Ovuakporie-Uvo et al. (2019) Can J Biotech 2(4): 124-131
https://doi.org/10.24870/cjb.2019-000126

Canadian Journal of Biotechnology

ISSN 2560-8304

ORIGINAL RESEARCH PAPER **OPEN ACCESS**

Toxicogenetic Studies of *Desplatsia dewevrei* using Gene Expression of Blood, Pancreatic, and Intestinal Genes in Wistar rats

Oghale Ovuakporie-Uvo[1], MacDonald Idu[2], and Omotuyi Idowu Olaposi[3]

[1]Department of Biological and Chemical Sciences, Faculty of Natural and Applied Sciences, Michael and Cecilia Ibru University, Agbarha-Otor, PMB 100, Ughelli, Delta State, NIGERIA

[2]Department of Plant Biology and Biotechnology, University of Benin, Benin City, PMB 1154, Edo State, NIGERIA

[3]Center for Bio-computing and Drug Development, Adekunle Ajasin University, Akungba Akoko, Ondo State, NIGERIA

Received: Feb 07, 2019; Revised: Mar 19, 2019; Accepted: Apr 16, 2019

Abstract

Background: Toxicity studies are relevant in assessing the side effects of chemical substances before they are incorporated into the process of drug development.
Introduction: *Desplatsia dewevrei* is a scarce forest species believed by natives to be nutritive and therapeutic, without scientific evidence though. Thus, this study was aimed at investigating the possible toxicity of short- and long-term oral administration of *D. dewevrei* using Wistar rats.
Methods: 0, 30 100, and 1000 mg/kg of *D. dewevrei* were daily administered p.o for 3 and 28 days to Wistar rats consisting of four animals (two females, two males) per group. Hemotoxicity and liver function tests were done using automated machines from ERMA Inc. RT-PCR method was used to study the regulation of intestinal glucose transporter 4 (GLUT4), glucose transporter 2 (GLUT2), glucagon-like peptide-1 (GLP-1), pancreatic insulin, KCJN5, and L-type voltage-gated calcium channel genes (CACNAIA).
Results: No morphological or hematological signs of toxicity were observed. Liver function test showed an elevated level of high-density lipoprotein (HDL-C) in the treatment group (100 mg/kg). The lethal dose (LD_{50}) of *D. dewevrei* extracts were above 1000 mg/kg as no mortality was observed at the highest regimen dose used. Up-regulation of pancreatic insulin and down-regulation of intestinal GLUT-2 suggest that the plant may contain therapeutic constituents.
Conclusion: Short- or long-term administration of *D. dewevrei* is relatively safe.

Keywords: *Desplatsia dewevrei*, Toxicity, Gene expression, Blood, Pancreas, Intestine

Introduction

Adverse Drug Reactions (ADRs) are horrible side-shoots of remedial interventions, which are, in fact, life-threatening [1]. With the ever-increasing use of herb-derived medicines worldwide and the rapid growth of the global markets for these products, the safety and quality of medicinal plant materials and finished herbal products have become a major concern for health care providers [2]. Preliminary studies have to be done to evaluate possible risks, such as undesirable effects, overdose, or poisoning of natural plant products for the purpose of standardization [3]. Bioactivities of herbal preparations are usually determined in animal models before any further clinical trials are undertaken to appreciate the therapeutic potentials and safety in human subjects [4].

Desplatsia dewevrei (De Wild. & Th. Dur.) Burret is a therapeutically useful tree in managing pain, nasal infections, febrifuges, venereal diseases, heart diseases, paralysis, epilepsy, convulsions, and spasm [5,6]. Though a few claims of the traditional use of *D. dewevrei* have been scientifically validated [7], the toxicity at any level has not been reported. Thus, using Reverse Transcription-Polymerase Chain

*Corresponding author: Email: Oghale.uvo@mciu.edu.ng

Reaction (RT-PCR) method, this research was aimed at investigating the lethality of short- and long-term oral administration of the leaves extract of *D. dewevrei* using albino rats.

Materials and Methods

Plant collection and authentication

Fresh leaves of *D. dewevrei* were harvested from a forest in Ugbogiobo, Edo State. Plant materials were identified and authenticated at the Herbarium unit in the Department of Plant Biology and Biotechnology, University of Benin, Benin City and assigned a voucher number UBHm0283.

Plant preparation and extraction

Plant materials were thoroughly rinsed under running water, shade-dried for 3 weeks, and further dried in a hot air oven at 55°C for 1 h before blending using a mechanical blender. Dried and blended plant materials were extracted by macerating with water or methanol as solvents. The extract was concentrated to dryness using a rotary evaporator and freeze-drier (4°C) at the Biochemistry laboratory, Adekunle Ajasin University, Akungba Akoko, Ondo State.

