亡，顯示VUKA水萃凍乾樣品對小鼠口服急毒性之半致死劑量(LD_{50})每公斤大於15 g，且處理組之腦、心、肝、腎、脾及胸腺等重要臟器，均無因試驗物質引起肉眼及組織病理變化；血液學檢測中之白血球數、紅血球數、血紅素、血球容積比、平均紅血球體積、平均血紅素、平均血紅素濃度及血小板等相較於對照組均無影響(p>0.05)；血清生化檢測中之麩氨酸氨基轉氨酶、麩氨酸丙酸轉氨酶與肌氨酸等相較於對照組亦沒有影響(p>0.05)。

關鍵字：*Mondia whitei*、急性毒性、半致死劑量(LD_{50})。

前　言

VUKA在南非恩古尼語(Nguni)的英文解釋是“wake up, come alive”，爲蘿藦科(Asclepiadaceae)具有塊莖狀之木質藤本植物，學名爲*Mondia whitei* L. (Hook. f.) Skeels。VUKA是一種廣泛分布在東非與南非中部地區的特有植物，其根莖部所具有的特殊香氣物質特別受到青睞，南非的祖魯人(Zulu)早在19世紀前就已經大量使用作爲香辛料調味[4]，在傳統醫藥方面上，可以減緩腸胃氣脹、腹痛、便秘與頭痛等症候，並可以治療泌尿道的感染與腹泄徵狀[2]。此外，其根部更是常被當地男性服用以提升性慾及治療性功能障礙[11,13]，近來研究發現以VUKA根部水萃物餵食雄性大鼠(rat)，對於雄性荷爾蒙具有促進調控的效果[14]。目前VUKA中所具的活性成份物質可能與一些新的配醣體(easter and phenolic glucosides)、生物鹼(indole and carbazole alkaloids)或是甲氧基安息香醛(2-hydroxy-4-methoxybenzaldehyde)等化合物的存在有關[5,6,7,9,10]，但是詳細的作用機制尚不清楚。臺中區農業改良場於2002年始自南非引進栽培，迄今已經順利馴化適應臺灣的氣候環境並成功開花結果(圖一)，VUKA的外觀型態爲葉對生呈心型，葉柄長約2 cm，花瓣黃色呈五瓣星型，每年約6~10月開花，具四瓣相互對稱之蓇葖果未熟時呈綠色，完熟的果實呈褐色約10 cm長，整株具有特殊香氣。

---

[1] 臺中區農業改良場研究報告第 0643 號。

[2] 臺中區農業改良場國防訓儲研究助理、副研究員、場長。

[3] 國立中興大學獸醫病理學研究所助理教授。

圖一、VUKA 植株之葉片、花序與幼果。
Fig. 1. The leaf, inflorescence and immature fruit of VUKA.

材料與方法

一、樣品製備

於連續一週以上晴天挖取兩年生以上之VUKA地下部，洗淨後剪切成小段於40℃烘箱中連續乾燥約60 hr，(乾燥後約爲鮮重50%，含水率約爲11%)，以70℃的一次蒸餾水(1:10, w/v)浸泡8 hr後，再煮沸2 hr以獲得初萃液，過濾收集濾液，並將殘留物再以一次蒸餾水(1:6, w/v)煮沸2 hr後過濾，收集並混合所有濾液，於55℃減壓濃縮至固形物約10% (w/v)，零下80℃冷凍後，以真空冷凍乾燥法獲得VUKA地下部水萃冷凍乾燥粉劑。

二、實驗動物

4週齡小鼠(ICR品系)，購自樂斯科生技園區實驗動物培育及研發中心(宜蘭，臺灣)，動物房溫度爲20~22℃及光照12 hr/黑暗12 hr之光照週期。以鼠專用粒狀飼料(LabDiet® 5001 Rodent diet, Purina Mills LLC, St. Louis, MO, USA)及逆滲透水採自由進食供應，經1週適應期後進行試驗，實驗動物之使用操作均依照中華實驗動物學會之「實驗動物管理與使用指南」規範進行[15]。

