IAA Journal of Biological Sciences 9(1):146-158 2022. ISSN:2636-7254
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Evaluation of the Acute Toxicity and Hematological Effect of Aqueous Extract of *Albizia chinensis* (Osbeck) Merr Stem Bark in Streptozotocin-induced Diabetic Wistar rats

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ABSTRACT

Acute toxicity and hematological effect of aqueous extract of *Albizia chinensis* (Osbeck) Merr stem bark in streptozotocin-induced diabetic Wistar rats were evaluated. The aqueous stem bark of extract of *Albizia chinensis* obtained by cold maceration was tested for acute toxicity in Wistar rats using Lorke's method followed by sub-acute toxicity and hematological assessment. The LD_{50} was found to be above 5000mg/kg although the extract showed signs of sedation and reduced activity. There was a statistically significant effect on hematological levels with RDW-SD (p=0.0016), mean platelet volume MPV (p=0.0022) and procalcitonin (p-value = 0.0056). In conclusion, the aqueous extract of the stem bark of *Albizia* is relatively safe for use at acute and subacute levels although more research needs to done to establish the chronic effects of the plant extract.

Keywords: Acute toxicity, Hematological effect, *Albizia chinensis* and streptozotocin

INTRODUCTION

An ethnobotanical survey carried out in three selected districts in Uganda by [1], to establish the plants traditionally used in the management of diabetes mellitus (DM), reported *Albizia chinensis* as the 7th most used plant out of the 18 plants that were reported to be used by the population to manage DM in Kayunga, Mukono and Wakiso districts of Uganda. In all these districts, *Albizia chinensis* was locally called '*Omugavu*' [1]. There are also undocumented reports of the plant use for the same purpose in Bushenyi District in Western Uganda but this time using stem bark instead of leaves. However, there is no scientific documentation about either the toxicity profiles of the plant or its hypoglycemic activity as claimed by the population. Furthermore, even though *Albizia chinensis* (Osbeck) Merr has been reported as being used in selected parts of Uganda for treatment of DM, no efficacy studies have been done to demonstrate whether the plant is efficacious or safe for use hence this proposal to undertake a study of this nature. Despite the fact that there is high level use of medicinal plants, not only are there questions about their efficacy, but also about their safety of use among the affected individuals. Below are some of the methods used to scientifically ascertain their safety. The Globally Harmonized System (GHS) defines acute toxicity as, "those adverse effects occurring following oral or dermal administration of a single dose of a substance or multiple doses given within twenty-four hours or an inhalational exposure of four hours." According to OECD (Organization for Economic Cooperation and Development), test guideline 423, acute oral toxicity refers to the unfavorable effects that occur after a one off dose of a chemical is taken or repeated doses are taken within 24 hours. In this aspect delayed death occurs when an animal does not die or appear moribund within 2 days (48 hours) of being observed, but instead death occurs later throughout the 2 weeks (14-days) period. Acute toxicity tests are usually the first tests performed to offer crucial information on the relative toxicity anticipated to result from a single brief exposure, and they are used to calculate the LD50. For oral and inhalational testing in rats, as well as

cutaneous testing in rats and rabbits, standardized tests are available. Fixed dose procedure (OECD test guideline-420), acute toxic class method (OECD test guideline-423), up and down procedure (OECD test guideline-425), acute dermal toxicity (OECD test guideline-402), and acute inhalational toxicity (OECD test guideline-403) are the most common OECD elaborative toxicity guidelines for describing acute systemic testing [2].

Aim of the study

The aim of this study is to assess the acute toxicity and hematological effect of aqueous extract of *Albizia chinensis* (Osbeck) Merr stem bark in streptozotocin-induced diabetic Wistar rats.

METHODOLOGY

Study design

This study was conducted using an experimental approach. The study experimental setups started with *Albizia chinensis* plant material collection, which was followed by drying, pulverizing and extraction. Acute toxicity testing of the extract followed Lorke's method [3] while the sub-acute toxicity tests were conducted according to the [4] regulations for 28-day toxicity studies following repeated oral substance administration in rats. Hyperglycemia was induced using streptozotocin following a practical guide for induction of type-2 diabetes in rats as suggested by [5]. The oral hyperglycemic activity of aqueous extract of *Albizia chinensis* stem bark in the diabetic rats was then evaluated using metformin as the comparator for oral hypoglycaemic activity.

