

Effect of the aqueous leaf extract of *Anchomanes difformis* on the glucose level and organ/body weight ratio of Wistar rats

Ovuakporie-Uvo Oghale, MacDonald Idu*

Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin, Africa

Received: 24.07.2015

Revised: 15.08.2015

Accepted: 16.08.2015

Published: 16.08.2015

*Address for

Correspondence:

MacDonald Idu, Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin, Africa. E-mail: mcdonald.idu@gmail.com

ABSTRACT

Anchomanes difformis (Araceae) is a plant with many reported therapeutic properties. This study is aimed at testing the blood sugar level and organ-to-body weight ratio effect of the aqueous leaf extracts of *A. difformis*, as measures of toxicity of the plant in a short term. Blood sugar test was done using a handy glucometer while organ-to-body weight ratio analysis was carried out following standard methods. Results got show that *A. difformis* had no effect on the blood glucose level and organ-to-body weight ratio on rats. A robust toxicity analysis of *A. difformis* aqueous leaf extract to further find out more proofs that the plant is safe for human subjects is recommended.

KEY WORDS: *Anchomanes difformis*, blood sugar level, glucose, organ-to-body weight ratio

INTRODUCTION

Using herbal medicines as complements or alternatives to orthodox medicines has been on the increase due to the accessibility, availability, and low cost of these natural medicines (Ozolua *et al.*, 2009). Organ weights are accepted in testing test article-associated toxicities (Black, 2002; Bucci, 2002; Wooley, 2002). Organ weight changes are often associated with treatment-related effects. The choice of organs to weigh in toxicology studies involves understanding the test article's mechanism of action, metabolism, and toxicokinetics (Sellers *et al.*, 2007). The Society of Toxicologic Pathology suggests vital organs should be weighed in multi-dose for general toxicology studies (Sellers *et al.*, 2007). A change in liver weight may suggest treatment-related changes including hepatocellular hypertrophy (Greaves, 2000; Amacher *et al.*, 2006; Juberg *et al.*, 2006). Liver weights may be increased in studies of <7 days duration for potent hepatic enzyme-inducing compounds. Increased heart weight may be the only evidence of myocardial hypertrophy that is often difficult to recognize (Greaves, 2000; Thiedemann, 1991). Changes in kidney weight may show renal toxicity, tubular hypertrophy or chronic progressive nephropathy (Greaves, 2000). The Society of Toxicologic Pathologists considers lung weights to be standard and valuable

endpoints in inhalation studies (Sellers *et al.*, 2007). The organ-to-body weight ratio is often used to clarify treatment-related organ weight changes in non-rodents (Wooley, 2002). Studies show that evaluation of organ-to-body weight ratios may be more proper for evaluation of liver and thyroid gland weights (Bailey *et al.*, 2004). The glucose tolerance test is a standard used to find out if a mammal produces enough insulin to promote the uptake of glucose from the blood after given a high glucose feeding (Butler, 1995). Amongst a number of common factors responsible for the increase or decrease of blood glucose levels are food; what, when, and how much, time you eat, type and amount of carbohydrate consumed (Example: bread, pasta, cereals, starchy vegetables, fruit, and milk), exercise or physical activity an individual is involved in, illness and pains, diabetes medication, alcohol, emotional stress and other medications (Anonymous, 2012; 2013). Testing blood glucose level is important to tell when to seek the advice of a diabetes health team about adjusting insulin, diabetes tablets, meal or snack planning when blood glucose targets are not being met Anonymous, 2012) *Anchomanes difformis* (Blume) is a member of the family Araceae (Burkil, 1985). In the South-West part of Nigeria, a decoction of the tuber is used to treat cough and ulcer and the peeled tuber is soaked in water to treat cases of dysentery (Okpo *et al.*, 2011). Peeled root soaked in water

is used in treating cases of dysentery (Oyetayo, 2007). The rhizome of *A. difformis* has analgesic and antipyretic activities (Eke *et al.*, 2013). The aim of this study is to test the effect of the aqueous leaf extract of *A. difformis*, on the glucose level and organ-to-body weight ratio in Wistar rats, after 21 days daily administration.

MATERIALS AND METHODS

Plant Material and Preparation of Extract

Fresh leaves (2,500 kg) of *A. difformis* were got from a bush in Ekosodin village in Ovia North East of Edo state. The plant was authenticated by Professor MacDonald Idu of the Department of Plant Biology and Biotechnology. The fresh leaves were washed in distilled water and blended using a kitchen blender. The filtrate was dried using an FD-10M freeze dryer at a temperature of -4°C at the National Centre for Energy and Environment (NCEE), Benin City. The dried filtrate amounted to 42% yield.