三、試驗步驟

試驗依據衛生署(口服急毒性試驗)[1]、美國環保署(USEPA) (Health Effects Test Guidelines; Acute Oral Toxicity; Harmonized Test Guidelines)[3]與經濟合作暨開發組織(OECD Guidelines for Testing of Chemicals; Acute Oral Toxicity-Acute Toxic Class Method)[12]等試驗規範進行口服急毒性試驗。試驗分爲15 g/kg body weight 劑量組及餵食蒸餾水之對照組，每組20隻小鼠(雌雄各半)，並以飽和苦味酸染劑於背部作編號標識。

將VUKA冷凍乾燥粉劑以蒸餾水配製濃度爲0.75 g/ml (pH值約爲5.7)，以不鏽鋼胃管依體重經口餵食每隻灌食劑量與體積量比例之上限爲10 ml/kg，間隔4 hr餵食2次，最終劑量爲15

g/kg body weight (BW)，處理後每日觀察並每週秤體重1次，至處理後第14天為止。試驗結束後，以2%異氟烷(isoflurane)麻醉後經眼窩血竇放血後犧牲進行大體解剖，並檢查體內臟器之肉眼及組織病理變化。

四、血液學檢測

將自眼窩血竇採集之全血放入含EDTA抗凝血劑試管(K3 EDTA syringes, Vacutainer, NJ, USA)，於血球計數儀(Sysmex K-4500, Toa Medical Electronics Co., Ltd., Kobe, Japan)檢測血液相 (complete blood count, CBC)，包括白血球數(white blood cell count, WBC count)、紅血球數(red blood cell count, RBC count)、血紅素(hemoglobin, HGB)、血球容積比(hematocrit, HCT)、平均紅血球體積(mean corpuscular, MCV)、平均血紅素(mean corpuscular hemoglobin, MCH)、平均血紅素濃度(mean corpuscular hemoglobin concentration, MCHC)及血小板(platelet, PLT)等項目。

五、血清生化檢測

將自眼窩血竇採集之全血以775 xg離心15 min後取上清液血清(serum)，以血清生化儀(Chiron Diagnostics Corporation, Oberlin, OH, USA)檢測肝腎血清酵素值，包括麩氨酸氨基轉氨酶(aspartate aminotransferase, AST)、麩氨酸丙酸轉氨酶(alanine aminotransferase, ALT)、尿素氮(blood urea nitrogen, BUN)、肌氨酸(creatinine)等項目。

六、臟器病理檢測

以2%異氟烷麻醉小鼠後經放血及解剖，秤腦、心、肝、腎、脾及胸腺等臟器重量(g)，並以最後一週之最終體重(g)，作為體內臟器重量比率(%)之計算。觀察肉眼病理變化，將臟器浸泡於10%中性福馬林溶液中固定1週，經組織粗修與石蠟包埋後，以石蠟組織切片機(Leica RM 2145, Nussloch, Germany)製成2 μm之切片，經Hematoxylin & Eosin (H&E)染色後，以光學顯微鏡觀察各切片之組織病理變化。

七、結果分析

試驗期間各組之體重變化，依體重(g)或增重(g或%)變化，以統計分析軟體Microsoft Excel進行Pair Student's *t*-test，或以單向變方分析法(One-way ANOVA)之Ducan's test或Least Significant Difference test (LSD，最顯著差異)進行組間比較分析，其組間顯著差異水準為$p<0.05$。試驗結束後，若處理組動物死亡數超過處理動物數半數(50%)，則統計方式求取劑量與死亡率之迴歸方程式，計算動物半致死劑量(LD_{50}值)及其95%可信賴區間(confidence limit)。