Study setting

Plant material drying, pulverizing and extractions were performed in KIU-WC Pharmacology laboratory with the animals being kept under observation in the animal facility at KIU-WC Pharmacology laboratory. All experimental activities were performed at KIU-WC Pharmacology and KIU–TH Hematology laboratories.

Plant collection and identification

The plant stem bark of *Albizia chinensis* was collected from Orushenyi village, in Bushenyi -Ishaka Municipality in Bushenyi District. This area was specifically selected as a site of plant material collection because there was undocumented use of the plant for DM management by the population. Once the plant stem bark was collected, a sample with other associated plant parts were carefully packaged and transported to a plant taxonomist at Mbarara University of Science and Technology for identification and authentication, for which an identification numbers of MW007 was provided.

Storage, drying and pulverization

The stem bark sample of *Albizia chinensis* was stored and dried in a shade to constant weight. The stem bark shavings were spread on a dry cemented tables in an isolated room and changed daily until constant weight was achieved. Dried stem bark material was coarsely powdered, sieved with a size #40 mesh sieve and then stored in an air tight container at room temperature after weighing until extraction time.

Extraction

In the extraction process used, I tried as much as possible to simulate the approach used by the respondents in the study by [1]. A total of 540g of powdered sample material was macerated in 5 liters of water that was already distilled, at 25^0C followed by shaking at intervals up to 2 days (48 hours) and then the mixture was strained with cotton wool and then filtered by use of a #1-Whatman filter paper. Then, using a rotary evaporator at 40°C under reduced pressure, the filtrate was then concentrated, then drying at room temperature was allowed to take place.

The percentage yield (%) of the extract was calculated using the equation below:

Percentage yield (% age yield) = Weight of concentrated extract divided by Weight of the plant powder multiply by 100

The dried crude extract was then stored in a refrigerator at 4°C until it was used for actual animal experiments.

Stock solution preparation

Stock solution preparation was performed through dissolving 5g of the extract in ten milliliters of water free from impurities to obtain a concentration of stock solution of 500mg/ml. Preparing the stock solution

was only done at the time it is was needed to be administered to the animals. The doses of the extract to give orally to the animals were arrived at based on calculation using Ghosh's formula [6];

Volume given to each animal (ml) = Weight of the animal (kg) divide by Extract concentration (mg/ml) multiply by the dose [mg/Kg]

Experimental animals and procedure

All procedural handling of animals was performed according to [7] and humane care and use of guidelines on laboratory use of animals [2]. Wistar rats, both male and female aged 7-8 weeks and weighing about 150-220g, were used for this study. Only non-pregnant females that had never given birth were considered. Standard cages were used to accommodate the animals throughout the study and they were fed on a standard regulations prescribed feeds and hydrated with water given for as much as was necessary, with 12 hour of access day light and 12 hour of night time. These selected experimental animals were kept under the stated conditions for five days in order to allow them time to acclimatize with the environmental conditions before initiation of experiments.

The animals were divided into five groups of five animals each as indicated below:

Table 1: Grouping and Animal treatments

Group	Treatment
Group I	These were untreated diabetic rats which received 1 ml of distilled water (Negative control)
Group II	The positive control group that was treated with the oral hypoglycemic drug (metformin)
Group III	Was the diabetic model group containing STZ- treated surviving diabetic rats which were treated with the bark extract of *Albizia chinensis*
Group IIIa:	Diabetic rats treated with low dose (200mg/kg Bwt) of *Albizia chinensis* bark extract
Group IIIb	Diabetic rats treated with medium range dose (400mg/kg) of *Albizia chinensis* bark extract
Group IIIc:	Diabetic rats treated with high dose (800mg/kg) of *Albizia chinensis* bark extract