Animals

Rats of either sex weighing 165 ± 2.51 (mean $\pm$ standard error of mean [SEM]) were got from the livestock market, Aduwawa, Benin City. They were allowed to acclimatize for 2 weeks in the Animal House and fed with grower's mash and drinking water *ad libitum* for the 21 days. Experimental methods followed the recommendations provided in the "Guide for the care and use of laboratory animals" (National Academy Press, 1996).

Toxicity Studies

Rats weighing between 120 and 225 g were used in this study. They were divided into four groups of five animals each; two males and three females. The extract of *A. difformis* was administered orally at varying doses of 500, 1,000 and 2,500 mg/kg to the animals. They were observed for any mortality during experimentation on a daily basis for the 21 days of this study. A group of animals treated with distilled water served as the control group. On the 21st day, vital organs (heart, lungs, kidney, liver, and spleen) from animals in each test and control group were isolated and weighed. Blood was obtained from the abdominal aorta with a 1 ml syringe. Droplets of the blood were placed on glucose strips and values got using the ACCU-CHEK glucometer.

Data Presentation and Statistical Analysis

The results are presented as mean $\pm$ SEM and *n* is the number of animals used in each experiment. Raw data were analyzed using column statistics, ordinary one-way

ANOVA and multiple comparison tests with the aid of Graph pad computer software version 6.0. $P < 0.05$ shows significant difference.

RESULTS

The effect of *A. difformis* aqueous leaf extract on organ-to-body weight ratio is shown in Table 1. No significant differences were noted when control was compared with treatment groups.

Effect of the aqueous leaf extract of *A. difformis* on blood sugar level after 21 days oral daily administration is shown in Table 2.

DISCUSSION

An important need in toxicological experiments is the ability to assess the effects of xenobiotics on specific organs (Bailey *et al.*, 2004). It is done through macroscopic examination of the organs, measuring histopathology of the tissues and organ weight. Organ weight may be the most sensitive indicator of the effect of an experimental compound. A disparity in organ weights between treated and untreated (control) animals can occur in place of any morphological changes (Bailey *et al.*, 2004). When organ weight changes are significant or outstanding from the control values in any way; the interpretations derived, should distinguish treatment related findings from incidental findings and give perspective on the reasons for these distinctions (Sellers *et al.*, 2007).

Blood sugar level and organ-to-body weight ratios are indices often used in toxicological evaluations (Michael *et al.*, 2007), but they do not show lesions (Ozolua *et al.*, 2010). Self-monitoring blood glucose (SMBG) can aid both patients and their health care professionals better adjust to therapy and evaluate the responses to therapy. Benefits of SMBG include; patients can immediately assess the impact of an action on blood glucose levels and consequently undertake prompt interventions designed to counter the increase or reduction in blood glucose concentration (Galvin, 2007). Elevated glucose concentration in the blood is a characteristic of diabetes mellitus syndrome due to deficiency or decreased effectiveness of insulin (Davidson, 1979). Glucose spill into the urine is associated with polyuric and other diseases (Iweala *et al.*, 2005). In this study, treatment groups showed reduced blood glucose indices; (not in a definite dose-dependent manner) at 500 mg/kg (78.20 ± 12.59), 1000 mg/kg (88.60 ± 13.63) and 2500 mg/kg (69.20 ± 14.62) as compared to the control group (94.20 ± 13.98).

Table 1: Organ-to-body weight indices following oral treatment with varying concentrations of aqueous leaf extract of *A. difformis* extract

Concentrations (mg/kg)	Organ-to-body weight indices				
	H: BW	K: BW	Li: BW	L: BW	S: BW
Control	0.004±0.000	0.004±0.000	0.034±0.002	0.008±0.000	0.005±0.000
500	0.004±0.000	0.003±0.000	0.034±0.003	0.009±0.001	0.005±0.000
1000	0.004±0.000	0.004±0.000	0.035±0.002	0.008±0.000	0.005±0.000
2500	0.004±0.000	0.003±0.000	0.033±0.003	0.007±0.001	0.005±0.000

H: BW: Heart to body weight, K: BW: Kidney to body weight, Li: BW: Liver to body weight, L: BW: Lung to body weight, S: BW: Spleen to body weight. $n=5$, mean±SEM. No statistical differences between control and treatment groups ($P>0.05$), SEM: Standard error of mean, *A. difformis*: *Anchomanes difformis*

Table 2: Effects of 21 days oral daily treatment with the aqueous leaf extract of *A. difformis* on Wistar rats

Concentrations (mg/kg)	Glucose index
0 (control)	94.2±13.98
500	78.20±12.59
1000	88.60±13.63
2500	69.20±14.62

$n=5$, values are mean±SEM. No statistical differences between control and treatment groups ($P>0.05$), SEM: Standard error of the mean, *A. difformis*: *Anchomanes difformis*

This result although not statistically significant, gives an idea tending to the belief that *A. difformis* aqueous leaf extract may have the potency of reducing blood glucose level. The organ-to-body weight ratios and blood glucose concentrations were not altered when compared with the control groups. This infers that a long-term use of *A. difformis* may not be toxic on organ-to-body weight ratios and blood glucose concentration. There's need for a comprehensive toxicological analysis of the plant extract to figure out the functional groups the extract contains and its mechanism of action in human subjects in particular, those suffering from diabetes mellitus syndrome.