Animal studies

A total of 35 subjects of both sexes having an average weight of 119.28 ± 0.05 kg b.w were involved in this study. Subjects (Wistar rats) were randomly grouped according to their weight into seven groups of five animals each. Group 1 served as control and was administered animal chow and drinking water ad libitum. Groups 2–4 were orally administered 1000, 100, and 30 mg/kg of the aqueous leaf extract pf *D. dewevrei* with the aid of an oral gastric tube, respectively. Similarly, Groups 5–7 were administered with 1000, 100, and 30 mg/kg of the methanol leaf extract of *D. dewevrei* p.o, respectively. At the end of the 28 days, only three animals per group were selected and analyzed for biochemical, hematological parameters, and toxicogenetic studies using gene expression techniques.

Biochemical and hematological assays

Blood sample taken from animals in each experimental group (control and treatment groups) were stored in EDTA and lithium heparin bottles prior to sending them to the Hematology laboratory of Inland Medical Center, Ikare. In the Hematology laboratory, the samples were analyzed using an automated machine from ERMA Inc. (Tokyo, Japan) after centrifuging at 3000 rpm to separate serum from plasma. Lipid profile and liver function tests were carried out using Elitech clinical systems at the University of Benin Teaching Hospital.

Molecular toxicity studies

Acute and chronic toxicity assessments were done on blood, pancreas, and small intestine of albino rats using a 3-day and 28-days treatment regimen by gene expression. The genes under investigation included β-actin, tumor necrosis factor-alpha (TNF-α), Insulin, L-type voltage-gated calcium channel genes (CACNAIA), glucagon-like peptide-1 (GLP-1), KCJN5, glucose transporter 2 (GLUT-2), and glucose transporter 4 (GLUT-4). The primer sequences for these genes are shown in Table 1.

Animal sacrifice and tissue harvesting

At the end of the 28th day period of stable administration, animals were subjected to fasting over-night and were sacrificed the next morning. Pancreas and intestinal crypt were excised into Eppendorf tubes containing 100 µl RNA snap™ kit (Beckman Coulter, Indianapolis, IN, United States) reagent across the groups.

RNA isolation, RT-PCR magnification

RNA was isolated and digested (n=3) as previously described [8,9]. PCR amplification, gel electrophoresis, and image processing methods used were adopted by Omotuyi et al. [10].

Statistical analysis

Data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analyses were carried out using one-way analysis of variance (ANOVA). Multiple comparisons were

Table 1: Primer sequences.

Genes used	Forward 5'-3'	Reverse 5'-3'
β-actin	ACACTTTCTACAATGAGCTGCG	ACCAGAGGCATACAGGACAAC
KCNJ5	CTGGGAGATGTCTCGTGCTC	CATGCCTGTGGCTTCTACCA
Insulin	GAGGCTCTGTACCTGGTGTG	ACCTCCAGTGCCAAGGTTT
TNF-α	CATCCGTTCTCTACCCAGCC	AATTCTGAGCCCGAGTTGG
GLUT-4	TCTCCGGTTCCTTGGGTTGT	TTCCCCATCTTCAGAGCCGAT
CACNIA	CGCATTTAAGCCCAAGCACT	TCAACGACGCTACCGACAAC
GLUT-2	CCTGGCGTCTTCAGAGAGGTG	ACCGAGGAAGGAATCGGTTT
GLP-1	ACCGTTTACATCGTGGCTGG	CCCTGTGAATGGCGTTTGTC

done using Duncan multiple range tests (SPSS version 23), Tukey's multiple range tests (GraphPad Prism 6), and Microsoft Excel software package. Significant levels were determined at $p < 0.05$.

Results

Toxicity (acute and sub-acute) studies

No mortality, loss of agility/cognitive ability, or any physical morphology associated toxicity was observed in all the groups of rats treated with 30–1000 mg/kg doses of the aqueous and methanol leaf extracts of *D. dewevrei* (Table 2).

After 72 h of daily oral administration, the change in adverse body weight was observed in the animal group treated with 1000 mg/kg of the aqueous leaf extract (–14.36) of *D. dewevrei*. Methanol leaf extract at doses 100 (–1.74) and 1000 (–9.81) mg/kg also showed weight reduction in comparison with the initial weight of animals on the first day of the experiment (Table 3). In the sub-acute toxicity studies, a percentage weight loss of –3.119 was observed in the animal groups administered with 1000 mg/kg of the methanol leaf extract.

From the results, the administration of aqueous and methanol extracts of *D. dewevrei* up to 1000 mg/kg b.w was not associated with hemotoxicity in Wistar rats. Neither the population of blood cells nor the concentrations of oxygen-carrying hemoglobin were significantly altered in comparison with the control in the 72 h and 28 days of toxicity studies as presented in Figure 1 and Table 4. Lipid profile and serum biochemistry of laboratory rats treated with the leaf extracts of *D. dewevrei* showed no significant difference in the levels of aspartate aminotransferase (AST), alanine transferase (ALT), gamma-glutamyl transferase (GGT), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL) in the treatment groups in comparison with the control group as presented in Table 5. However, high-density lipoprotein cholesterol (HDL-C) was slightly elevated at 100 mg/kg of aqueous extract, which is a positive response to treatment.