Source: [8]

Streptozotocin induction of diabetes mellitus

Diabetes was induced following the method by [9]. Animals were administered 3 (multi dose) intra-peritoneal injections of streptozotocin (STZ) solution (Agscientific brand; T 858 452 9925, San Diego, CA 92121). The STZ solution was prepared at the very time for the purpose and administered at a dose of 35mg/kgb.w. in 0.1M cold citrate buffer & pH 4.5. Animal blood samples were collected by bleeding the animals via the tails after 72 hours, to determine blood glucose level using a glucometer (On-Call® Plus Model Number: G113-111). Animals were considered to be diabetic if the blood glucose levels were consistently above 250 mg/dl over a range of time [9].

Acute and sub- acute toxicity study of the extract

Lorke's method and OECD 407 guidelines were followed to determine acute oral toxicities of *A. chinensis* extract. Wistar rats, both male and female, were randomly selected from the experimental pool to perform this test [3; 10].

Acute toxicity studies

Lorke's method [3] was performed through a two-phase process. In Phase one, 9 animals were used. Divided into three groups of 3 animals with each group being administered a different dose (100, 500 and 1,000 mg/kg) of plant extract. The animals were then observed over a 24 hours period to monitor their specific behavior as well as for mortality. In Phase Two, only 3 animals, were used having been distributed into 3 groups of one animal each. They were administered higher doses at (1600, 3200 and 5000

mg/Kg.bwt) of plant extract and then also observed for 24 hours for behavior as well as mortality [3]; as shown in Table 2 below.

Table 2: Lorke's method animal groupings for acute toxicity study

Phase	Animals S.No.	Body weight	Injectable Volume	Animal Death	Observations
Phase One	**Group 1 (100mg/kg)**				
	1	135.6	0.14		
	2	147.2	0.15		
	3	117.8	0.12		
	Group 2 (500mg/kg)				
	1	148.2	0.74		
	2	132.2	0.66		
	3	119.3	0.60		
	Group 3 (1000mg/kg)				
	1	155.8	1.60		
	2	116.9	1.20		
	3	137.7	1.40		
Phase Two	**Group 1 (1600mg/kg)**				
	1	160.5	1.30		
	Group 2 (3200mg/kg)				
	1	155.6	2.5		
	Group 3 (5000mg/kg)				
	1	166.4	4.1		

Sub- acute toxicity studies

The principle of this test according to OECD – 407 guidelines is that for a time period of 28 days, a test drug is administered orally in progressive dosages to multiple groups of experimental animals, with one dose level for each group [10]. The starting dose for sub-acute studies was selected based on OECD – 407 guidelines in consideration of the LD50 from the acute toxicity studies. The protocol guides that toxic effects, but not upto severe suffering or death, should be the goal of the greatest dose level. Following that, a descending dose-level sequence should be elected with the goal of showing any dosage-related response and no side effects at the least dose level [4].

Based on the OECD – 407 dose selection guidelines mentioned above, three descending doses where chosen; Low dose group - 200mg/kg bwt,; Medium dose group – 400mg/kg bwt; High dose group – 800mg/kg bwt. The animals were observed very closely every day for any toxicity signs.

Observations for toxic effects

Twice each day, all of the animals were checked for illness and mortality. Any aberrant physical or behavioral changes were also examined. Changes in the eyes, skin, mucus membrane, fur and autonomic effects such as pupil size, lacrimation, piloerection, and a typical patterns of breathing were also seen. If any such symptoms occurred, the time of onset, intensity, and duration were recorded. All animals had their eyes examined prior to the start of the tests and the day before they were euthanized. At least once a week, all of the animals were weighed. Food consumption was also measured at least once a week. All observations were performed as guided by OECD guidelines 423 (2001) [1].

Evaluation of hematological parameters

A method used in an earlier study by [11; 36] was applied in our study. Here, after elapse of our 28 day duration of study period, the animals were fasted overnight.