結果與討論

ICR小鼠之口服急毒性試驗以15 g/kg body weight最高毒性測試劑量濃度，試驗將VUKA冷凍乾燥粉劑以蒸餾水配製最高濃度為0.75 g/ml，以不鏽鋼胃管依體重經口餵食每隻灌食劑量與體積量比例之上限為10 ml/kg，間隔4 hr餵食2次，投予後連續觀察14天。結果顯示，灌食15 g/kg之VUKA處理組雌雄鼠第0~3天出現精神萎靡、毛髮粗糙、肛門及尾部可見淡褐色黏稠下痢便(diarrhea)，第4天後逐漸恢復正常，試驗期間全部試驗鼠隻均無死亡(表一)，顯示對

小鼠之口服急毒性LD_{50}值大於15 g/kg BW。另外如表二所示，VUKA處理組之雌鼠在第7天以及雄鼠在第14天時，相較於對照組之體重增加有顯著差異(p<0.05)。

表一、灌食 VUKA 水萃凍乾回溶劑對小鼠口服急毒性試驗之臨床症狀及死亡率觀察

Table 1. Clinical signs and time course of death of mice after gavaged with VUKA extracts in acute oral toxicity test

Sex/Dose (g/kg)	Animal No.	Clinical sign	Day after treatment								Mortality [1] (%)
			1	2	3	4	5	6	7	14	
Male											
0	10	Normal	10 [2]	10	10	10	10	10	10	10	0
15	10	Diarrhea [3]	10	10	10	-	-	-	-	-	0
		Normal	-	-	-	10	10	10	10	10	0
Female											
0	10	Normal	10	10	10	10	10	10	10	10	0
15	10	Diarrhea	10	10	10	-	-	-	-	-	0
		Normal	-	-	-	10	10	10	10	10	0

[1] Mortality (%)=Dead No./Treated No.)×100.
[2] Number of mice effected.
[3] Treated mice showed slight diarrhea after gavaged with test substance and then recovered gradually post 3 days treatment.

表二、灌食 VUKA 水萃凍乾回溶劑對小鼠口服急毒性試驗之體重及增重變化

Table 2. Changes of body weight and weight gain of mice after gavaged with VUKA extracts at 0, 7, and 14 days in acute oral toxicity test

Dose (g/kg)	Animal No.	Body weight (g)/gain (g)		
		0-day	7-day [1]	14-day [2]
Male				
0	10	24.2±1.1 [3]	32.3±1.9 (8.1±1.2)	33.8±2.2 (9.6±1.5)
15	10	23.8±1.1	32.0±1.5 (8.2±0.6)	31.7±1.7 (8.0±1.2)
Female				
0	10	19.6±1.7	25.7±2.1 (6.1±0.7)	27.9±2.9 (8.3±1.5)
15	10	19.1±1.0	24.5±1.3 (5.4±0.8) [4]	27.0±1.0 (7.8±1.0)

[1] Body weight (BW) gain on the 7th day (g)=(7th-oth) BW (g).
[2] Body weight (BW) gain on the 14th day (g)=(14th-oth) BW (g).
[3] Body weight (g) /gain (%) are expressed as the mean±SD (n=10).
[4] Significant difference between the control and treated groups at p<0.05.

試驗結束後，檢查VUKA處理組之血液相，結果顯示白血紅素(WBC)、紅血球數(RBC)、血紅素(HGB)、血球容積比(HCT)、平均紅血球體積(MCV)、平均血紅素(MCH)、平均血紅素濃度(MCHC)及血小板(PLT)等檢測項目相較於對照組之數值均介於正常值範圍內(p>0.05)(表三)，表示在血液學上不會造成顯著的變化。檢查肝腎血清酵素值結果顯示，麩氨酸氨基轉氨酶(AST)、麩氨酸丙酸轉氨酶(ALT)與肌氨酸(creatinine)等項目均正常無影響(p>0.05)(表四)，表示不會造成代謝物的累積現象，惟處理組雄鼠之尿素氮(BUN)值與對照組相較有顯著上升