REFERENCES

- Amacher DE, Schomaker SJ, Boldt SE, Mirsky M. The relationship among microsomal enzyme induction, liver weight, and histological change in cynomolgus monkey toxicology studies. *Food Chem Toxicol* 2006;44:528-37.
- Anonymous. Blood glucose monitoring. A diabetes information series from diabetes state/territory organizations, Australia; 2012.
- Anonymous. Why blood sugar is important. Personal solutions for everyday life, Lilly diabetes, USA; 2013.
- Bailey SA, Zidell RH, Perry RW. Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? *Toxicol Pathol* 2004;32:448-66.
- Black H. Preparation of the report for a toxicology/pathology study. In: Haschek WM, Rousseaux CG, Wallig MA, editors. *Handbook of Toxicologic Pathology*. San Diego: Academic Press; 2002.
- Bucci TJ. The practice of toxicologic pathology: Basic techniques. In: Haschek WM, Rousseaux CG, Wallig MA, editors. *Handbook of Toxicologic Pathology*. San Diego: Academic Press; 2002.
- Burkil HM. The Useful Plants of West Africa. Family *Anchomanes Difformis*. 2nd ed. Ibadan: Royal Botanic Gardens; 1985.
- Butler LK. Regulation of blood glucose levels in normal and diabetic rats. In: Goldman CA, editor. *Tested Studies for Laboratory Teaching*. Vol. 16 Proceedings of the 16th Workshop/Conference on Association for Biology Laboratory Education; 1995. p. 181-202.
- Davidson AC. *Biochemistry*. 2nd ed. New York: Academic Press; 1979.
- Eke IG, Obioha FC, Anaga AO. Evaluation of the methanolic rhizome extract of *Anchomanes difformis* for analgesic and antipyretic activities. *Int J Basic Appl Sci* 2013;2:289-96.
- Galvin JR. The importance of monitoring blood glucose. In: *Endocrine Diseases*. US: American Registry of Pathology; 2007.
- Greaves P. *Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation*. 2nd ed. Amsterdam: Elsevier Science; 2000.
- Iweala EE, Akubugwo EI, Okeke CU. Effects of ethanolic extracts of *Allium sativum* Linn. *Liliaceae* (Garlic) on serum cholesterol and blood sugar levels of albino rabbits. *Plant Prod Res J* 2005;9:105-18.
- Juberg DR, Mudra DR, Hazelton GA, Parkinson A. The effect of fenbuconazole on cell proliferation and enzyme induction in the liver of female CD1 mice. *Toxicol Appl Pharmacol* 2006;214:178-87.
- Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, et al. Evaluation of organ weights for rodent and non-rodent toxicity studies: A review of regulatory guidelines and a survey of current practices. *Toxicol Pathol* 2007;35:742-50.
- National Research Council. *Guide for the Care and Use of Laboratory Animals*. 7th ed. Washington, DC.: National Academy Press; 1996.
- Okpo SO, Ching FP, Ayinde BA, Udi OO, Alonge PO, Eze GO. Gastroprotective effects of the ethyl acetate

- fraction of *Anchomanes difformis* (Engl). Int J Health Res 2011;4:155-61.
- Oyetayo VO. Comparative studies of the phytochemical and antimicrobial properties of the leaf, stem and tuber of *Anchomanes difformis*. J Pharmacol Toxicol 2007;2:407-10.
- Ozolua RI, Anaka ON, Okpo SO, Idogun SE. Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* mill (Lauraceae) in rats. Afr J Tradit Complement Altern Med 2009;6:573-8.
- Ozolua RI, Idogun SI, Tafamel GE. Acute and sub-acute toxicological assessment of aqueous leaf extract of *Bryophyllum pinnatum* (Lam.) in Sprague-Dawley rats. Am J Pharmacol Toxicol 2010;5(3):145-51.
- Sellers RS, Morton D, Michael B, Roome N, Johnson JK, Yano BL, *et al.* Society of toxicologic pathology position paper: Organ weight recommendations for toxicology studies. Toxicol Pathol 2007;35:751-5.
- Thiedemann KU. Left ventricular hypertrophy. In: Jones W, Hunt RD, editors. Cardiovascular and Musculoskeletal Systems. New York: Springer-Verlag; 1991.
- Wooley A. Determination - General and reproductive toxicology. In: A Guide to Practical Toxicology Evaluation, Prediction and Risk. New York: Taylor and Francis; 2003.