The following morning, each animal was anaesthetized using halothane and seven milliliters of blood were collected by application through cardiac puncture method into 2ml vacutainers laced with heparin and non heparinised vacutainers (5mls). The heparinised samples of blood collected were analysed using an automated hematology analyser (PE 6000). White blood cells (WBC) count, hemoglobin (HgB) levels, red blood cells (RBC) count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW-CV), platelet (PLT) count, mean platelet volume (MPV), platelet distribution width (PDW) and procalcitonin (PCT) levels were analysed. The hematological evaluation was conducted at the Institute of Biomedical Research (IBR) Laboratory of Kampala International University Western Campus (KIU WC) and at Kampala International University -Teaching Hospital laboratories basing on methods by [12; 11; 36].

Data analysis

Data was entered in MS Excel, then exported to STATA (Version 15, Stata Corp LLC, College Station, Texas 77845-4512) software for statistical analysis. Experimental results were analyzed by one way analysis of variance (ANOVA). All results were expressed as Mean ± SD and a statistical significance was considered at $p \leq 0.05$. The Benforoni post-hoc test was used to identify the location of the differences across groups.

RESULTS

Percentage yield

Since the mass of dry extract = 173.3 g and the mass of powdered material used in the extraction was 540 g, the percentage yield (%) of the extract was calculated using the equation below:

$$\text{Percentage yield (\% age yield)} = \frac{\text{Weight of concentrated extract}}{\text{Weight of the plant powder}} \times 100$$

Implying that percentage yield (% age yield) = (173.3/540) x 100 = 32.1%

Acute toxicity studies

Lorke's method was used to conduct the acute toxicity investigation, and the findings are shown in Table 3 below.

Table 3: A table showing the observations for acute toxicity

Phases	Animals S. No.	Body wgt	Inj. Vol	Animal Death	Observations
	Group 1 (100mg/ kg.bwt)				
	1	135.6	0.14	No	• Reduced activity after 1.5 hours of drug administration
	2	147.2	0.15	No	
	3	117.8	0.12	No	
Phase One	**Group 2 (500mg/ kg.bwt)**				
	1	148.2	0.74	No	• Reduced activity after 1 hour of drug administration
	2	132.2	0.66	No	
	3	119.3	0.60	No	
	Group 3 (1000mg/ kg.bwt)				
	1	155.8	1.60	No	• Reduced activity
	2	116.9	1.20	No	• Sedation
	3	137.7	1.40	No	• Mild diarrhea
	Group 1 (1600mg/ kg.bwt)				
	1	160.5	1.30	No	• Reduced activity • Sedation • Diarrhea • Pilo erection
Phase Two	**Group 2 (3200mg/ kg.bwt)**				
	1	155.6	2.5	No	• Reduced activity • Sedation • Diarrhea • Pilo erection
	Group 3 (5000mg/kg.bwt)				
	1	166.4	4.1	No	• Reduced activity • Difficulty in breathing • Diarrhea • Pilo erection

After 24 hours of observing the test animals, there was no fatality recorded. All animals had regained normalcy at the end of 24 hours after plant extract administration.

Calculation of LD_{50}

Using Lorke's method, LD_{50} is calculated using the formula:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$

Where:

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality.

Therefore, with the highest dose used (5000mg/ kg. bwt) having caused no mortality, the LD_{50} of the plant extract was deemed to be greater than 5000mg/kg.bwt.

LD_{50} = >5000mg/kg.bwt

Sub – acute toxicity studies

After 28 days of experimentation, the animals were sacrificed to perform hematological, biochemical and histo-pathological evaluations on the blood and organ tissues and the results are presented in the subsections below.