(p<0.05)。由於尿素氮爲蛋白質代謝後的最終廢物並由腎臟所排泄，因此尿素氮值與蛋白質攝取量成正比而與其排泄速度成反比，所以腎臟機能若是良好，則血清中尿素氮值就會降低，但是整體來說本試驗尿素氮值結果仍在正常生理背景值範圍內[8]。另外檢查體內臟器重量結果顯示，對照組與處理組之腦、心、肝、腎臟及胸腺重量均與對照組無明顯差異，惟處理組雌鼠脾臟重量比對照組有顯著性增加(p<0.05)(表五)。所有鼠隻經解剖體內檢查臟器病變，結果顯示對照組與處理組之腦、心、肝、腎、脾及胸腺等重要臟器均無明顯肉眼病理變化(圖二)，各重要臟器切片結果亦無明顯病理組織變化(圖三)，顯示VUKA水萃凍乾粉劑對於體內重要器官不會造成遲發性之毒性反應。綜合以上試驗結果，VUKA水萃凍乾粉劑對小鼠口服急毒性之半致死劑量(LD_{50})每公斤大於15公克，相較於健康食品安全評估方法之單一劑量急毒性試驗，其給予測試最大劑量爲5公克試驗動物/公斤動物體重高出了許多，所以VUKA水萃凍乾粉劑在安全評估分類標準應是屬於無毒害或是低毒害之物質，若再考量小鼠體表面積折算人體的等效劑量以及濃縮萃取倍率(約10倍)來計算，對於人體服食後所產生的口服急毒性危害初步評估應是安全無虞的。

表三、灌食 VUKA 水萃凍乾回溶劑對小鼠口服急毒性試驗之血液學變化

Table 3. Hematological parameters of mice after gavaged with VUKA extracts in acute oral toxicity test

Dose (g/kg)	WBC (10^3/μl)[1]	RBC (10^6/μl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^3/μl)
Male								
0	1.8±1.0[2]	11.6±2.4	15.8±3.0	60.6±12.3	52.5±1.5	13.8±1.0	26.2±1.8	1509.3±473.0
15	2.2±1.5	10.3±1.6	15.3±0.8	56.2±9.1	54.3±2.0	15.0±2.0	26.5±4.4	1375.4±188.0
Female								
0	3.5±1.5	10.5±1.5	15.3±1.1	54.1±8.1	51.3±1.8	14.6±1.1	28.5±2.2	1325.7±521.5
15	2.4±0.7	12.4±3.0	15.3±0.5	65.5±16.0	52.7±1.9	12.8±2.4	24.4±4.7	4100.4±243.7

[1] WBC, white blood count; RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets.

[2] Data are expressed as the mean±SD (n=7-10).

表四、灌食 VUKA 水萃凍乾回溶劑對小鼠口服急毒性試驗之血清肝及腎臟功能指數變化

Table 4. Serum biochemistry changes in liver and renal function in mice treated with VUKA extracts in acute oral toxicity test

Dose (g/kg)	ALT (U/l)[1]	AST (U/l)	BUN (mg/dl)	Creatinine (mg/dl)
Male				
0	99.4±24.2[2]	41.7±12.0	18.8±1.6	0.2±0.0
15	99.0±28.1	50.6±12.4	22.5±2.2[3]	0.2±0.0
Female				
0	101.1±21.3	39.9±12.6	18.0±3.7	0.2±0.1
15	99.3±16.2	31.5±5.0	19.5±2.4	0.3±0.1

[1] ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

[2] Data are expressed as the mean±SD (n=10).

[3] Significant difference between the control and treated groups at p<0.05.