Further molecular investigation for any toxic effect associated with the administration of *D. dewevrei* to experimental animals at treatment doses up to 1000 mg/kg showed no deleterious effect on pancreatic and intestinal genes of Wistar rats. The oral administration of *D. dewevrei* for 28 days caused no harmful effect on the pancreas as evident by the downward regulation of TNF-α, CACNAIA and the upward regulation of insulin and GLP-1 in the pancreas of experimental animals. The small intestine also showed no signs of toxicity as markers

Table 2: Lethal effect of the aqueous and methanol leaf extracts of *Desplatsia dewevrei* on rats after 72 h and 28 days of daily oral administration.

Groups	Agility	Cognition	Other morphological signs of toxicity (e.g., grooming, writhing, nausea, etc.)	% Mortility
Control	Normal	Normal	Nil	Nil
DDAE 1000 mg/kg	Normal	Normal	Nil	Nil
DDAE 100 mg/kg	Normal	Normal	Nil	Nil
DDAE 30 mg/kg	Normal	Normal	Nil	Nil
DDME 30 mg/kg	Normal	Normal	Nil	Nil
DDME 100 mg/kg	Normal	Normal	Nil	Nil
DDME 1000mg/kg	Normal	Normal	Nil	Nil

n = 3 replicates; control received 3 ml/kg distilled water. DDAE = *D. dewevrei* aqueous extract; DDME = *D. dewevrei* methanol extract.

Table 3: The Percentage change in weight of Wistar rats in acute (72 h) toxicity of *Desplatsia dewevrei*.

Groups	Initial	Final	% b.w. change
Control	87.2 ± 8.80	103.1 ± 3.85	18.23
DDAE 1000 mg/kg	116.3 ± 8.60	99.6 ± 0.20	–14.36
DDAE 100 mg/kg	102.9 ± 0.55	109.4 ± 1.45	6.32
DDAE 30 mg/kg	101.6 ± 0.20	113.4 ± 9.15	11.61
DDME 30 mg/kg	135.9 ± 0.35	148.8 ± 10.8	9.49
DDME 100 mg/kg	140.45 ± 0.05	138.0 ± 3.35	–1.74
DDME 1000 mg/kg	151.35 ± 7.55	136.5 ± 3.55	–9.81

Values are Mean ± SEM. n = 3. DDAE= *D. dewevrei* aqueous extract; DDME= *D. dewevrei* methanol extract.

indicating early irritation (GLUT-2) to the small intestine were minimally expressed; however, the upward regulation and expression of GLP-1 and GLUT 4 confirmed no adverse effect on the small intestine of test animals (Figure 2).