Hematological evaluation

The results for white blood cell (WBC) count, hemoglobin (HgB) levels, red blood cells (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW-SD) and red blood cell distribution width (based on both width of distribution curve and mean cell size)(RDW-CV), platelet (PLT) count, mean platelet volume (MPV), platelet distribution width (PDW) and procalcitonin (PCT) levels are presented in Table 4 below:

Table 4: The descriptive analyses of hematology parameters

Blood parameters	p-value	Control Group (1 ml DW) Mean ± SEM	Low dose (200mg/kg) Mean ± SEM	Medium Dose 400mg/kg Mean ± SEM	High Dose 80mg/kg Mean ± SEM
WBC	0.8444	8.14±1.17	7.42±1.12	8.57±1.06	8.18±1.12
HgB	0.2442	12.38±1.13	14.44±1.03	14.75±1.01	14.19±1.01
RBC	0.4827	10.47±1.44	8.23±1.03	8.03±1.02	7.18±1.02
HCT	0.8156	45.6±1.07	44.11±1.02	45.78±1.01	44.09±1.02
MCV	0.375	48.14±1.25	59.11±1.01	57.12±1.01	61.53±1.02
MCH	0.3067	21.46±1.12	19.32±1.01	18.35±1.01	19.73±1.01
MCHC	0.4572	27.01±1.2	32.70±1.01	32.18±1.00	32.14±1.01
RDW-CV	0.9409	15.18±1.19	14.37±1.02	15.52±1.01	15.03±1.01
RDW-SD	**0.0016**	30.80±0.28	32.8±0.54	**33.14±0.54**[a]	**34.26±0.60**[b]
PLT	0.0718	712.2±35.8	792.0±56.8	829.8±28.8	700.4±13.1
MPV	**0.0022**	6.37±1.03	**6.96±1.01**[c]	**7.10±1.01**[d]	**6.88±1.02**[e]
PDW	0.1521	15.46±0.09	15.46±0.08	15.36±0.06	15.66±0.12
PCT	**0.0056**	0.45±0.03	0.55±0.04	**0.59±0.02**[f]	0.48±0.01

Values are expressed as Mean ± SEM for *N*= 5. Values were considered significant if (*P* < 0.05).

When one-way analysis of variance (ANOVA) was performed, RDW-SD (p=0.0016), MPV (p=0.0022) and PCT (p=0.0056) showed statistically significant mean differences. This indicated that the extract at 200mg/kg bwt and above, caused a statistically significant increase in mean platelet volume (MPV) that at 400mg/kg bwt and above, caused a statistically significant increase in red blood cell distribution width (RDW-SD) while that 400mg/kg caused a statistically significant increase in procalcitonin (PCT) levels. When a Bonferroni post-hoc test was performed to determine where the differences truly came from; **a.**-p=0.029; **b.**-p=0.001; **c**.-p=0.014; **d.**-p=0.002; **e**.-p=0.038; **f.**-p=0.009; there were statistically significant mean differences between the control group and the treatment group. (Adjusted p-values where the post hoc test was used). This implies that the observed differences of RDW – SD of the medium and high dose and the MPV of all groups as compared to the untreated group is very unlikely to be due to chance.

DISCUSSION

Yield of the extraction process

A technique for extracting high-yield extracts with little modifications to the extract's functional characteristics would be ideally the most preferred extraction technique as biological activity of extracts generated utilizing various extraction procedures have been described in several investigations [12, 13].

In this study, we used aqueous extraction technique of the *Albizia chinensis* stem back in an effort to mimic the extraction of the plant for use in the management of diabetes mellitus by the communities as reported by Ssenyange and colleagues [1]. Having obtained a percentage yield of 32.1%, it is considered to be a relatively good out considering the fact that aqueous extraction of phytochemicals does not usually give good yields [14].

Further still, the method of extraction used has been reported before to be able to achieve extraction of water soluble phytochemicals like flavonoids, saponins, glycosides, alkaloids and terpenoids [14; 15]. Therefore, having obtained positive antidiabetic effect of the plant extract in the study, and all those five

phytochemicals compounds having been confirmed to elicit antidiabetic activity [16; 17; 18], its highly likely that the technique used for extraction was able to obtain some or all of the water-soluble compounds that are known to have antidiabetic effect, although this cannot be definitely ascertained without further studies. A good yield from the plant implies that it has good potential for commercialization by the community, following confirmation of efficacy and safety. This is because many times bioprospecting for drugs from plant sources tends fail due to limited yield when the locally used extraction techniques are simulated.