表五、灌食 VUKA 水萃凍乾回溶劑對小鼠口服急毒性試驗之臟器重量變化

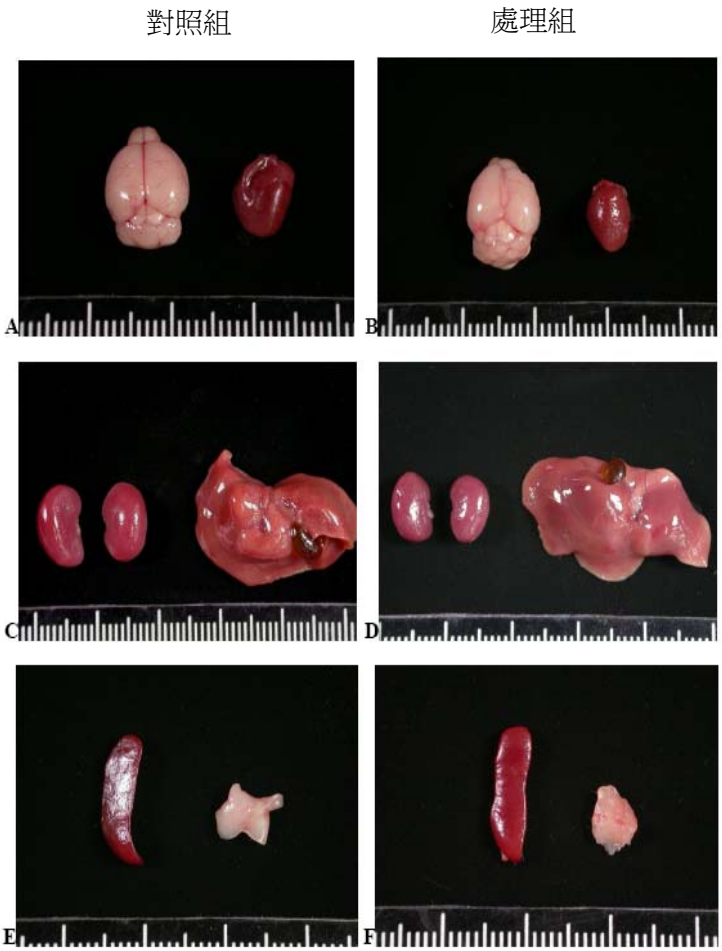
Table 5. Organ weight changes of mice treated with vuka extracts in acute oral toxicity test

Dose (g/kg)	Brain (%)[1]	Heart (%)	Liver (%)	Kidney (%)	Spleen (%)	Thymus (%)
Male						
0	1.39±0.09[2]	0.45±0.05	4.57±0.45	0.24±0.04	1.51±0.14	0.13±0.04
15	1.48±0.10	0.46±0.07	4.42±0.38	0.26±0.04	1.61±0.16	0.15±0.03
Female						
0	27.90±0.19	1.70±0.04	0.41±0.51	4.07±0.03	0.26±0.25	0.17±0.06
15	26.98±0.10	1.75±0.06	0.45±0.38	4.18±0.07	0.32±0.14[3]	0.23±0.07

[1] Organ weight (%) = [organ weight (g) / final body weight (g)] × 100.