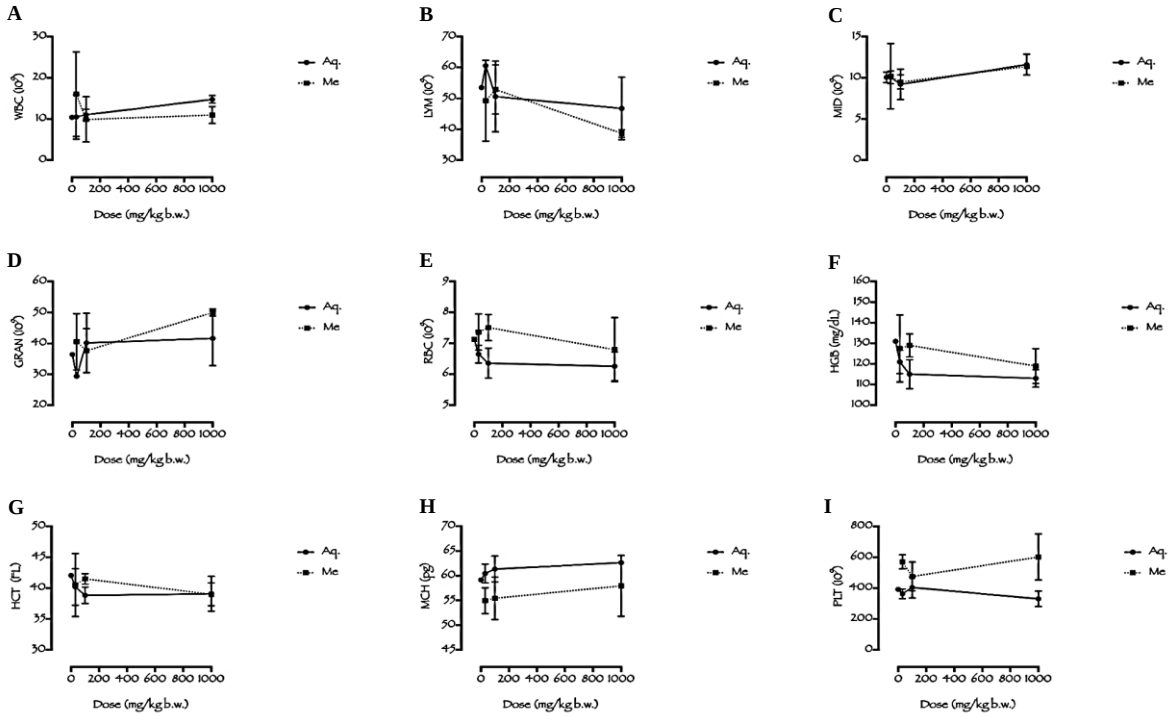

Figure 1 (A–I): Acute hemotoxicity of *Desplatsia dewevrei* leaves.
Aq = Aqueous extract; Me = Methanol extract; WBC = White blood cell indices; LYM = Lymphocyte; MID = Minimum inhibitory dilution (A measure of rare cells and a number of precursor white cells, for example, Basophils, Blasts, Eosinophils, Monocytes, etc.); GRAN = Granulocytes; RBC = Red blood cell count; HGB = Hemoglobin; HCT = Hematocrit; MCH = Mean corpuscular hemoglobin concentration; PLT = Platelet count.

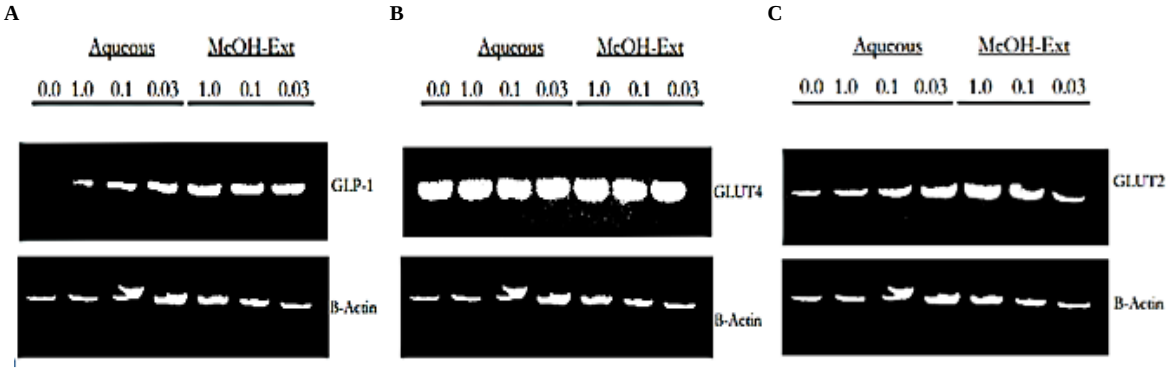

Figure 2: Gene expression. (A–C) Regulation of glucagon-like peptide-1 (GLP-1), glucose transporter 4 (GLUT4), and glucose transporter 2 (GLUT2) genes in the small intestine of albino rats after the daily administration of the leaf extracts of *D. dewevrei* for 28 days. Beta-actin was used as a loading control.

Table 4: Sub-acute (28 days) toxicity of Hematology parameters in Wistar Rats treated with *Desplatsia dewevrei* extracts.

Parameters	Concentrations						
	Control	Aqueous 1000 mg/kg	Aqueous 100 mg/kg	Aqueous 30 mg/kg	Methanol 1000 mg/kg	Methanol 100 mg/kg	Methanol 30 mg/kg
WBC ($\times 10^3$/ µl)	10.4 ± 0.00	10.50 ± 3.80	11.00 ± 1.00	14.75 ± 0.65	16.05 ± 7.25	9.90 ± 3.90	10.95 ± 1.45
LYM ($\times 10^3$/ µl)	5.60 ± 0.00	6.35 ± 2.35	5.50 ± 0.40	6.95 ± 1.35	7.25 ± 2.05	4.85 ± 1.65	4.25 ± 0.45
MID ($\times 10^3$/ µl)	1.05 ± 0.05	1.05 ± 0.35	1.05 ± 0.25	1.75 ± 0.05	1.85 ± 1.15	0.95 ± 0.45	1.25 ± 0.15
GRAN ($\times 10^3$/ µl)	3.75 ± 0.05	3.10 ± 1.10	4.45 ± 1.15	6.05 ± 0.65	6.95 ± 4.05	3.95 ± 1.95	5.45 ± 0.85
RBC ($\times 10^6$/ µl)	7.13 ± 0.015	6.65 ± 0.20	6.36 ± 0.35	6.20 ± 0.33	7.37 ± 0.42	7.51 ± 0.30	6.80 ± 0.74
HGB (g/dL)	131.0 ± 0.00	121. 0 ± 4.00	115.00 ± 5.00	113.0 ± 3.00	127.50 ± 11.50	129.0 ± 4.00	119.0 ± 6.00
HCT (%)	42.05 ± 0.05	40.2 ± 2.10	38.85 ± 0.95	39.10 ± 2.00	40.50 ± 3.60	41.50 ± 0.60	39.0 ± 1.30
MCV (fL)	59.15 ± 0.05	60.45 ± 1.35	61.35 ± 1.85	62.65 ± 0.05	54.95 ± 1.85	55.45 ± 3.05	57.95 ± 4.35
MCH (Pg)	18.35 ± 0.05	18.15 ± 0.05	18.10 ± 0.20	18.05 ± 0.45	17.20 ± 0.60	17.20 ± 1.20	17.60 ± 1.00
PLT ($\times 10^3$/ µl)	392.5 ± 2.50	363.00 ± 22.0	404.50 ± 48.50	331.0 ± 35.0	570.50 ± 31.50	475.0 ± 66.0	602.00 ± 106.00
PCT (%)	0.24 ± 0.00	0.23 ± 0.01	0.25 ± 0.02	0.24 ± 0.005	0.38 ± 0.02	0.34 ± 0.05	0.39 ± 0.09