Acute toxicity of crude aqueous stem bark extract of *Albizia chinensis*

The main goal of analyzing the safety of any medicinal plant is to determine the nature and significance of any adverse effects, as well as the exposure level at which they occur [19]. The acute toxicity test results of this study show that the aqueous extract of *Albizia chinensis* stem bark given orally to rats at concentrations of 100, 500, 1000, 1600, 3200, and 5000 mg/kg using Lorke's method did not induce any mortality in the rats implying that the LD_{50} of *Albizia chinensis* stem bark is above 5000mg/kg. This implies that the plant has a high safety range making it safe for use at least in terms of acute toxicity. This finding is consistent with other studies that have reported high LD_{50} values for other members of the Genus *Albizia* with species like *Albizia falcataria, Albizia chevalieri, Albizia odoratissima, Albizia saman, Albizzia lebbeck, Albizia gummifera* and *Albizia coriaria* [20-,21,22,23]. However, there were signs of some negative acute effects at all doses with reduced activity of the animals being observed from over one hour of administration and lasting close to an hour, coupled with signs of sedation. Observation of these toxic signs may be attributed to the confirmed presence of high levels of flavonoids and saponins in *Albizia chinensis* having been reported in previous studies [15].

Flavonoids and saponins have been confirmed to elicit sedative – hypnotic effects on the human system [24], and therefore, observing sedative – hypnotic effects of the stem bark extract of *Albizia chinensis* in this study may be attributed to the presence of these phytochemicals. This implies that the plant extract has some central nervous system effects especially in terms of enhancing the inhibitory pathways such as the GABA by either acting on the GABA binding site or the allosteric site for GABA agonists. Alternatively, the CNS depressant effects could be a result of antagonistic effects against the excitatory neurotransmitters such as glutamic acid and aspartate through inhibition of the N-methyl-D-aspartate receptors or by suppressing monoamine activity, among other CNS effects [24,25]. Considering the fact that a number of clinically used drugs is known cause sedative hypnotic effects, this might not be a hinderance to the use of the plant extract for antidiabetic activity as long as the sedative effect is transient. However, this can be a cause of concern since sedative-hypnotic drugs are known to cause addiction when used for a long time [25,26].

Also, to note are the signs of piloerection observed in the animals at all doses above 1000mg/kg bwt (In 1600mg/kg bwt, 3200mg/kg bwt and 5000mg/kg bwt). Piloerection is an autonomic nervous system (specifically, sympathetic or adrenergic nervous system) reaction to thermal regulation or in response to an “adrenaline rush” (the “fight-or- flight” response) that comes as a result of contraction of the *musculi arrectores pilorum* smooth muscle that stretches from the dermis' fibrils into the hair follicle's connective tissue investment [26]. Piloerection at high doses that was not observed in the lower doses could be as a result of autonomic nervous system toxicity that triggers off the physiological mechanism that causes the effect. This calls for further investigation to ascertain the real cause of this reaction.

Similarly, diarrhea was observed in the animals at all doses above 1000mg/kg bwt. Available literatures shows that diarrhea is one of the Functional Bowel Disorders that occurs as a result of dysfunction of the autonomic nervous system [27]. Therefore,

it is highly likely that the observed diarrhea is as a result of toxicity of the autonomic nervous system as also suggested by other scholars [28; 27]. Diarrhoea can be induced by causing the release of nitric oxide thereby increasing permeability of the gastrointestinal membrane to calcium ions; by stimulating prostaglandin synthesis or release thereby increasing fluid and electrolytes int the lumen of the intestine; and by increasing GIT motility (peristaltic movements) thereby reducing the chances for water absorption. This also requires further investigation to establish the mechanism by which the extract causes diarrhoea and which particular phytochemicals are responsible before a definite recommendation can be made about the plant.

Sub-acute acute toxicity of the crude aqueous stem bark extract of *Albizia chinensis*

The implications of the results of hematological, biochemical and histopathological assessments following 28 days of repeated administration of the crude aqueous stem bark extract of *Albizia chinensis* to normal Wistar rats in varying doses are discussed in the sections that follow.

