Sub-Chronic Toxicological Evaluation of the Sesquiterpene Lactone-Enriched Fraction of *Tithonia diversifolia* (Hemsley) A. Gray in Experimental Rats

Daniel K. Egbule, Akudo P. Oji and Charles O. Nnadi

Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria Nsukka,, Nigeria,.

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INTRODUCTION

The growing interest in herbal and alternative medicines demands information on the toxicity risk assessment of the various plant extracts used in traditional medicines. The rich presence of sesquiterpene lactone, a potential toxic phytochemical, in *Tithonia diversifolia* necessitates toxicological evaluation of its biologically active constituents. Like many other plants in the Asteraceae family, *Tithonia diversifolia* (**Figure 1**) richness in sesquiterpene lactones (STLs) has continued to generate potentiality of toxicity as well as diverse pharmacological activities, SARs, and pharmacokinetics[1]. Studies have shown that through non-selective off-targets binding as a Michael acceptor, the interaction of the thiol (-SH) of proteins and enzymes with the nucleophilic α-methylene-γ-lactone of STLs could be responsible for their toxicities[2]. The study evaluated the in vivo sub-chronic toxicity of the moderately polar fractions of *T. diversifolia* in a rat model.

METHODS

The ethyl acetate soluble portion from the methanol extract was separated by vacuum liquid chromatographic method. Three-dose levels- an observed adverse effect level (OAEL) of 2000 mg/kg (group 1), a no-observed adverse effect level (NOAEL) of 80 mg/kg (group 3) and an intermediate dose of 500 mg/kg (group 2) body weight of rats per day- were selected for a 28-day repeated dosing for the sub-chronic toxicological evaluation of the liver section.(**Figure 3** and **Table 1**)[1-3]. The group 4 represents the control (untreated)

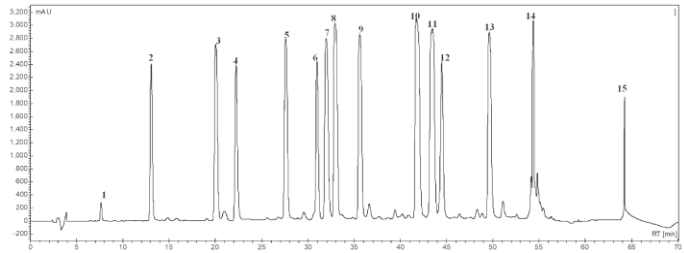

Figure 2: UHPLC-MS of VLC-STLs showing possible STLs; peaks represent base peak chromatograms; c= 10 mg/mL; m/z 50-1500 Da. Orizatin (**3**), tagitinin A (**5**), tagitinin E (**6**), tagitinin C (**7**), diversifolin (**8**), tagitinin F (**10**), tagitinin D (**11**), woodhousin (**12**), diversifolin methylether (**13**).

RESULTS

The LC-MS dereplication of the STLs fractions showed the presence of sesquiterpene lactones such as diversifolin, diversifolin methylether, tagitinin A, tagitinin C-F, woodhousin, and orizatin and many unidentified peaks (**Figure 2**).

There was a significant reduction ($p < 0.05$) in the weights of and food consumption by the rats dosed with OAEL of the fraction on week 1 which normalized during the subsequent weeks of the study.

The histopathological examination showed a mild necrosis and degeneration of hepatocytes in the centrilobular areas of the rats treated with OAEL of the active VLC fraction (**Figure 3**)

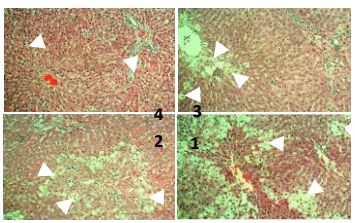

Figure 3. Liver section of rats in groups 4 (normal), 3 (mild degeneration), 2 (mild necrosis), and 1 (marked degeneration

Figure 1. Aerial part of *T. diversifolia*

Table 1.. Clinical pathological effects of STLs fraction on experimental rats

Parameter/ Groups	1	2	3	4
AST (i/uL)	11.85±0.05^{a}	11.47±0.08^{a}	10.90±0.46^{b}	11.93±0.06^{a}
ALT (i/uL)	11.77±0.17^{a}	11.49±0.02^{b}	10.92±0.16^{c}	11.97±0.09^{a}
ALP (iu/L)	51.79±1.81^{a}	47.22±2.41^{b}	42.76±3.64^{c}	53.33±3.19^{a}
PCV (%)	40.67±1.15^{a}	39.33±1.15^{a}	38.67±1.53^{a}	40.67±1.15^{a}
RBC (x10^{6}/µL	7.33±0.58^{a}	7.43±0.60^{a}	6.67±0.42^{b}	7.50±0.50^{a}
Hb (g/dL)	10.24±0.35^{a}	9.91±0.22^{a}	9.05±0.89^{b}	10.47±0.11^{a}
WBC (x10^{6} /µL)	9.00±0.87^{a}	7.73±0.50^{b}	7.27±0.64^{b}	9.40±0.53^{a}

Data are expressed as mean ± SEM (n= 5). ^{a,b,c}*Values across the row with the same superscript are not statistically different. ($p > 0.05$) when compared with the untreated group*

CONCLUSION

- Dereplication identified several STLs from STL-containing fractions
- There was no *T. diversifolia*-related adverse toxicological events in rats with a 2000 mg/kg/day when dosed orally for 28 days

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Original Research Article

Sesquiterpene Lactone-Rich Extract of *Tithonia diversifolia* (Hemsley) A. Gray (Asteraceae) suppresses *Trypanosoma brucei brucei* in both *In Vivo* and *In Vitro* Experimental Models

Edith O. Diovu[1], Chibuzo O. Osuh[2], Kelvin E. Odo[3], Hefyinwa N. Amaechina[1], Uchechukwu D. Akpadolu[1], Adanne Judith Nwodo[3], Chinedu A.T. Chah[3], Chidiogor M. Akupue[3], Charles O. Nnadi[3]*

[1]Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, 410001 Enugu State, Nigeria
[2]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, 410001 Enugu State, Nigeria
[3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, 410001 Enugu State, Nigeria

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ABSTRACT

Tithonia diversifolia has continued to play vital roles in phytomedicine due to its ethnopharmacological relevance, medicinal properties and agricultural applications. Its richness in sesquiterpene lactones has also continued to generate interest due to its diverse pharmacological activities, structure-activity relationships, and pharmacokinetics. The study evaluated the anti-trypanosomal and cytotoxic activities of the sesquiterpene lactone (STL)-