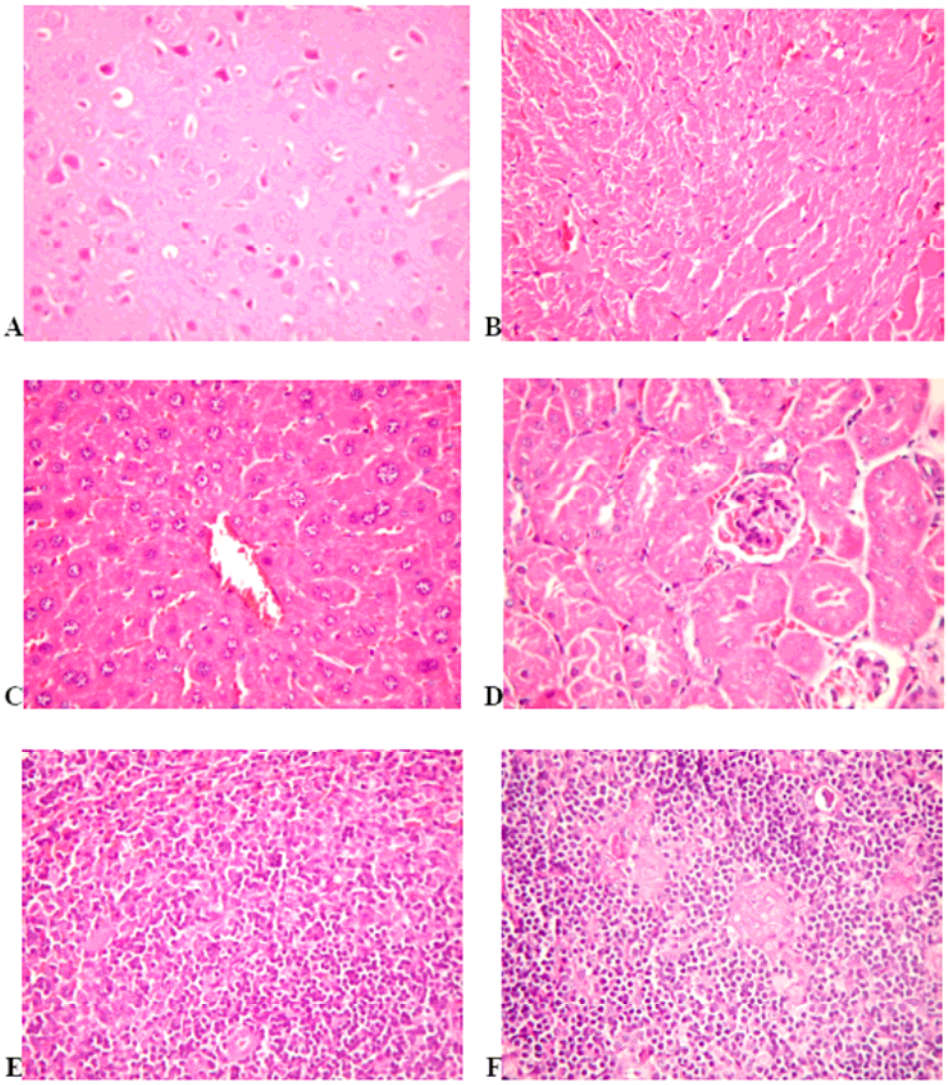
[2] Data are expressed as the mean±SD (n=10).

[3] Significant difference between the control and treated groups at p<0.05.



圖二、灌食 VUKA 水萃凍乾回溶劑對小鼠體內臟器之肉眼病理觀察。對照組及處理組之腦及心臟(A, B)；肝臟及腎臟(C, D)；脾及胸腺(E, F)等重要臟器均無明顯肉眼病理變化。

Fig. 2. Gross findings of mice after gavaged with vuka extracts in acute oral toxicity test. No significant lesions of brain, heart, liver, kidney, spleen and thymus were found in the control (A, C, E) and treated (B, D, F) groups.



圖三、灌食VUKA處理組小鼠體內臟器之組織病理觀察。處理組小鼠之腦(A)、心臟(B)、肝臟(C)、腎臟(D)、脾(E)及胸腺(F)等重要臟器均無明顯組織病理變化(H&E stain, 400x)。

Fig. 3. Histopathological findings of treated mice gavaged with vuka extracts in acute oral toxicity test. No significant lesions of brain (A), heart (B), liver (C), kidney (D), spleen (E) and thymus (F) were found in the treated mice (H&E stain, 400x).

參考文獻

1. 衛生署　1999　口服急毒性及28天餵食毒性試驗　「健康食品安全及功效評估方法」　衛署食字第8803780號　臺北。
2. Adjanohoun, E. C., N. Aboubakar, K. Dramane, M. E. Ebot, J. A. Ekpere and E .G. Enow-Orock. 1996. Traditional medicine and pharmacopoeia. Contribution to ethnobotanical and floristic studies in Cameroon. Lagos: OUA/STRC. 301 pp.