Hematological evaluation

The hematological parameters analysed were WBC, HgB, RBC, HCT, MCV, MCH, MCHC, RDW-CV, PLT, MPV, PDW and PCT. When one-way analysis of variance (ANOVA) was performed only three of the parameters namely; RDW-SD (p=0.0016), MPV (p=0.0022) and PCT (p=0.0056) showed statistically significant mean differences. When a Bonferroni post-hoc test was performed to determine where the differences truly came from; medium dose (p=0.029) and high dose (p=0.001) for RDW-SD; p=0.009 for PCT in medium dose and they were statistically significant mean differences between the negative (non-treated) control group and the treatment groups across the board for MPV. The significant difference of RDW-SD shown by *Albizia chinensis* in this study is consistent with effects of *Albizia mahalao* and *Albizia chevalieri* on red blood cells of test animals in two previous studies [29; 20].

Since too high RDW has long been thought to be a sign of iron, folate, or vitamin B-12 deficiency, this result could potentially indicate macrocytic anemia, which occurs when the body creates too many big red blood cells rather than enough normal red blood cells. A high RDW is a sign of systemic inflammation in the body, as well as a symptom of various kinds of anemia [29]. It has also been reported that variation of RDW-SD and RDW-CV may significantly affect the breathing capabilities of an animal [30]. Therefore, with variation of this parameter being consistent with increase in the dose of administration, it may be probable that the difficulty in breathing observed with the highest dose administered (5000mg/kg.bwt) during acute toxicity studies may have been as a result of the significant variation of RDW-SD. Toxic effects that involve alteration of the functioning of the immune system as well as the respiratory system a cause for concern since they can have very serious consequences on a diabetic patient, a condition that tends to compromise immunocompetence. This calls for more studies to determine the mechanisms involved in this observed effect and the dose-response relationships so that measures can be devised against the toxicities before a definite recommendation can be made about the use of the plant.

Mean platelet volume showed a significant mean difference in this study and the Benforoni post hoc tested showed the difference was exhibited across all treatment groups. This significant effect of *Albizia chinensis* extract on the MPV parameters of the animals is consistent with the reporting of the same effects of *Albizia julibressin* on platelets of animals [31,32,33,34,35,36]. This significant effect of *Albizia chinensis* may be attributed to the confirmed presence of flavonol glycosides isomers quercitrin and isoquercitrin [15,37,38,39,40,41], which were reported to have anti-platelet activity [32,33,34, 39,40,41]. Antiplatelet activity implies that the extract can interfere with the blood clotting process which may present a risk to the patient on the product derived from this plant. Again, there is

need to carry out further investigation to ascertain the mechanism of antiplatelet activity as well as the exact chemical causing it before a definite recommendation can be made about the plant.

Pre-calcitonin (PCT) levels also showed a statistically significant mean difference (p=0.0056) with the difference being located in the medium (400mg/kg) dose (f – p=0.009). Pre-calcitonin is a known amino acid precursor that mainly is an indicator of tissue damage. This PCT variation in this study is further supported by the inflammation observed in the photomicrographs of this particular treatment group which points to the fact that PCT levels may increase in cases of tissue inflammation [35]. However, a conclusion in this study about the cause of this variation is not possible as the difference was shown to be exhibited by only the medium dose of which the tissue inflammation which could cause the variation of the PCT levels cannot be attributed to the dose for now. Another reason further studies are needed in this respect.

CONCLUSION

The results of this study have shown that the aqueous stem bark extract of *Albizia chinensis* has an LD_{50} above 5000mg/kg, which puts it in the safe range although some acute toxic effects like reduced activity, sedation and autonomic effects such as diarrhea and piloerection were observed. The study has further shown that the aqueous stem bark extract of *Albizia chinensis* is relatively safe at sub-acute level with no significant effects on the studied hematological parameters. Although at high doses the plant may affect the hematological profile affecting red blood cell parameters, mean platelet volume and procalcitonin levels and showing some relative inflammatory infiltration which may lead to tissue damage.

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