Values are n = 3; No significant difference between treatment and control groups.
WBC = White blood cell count; LYM = Lymphocyte count; MID = Minimum inhibitory dilution (A measure of rare cells and a number of precursor white cells, for example, Basophils, Blasts, Eosinophils, Monocytes, etc.); GRAN = Granulocytes; RBC = Red blood cell count; HGB = Hemoglobin; HCT = Hematocrit; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; PLT = Platelets; PCT = Platelet crit.

Table 5: Liver function and serum lipid test in the sub-Acute (28 days) toxicity studies of *Desplatsia dewevrei.*

Parameters	Control	1000 mg/kg DDAE	100 mg/kg DDAE	30 mg/kg DDAE	1000 mg/kg DDME	100 mg/kg DDME	30 mg/kg DDME
AST (IU/L)	128.0 ± 50.00	96.0 ± 6.00	86.0 ± 19.00	70.50 ± 34.50	110.00 ± 2.00	139.00 ± 9.00	118.00 ± 2.00
ALT (IU/L)	36.00 ± 7.00	30.00 ± 4.00	22.50 ± 1.50	21.50 ± 3.50	44.50 ± 5.50	40.00 ± 10.00	38.50 ± 9.50
GGT (U/L)	45.15 ± 10.35	16.10 ± 11.50	13.25 ± 7.55	33.30 ± 69.50	27.75 ± 20.85	21.55± 59.05	24.25 ± 10.45
TG (mmol/L)	73.75 ± 18.25	107.55±13.05	71.50±10.20	73.80 ± 6.70	67.35 ± 41.85	75.40 ± 19.20	63.85 ± 10.25
TC (mmol/L)	50.95 ± 12.15	62.10 ± 8.20	72.15±17.55	46.25 ± 5.05	67.80 ± 4.90	64.15 ± 2.35	44.80 ± 3.60
HDL-C (mg/dL)	19.50 ± 1.80	18.00 ± 1.50	30.20±1.80*	18.10 ± 1.40	19.20 ± 2.10	17.70 ± 1.90	16.00 ± 1.80
LDL-C (mmol/L)	17.60 ± 16.70	22.60 ± 4.10	27.65±21.35	13.40 ± 5.10	35.15 ± 11.15	34.20 ± 3.20	16.05 ± 3.85
VLDL (mmol/L)	14.75 ± 3.65	21.50 ± 2.60	14.30 ± 2.00	14.75 ± 1.35	13.45 ± 8.35	12.25 ± 1.05	12.75 ± 2.05

Values are Mean ± SEM; * Mean difference is significant at the 0.05 level
DDAE = *D. dewevrei* aqueous extract; DDME = *D. dewevrei* methanol extract; AST = Aspartate aminotransferase; ALT = Alanine transferase; GGT = Gamma glutamyl transferase; TG = Triglycerides; TC = Total cholesterol; HDL-C = High-density lipoprotein cholesterol; LDL-C = Low-density lipoprotein cholesterol; VLDL = Very low-density lipoprotein cholesterol.

Discussion

Acute toxicity defines the antagonistic effects of a substance that results either from a single exposure or from multiple exposures in a short period of time. The adverse effects in an ideal acute toxicity study must happen within 14 days of the administration of the substance [11,12]. Morphological signs of toxicity, increased motor activity, anesthesia, tremors, arching and rolling, convulsions, cyanosis, and analgesia are worthy of notes for caution on drug side effects [13]. In this study, Table 2 shows that morphological signs of toxicity, as described by Bhardwaj and Gupta [13], were not observed during the period of the acute toxicity studies. According to Zhu et al. [12], chemical toxicity can be associated with many hazardous biological effects, such as gene damage, carcinogenicity, or induction of lethal rodent or human diseases. However, in this study, inference from Table 1 reveals that no mortality is associated with the administration of *D. dewevrei* leaves over a short or long period of time. Thus, it can be concluded that the lethal dose (LD_{50}) of the aqueous and methanol leaf extracts of *D. dewevrei* is greater than 1000 mg/kg as no death was recorded up to this dose.