3. Environmental Protection Agency (EPA), Office of Prevention, Pesticides and Toxic Substances. 1998. Acute oral toxicity. In: OPPTS Harmonized Test Guidelines, Series 870.1100, EPA 712-C-98-190, 10 pp. Washington, DC.
4. Hooker, J. D. 1871. *Chlorocodon whiteii*- Curtis. Botanical Magazine. 5898 pp.
5. Kerharo, J. and J. G. Adam. 1974. La Pharmacopée sénégalaise traditionnelle: Plants médicinales et toxiques. Paris: Edition Vigot et Frères. 489 pp.
6. Koorbanally, N. A., D. A. Mulholland and N. R. Crouch. 2000. Isolation of isovanillin from aromatic roots of the medicinal African liane, *Mondia whitei*. Journal of Herbs, Spices & Medicinal Plants. 7(3): 37-44.
7. Kubo, I. and I. Kist-Hori. 1999. 2-hydroxy-4-metyoxybenzaldehyde: a potent tyrosinase from African medicinal plants. Planta Medica. 65: 19-22.
8. Liang, C. T., M. H. Chang, C. C. Hong and K. J. Huang. 1999. Established the blood chemistry reference values for SPF rats and mice. J. Chin. Soc. Sci. 25: 55-68.
9. Msonthi, J. D. 1991. A novel phenolic glycoside from *Mondia whitei* Skeels. Bull. Chem. Soc. Ethiop. 5: 107-110.
10. Mukonyi, K. W. and I. O. Ndiege. 2001. 2-hydroxy-4-metyoxybenzaldehyde: aromatic taste modifying compound from *Mondia whitei* Skeels. Bull. Chem. Soc. Ethiop. 15: 137-141.
11. Noumi, E., Z. P. H. Amvam and D. Lontsi. 1998. Aphrodisiac plants used in Cameroon. Fitoterapia 69(2): 125-134.
12. Organization for Economic Cooperation and Development. 2001. Acute Oral Toxicity-Acute Toxic Class method. In: OECD Guidelines for Testing of Chemicals. Section 4: Health effects. No. 423, 14 pp. Adopted: 17th December, 2001.
13. Oryem-Origa, H. E. K. Z. Kakudidi, A. B. Katende and Z. R. Bukenya. 1995. Preliminary ethno botanical studies of the Rwenzori Mountain forest area in Bundibugyo District, Uganda. Bothalia. 25 (1): 111-119.
14. Watcho, P., P. Kamtchouing, S. D. Sokeng, P. F. Moundipa, J. Tantchou, J. L. Essame and N. Koueta. 2004. Androgenic effect of *Mondia whitei* roots in male rats. Asian Journal of Andrology. 6: 269-272.
15. Yu, J. Y. L., C. K. Cheng, B. J. Chen, M. J. Cheng, H. H. Cheng, W. J. Chang, H. H. C. Chen, C. C. Hong, P. J. Lee, S. C. Liang, K. S. Sheu, Y. Y. Sung, C. N. Weng, C. W. Tsai, C. S. Wang, M. H. Wang, L. S. Yen, C. K. Yu and J. Y. L. Yu. 2004. A Guideline for the Care and Use of Laboratory Animals. Chinese Society for the Laboratory Animals Science, 2nd Edition, 207 pp. Taipei, Taiwan, ROC.

Safety Evaluation of Feeding A Newly Introduced Medicinal Plant VUKA (*Mondia whitei* L.) to Mice[1]

Xhao-Kai Kuo[2], Long-Zen Chang[2], Yung-Wu Chen[2] and Jiunn-Wang Liao[3]

ABSTRACT

For the purpose to investigate the safety of utilizing VUKA (*Mondia whitei* L.), we processed the acute oral toxicity test in ICR mice. There was no mouse dead after given with VUKA extracts during the time course, so the LD_{50} of water-extracted and freeze-dried powder of VUKA was estimated over 15 g/kg body weight for mice. No significant lesions of brain, heart, liver, kidney, spleen and thymus were found at gross and histopathological observation in the treated mice. Hematological parameters included of WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT values were as normal as control. Besides, serum biochemistry changes in liver and renal function included of ALT, AST and creatinine values had no significant difference (p>0.05) with control.

Key Words: VUKA (*Mondia whitei*), acute toxicity, semi-mortality (LD_{50}).

---

[1] Contribution No. 0643 from Taichung DARES, COA.

[2] DIRDS Research Assistant, Associate Agronomist and Director of Taichung DARES, COA.
[3] Assistant Professor, Graduate Institute of Veterinary Pathology, National Chung Hsing University.