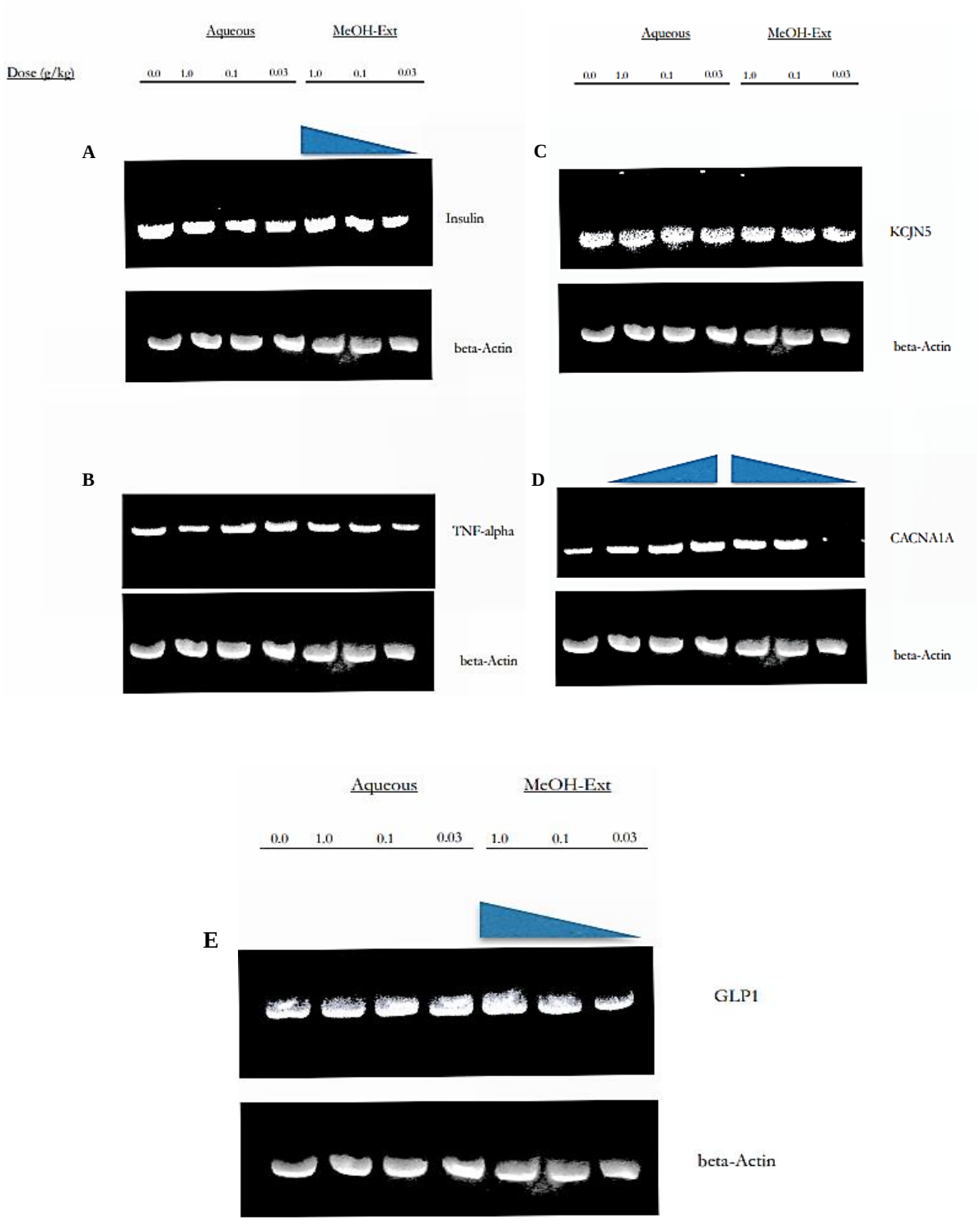

Figure 3: Gene expression. (A and C) Upward regulation of insulin and KCJN5 genes, **(B and D)** Downward regulation of tumor necrosis factor-alpha (TNF-α) and L-type voltage-gated calcium channel (CACNAIA) genes of the pancreas after the administration of the leaf extracts of *D. dewevrei* for 28 days, and **(E)** Upward regulation of Glucagon-like peptide 1(GLP-1) in the pancreas after the administration of the leaf extracts of *D. dewevrei* for 28 days. Beta-actin was used as a loading control.

Reduction in body weight and internal organ weights are simple and sensitive indices of toxicity after exposure to a toxic substance [14,15]. However, according to reports of the national council of fiber, Palmer et al. [16], and Anonymous [17], high fiber containing foods have low calorific value which makes them good for weight loss, lower cholesterol, improve blood glucose in the body, promote regularity, and eliminating and minimizing constipation thereby enhancing immune system to fight infection and chronic diseases. In this study, there was no appreciable weight loss in the treatment groups as compared with the control group except in the group of animals treated with 1000 mg/kg of both extracts in the 72 h and 28-days toxicity study (Table 3). However, this may be considered a positive physiological response to treatment as the plant extracts may be useful in weight loss, especially for obese people, because of the high fiber content.

Serum enzyme levels are useful tools in toxicological studies [18]. In the acute and sub-acute hemotoxicity studies in this research, blood parameters tested in the hemotoxicity test were all not significantly different between the treatment groups and control (Figure 1 and Table 4).

Any significant change in the functional capacity of the liver, kidney, and serum may impair the normal function of the organs resulting in the pathogenesis of any sort [19]. Using biochemical markers, such as AST, ALT, GGT, TG, TC, LDL-C, VLDL, and total proteins, any impairment in the physiology of the liver can be detected [20]. The earlier listed biomarkers were not significantly different in the treatment groups when compared with the control in the lipid profile and serum biochemistry aspect of this study (Table 5). Concentrations of HDL-C, LDL-C, and TG when altered can give vital information on lipid metabolism. The alteration can give useful information on the predisposition of the heart to atherosclerosis and other associated cardiovascular diseases [21]. In the study, the concentration of HDL-C was elevated in the treatment group administered with 100 mg/kg of the aqueous extract of *D. dewevrei* (Table 5). This is a physiological sign that cardiovascular disorders may be prevented at that dose as HDL-C, which is the good cholesterol, is primarily responsible for keeping cholesterol from building up in the arteries and reducing the risk of coronary heart disease and stroke [22]. HDL-C is the principal vehicle for the removal of surplus cholesterol from the extrahepatic and peripheral tissues for disposal in the liver, that is, reverse cholesterol transport [23,24]. Although most toxicity studies majorly target the liver and kidneys [25], the small intestine and pancreas are vital and worthy of assessment. Genes which are differentially up- or down-regulated in diseased tissue are the potential targets for drug development [26]. In this study, certain genes that predict tissue injuries in the small intestine (GLUT-2) and pancreas (TNF-α, CACNA1A, GLP-1) were found to be down-regulated after treatment with the leaf extracts of *D. dewevrei* as depicted in Figures 2 and 3, respectively. This further supports the results of the hematology and serum lipid profile.

Conclusion

In conclusion, short- or long-term administration of the leaf extracts of *D. dewevrei* poses no toxic effect to the blood platelets, pancreas, and small intestine of laboratory animals. Therefore, it can be considered to be relatively safe in drug development, especially of hypoglycemic agents, as speculated by the increased production of insulin in the pancreas (Figure 3) and intestinal mop up of GLP-1 and GLUT-2 genes (Figure 2).

Acknowledgments

The authors wish to heartily thank the Chief Medical Directors and laboratory staff of Inland Medical Center Ikare, Ondo State and University of Benin Teaching Hospital, Benin City, Edo State for their contributory role in the success of this research. We also thank the members of the Center for Bio-computing and Drug Development, Adekunle Ajasin University, Akungba Akoko for their help and support to make this research good. This research received no specific grant from any funding agency in the public, commercial, private, or not-for-profit sectors. The research was solely funded by the authors.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this manuscript.

References

[1] Adewale, J.O., Oludare, T.O., Olumide, K.I. and Olaposi, I.O. (2018) Evidence for the immune-toxicity of green tea polyphenols: A Computational Study. *SAJ Pharm Pharmacol* 5: 1–9. https://doi.org/10.13140/RG.2.2.20531.53287

[2] WHO 2007. The World Health Report: a safer future: global public health security in the 21st century. Geneva. 96P.

[3] Oyesola, T.O., Oyesola, O.A. and Okoye, C.S. (2010) Effects of aqueous extract of *Aspilia africana* on reproductive functions of female Wistar rats. *Pak J Biol Sci* 13: 126–131. https://doi.org/10.3923/pjbs.2010.126.131

[4] Idu, M. and Timothy, O. (2012) 'Plant extracts and biodiversity testing'. In Biological Techniques and Applications (Okhuoya JA, Okungbowa FI, Shittu HO, Eds). UNIBEN Press, Nigeria, 157–179.

[5] Burkill, H.M. (1985) The useful plants of West Tropical Africa. 2nd Edition. Volume 1, Families A–D. Royal Botanic Gardens, Kew, United Kingdom.

[6] Harris, D.J., Moutsamboté, J.M., Kami, E., Florence, J., Bridgewater, S. and Wortley, A.H. (2011) An Introduction to the trees from the North of the Republic of Congo. Royal Botanic Garden Edinburgh.

[7] Ovuakporie-Uvo, O., Idu, M., Obarisiagbon, P. and Abode, C. 2018. Analgesic, pro and anti-inflammatory activities of *Desplatsia dewevrei*; Cytokine gene expression using Wistar rats and mice. *J Phytopharmacol* 7: 185–190.

[8] Chen, L., Alam, T., Johnson, J.H., Hughes, S., Newgard, C.B. and Unger, R.H. (1990) Regulation of beta-cell glucose transporter gene expression. *Proc Natl Acad Sci USA* 87: 4088–4092.

[9] Franca, A, Freitas, A.I., Henriques, A.F. and Cerca, N. (2012) Optimizing a qPCR gene expression quantification assay for S. epidermidis biofilms: a comparison between commercial kits and a customized protocol. *PLoS One* 7: e37480. https://doi.org/10.1371/journal.pone.0037480

[10] Omotuyi, I.O., Ovuakporie-Uvo, O. and Idu, M. (2017) Regulation of intestinal GLP-1 and GLUT 2 genes underlie hypoglycemia in *Desplatsia subericarpa* (Bocq)-fed Wistar rats. *J Herbal Drugs* 8: 79–86.

[11] Akhila, J.S. Shyamjith, D. and Alwar, M.C. (2007) Acute toxicity studies and determination of median lethal dose. *Curr Sci* 93:917–920.

[12] Zhu, H., Martin, T.M., Ye, L., Sedykh, A., Young, D.M. and Tropsha, A. (2009) QSAR modeling of rat acute toxicity by oral exposure. *Chem Res Toxicol* 22: 1913–1921. https://doi.org/10.1021%2Ftx900189p

[13] Bhardwaj, S. and Gupta, D. (2012) Study of acute, sub acute and chronic toxicity test. *Intl J Adv Res Pharm Bio Sci* 2: 103–129.

[14] Raza, M., Al-Shabanah, O.A., El-Hadiyah, T.M. and Al-Majed, A.A. (2002) Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. *Sci Pharm* 70: 135–145.

[15] Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A. and Khetani, V. (2002) A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague-Dawley rats. *Toxicol* 179: 183–196. https://doi.org/10.1016/S0300-483X(02)00338-4

[16] Palmer, J.L., Trotter, T., Joy, A.A. and Carlson, L.E. (2008) Cognitive effects of Tamoxifen in pre-menopausal women with breast cancer compared to healthy controls. *J Cancer Surviv* 2: 275–282. https://doi.org/10.1007/s11764-008-0070-1

[17] Anonymous (2005) Institute of Medicine, "Dietary intakes and energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acid". National Academy Press. Washington, DC, United States. 380–382.

[18] Ashafa, A.O.T., Yakubu, M.T., Grierson, D.S. and Afolyan, A.J. (2009) Toxicological evaluation of the aqueous extract of *Felicia muricata* Thunb. leaves in Wistar rats. *Afr J Biotech* 8: 949–954.

[19] Afolayan, A.J. and Yakubu, M.T. (2009) Effects of *Bulbine natalensis* Baker stem extract on the functional indices and histology of liver and kidney of male Wistar rats. *J Med Food* 12: 814–820. https://doi.org/10.1089/jmf.2008.0221

[20] Yakubu, M.T., Akanji, M.A. and Oladiji, A.T (2005) Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. *Asian J Androl* 7: 399–404. https://doi.org/10.1111/j.1745-7262.2005.00052.x

[21] Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. (2008) Alteration in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia agrestis* stem. *Res J Med Plant* 2: 66–73. http://doi.org/10.3923/rjmp.2008.66.73

[22] Anonymous (2001) Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *J Am Medic Assoc* 285: 2486–2497. https://doi.org/10.1001/jama.285.19.2486

[23] Genest, J., Marcil, M., Denis, M. and Yu, L. (1999) High density lipoproteins in health and in disease. *J Investig Med* 47: 31–42.

[24] Vaziri, N.D. (2006) Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences. *Am J Physiol - Renal Physiol* 290: F262–F272. https://doi.org/10.1152/ajprenal.00099.2005

[25] Ovuakporie-Uvo O., Idu M., Eze, G.O. and Ozolua, R.I. (2015) Toxicological studies of *Anchomanes difformis* Blume (Araceae) using rats and mice. *Intl J Basic Clin Pharmacol* 4: 1228–1234. https://doi.org/10.18203/2319-2003.ijbcp20151364

[26] Rastogi, S.C., Mendiratta, N. and Rastogi, P (2015) Bioinformatics: methods and applications: (Genomics, proteomics and drug discovery). Forth Edition, published by Ghosh, A.K., PHI learning private limited, Rimjhim House, III, Patparganj Industrial Estate, Delhi-110092.;628.