Subchronic toxicity of aqueous extract of *Alstonia boonei* de wild. (apocynaceae) stem bark in normal rats

Barnabé Lucien Nkono Ya Nkono 1,2*, Selestin Dongmo Sokeng 2, Dzeufiet Djomeni Paul Désiré 3, Longo Frida 1, Pierre Kamtchouing 3

1 Department of Biological Sciences, Higher Teacher Training College, University of Yaounde 1, Cameroon
2 Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, Cameroon
3 Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1, Cameroon

*Corresponding author E-mail: luciennkono@gmail.com

Abstract

Methodology: Wistar rats were randomly assigned into eight groups of five animals each: four male groups and four female groups. Each sex group had a control group receiving distilled water and three test groups receiving 200, 500 and 1000mg/kg respectively. Animal’s body weights were recorded on the first day and once a week for the four experiment weeks. The hematological analysis included total WBC count, total RBC count, Hb, %HCT, MCV, MCH and MCHC. Biochemical/serum profile studies include TG, TC, ALT, AST, urea and TP. Tissue specimens of the liver, kidney and lung were subjected to histological examination using standard hematoxylin-eosin staining.

Results: In male rats, aqueous extract showed significant decreases in relative weight of liver with extreme significance P<0.001 at a dose of 200mg/kg (vs. control group), P<0.001 of lung at all the doses, P<0.05 (200 and 500mg/kg) and P<0.01 (1000mg/kg) in heart weight. In relative kidney weight, only the dose of 1000mg/kg showed a significant increase vs. normal control male rats. Unlike male rats, only relative kidney weight in female rats was significantly different from the control group in a dose-dependent manner. The aqueous extract treated male groups showed significant increases P<0.001 (1000mg/kg) of total WBC count and MCHC, significant decreases of %HTC (dose response manner), P<0.05 total RBC count (at doses of 500 and 1000mg/kg) and Hb P<0.01 (500mg/kg) vs. normal male rats. In female rats, the haematological study showed significant increase P<0.01 of total WBC count (at the doses of 500 and 1000mg/kg), significant decreases P<0.05 and P<0.01 of total RBC respectively at the doses of 200 and 1000mg/kg, significant decrease of Hb with extreme significance P<0.001 at the dose 1000mg/kg, %HTC also decrease dose response manner vs. control female rats. Biochemical study showed in male rats significant decreases in level of TG P<0.001 (at the doses of 200 and 500mg/kg) and urea, although it showed any dose-dependent effect vs. control male rats. AST also decreases (P<0.05) in male rats at a dose of 500mg/kg but significantly increase P<0.001 at the dose of 500mg/kg. In the female rats, biochemical study revealed significant increases in level of TG P<0.001 and urea P<0.01 at the dose of 200mg/kg and significant decreases in level of TG P<0.01, AST P<0.05 and urea P<0.05 at the dose of 500mg/kg (vs. control female rats). Microscopically, there were mild hepatic and renal tissue injuries supporting the hematological analysis.

Conclusion: The results indicated that aqueous extract of *Alstonia boonei* De Wild is toxic in high doses.

Keywords: *Alstonia boonei*; Aqueous Extract; Rats; Subchronic Toxicity.

1. Introduction

*Alstonia boonei* De Wild. belongs to the family of Apocynaceae, with about 50 species widely distributed in Africa, Asia and America’s continents (Iwu 1993). *A. boonei* possess antihyperglycemic and antioxidant properties (Nkono et al. 2014), and it has been reported that the stem bark is used in traditional medicine to treat fever, painful micturition, insomnia, chronic diarrhea, rheumatic pains, as antivenom for snake bites and in the treatment of arrow poisoning (Olivier-Bever 1986, Asuzu & Anaga 1991).

In as much as many health problems have been solved using medicinal plants, a number of them presents toxic effects when not properly prepared or dispensed (Dalsiel 1997). As such a scientific approach needs to be applied towards the use of plant extracts in managing ailments, especially in the developing countries where the level of literacy is low and the status of health management is poor, and about 80% of the population patronized herbal drugs.

In our previous studies (Nkono et al, 2014), a single administration of *A. boonei* aqueous extract showed no toxicity, and the LD50 value was greater than 5000mg/kg. In their own studies with ethanolic stem bark extract of *A. boonei*, Oze et al. (Oze et al. 2007) reported moderate nephrotoxicity in guinea pigs. The kidney is the primary organ for clearance and excretion of xenobiotics, including drugs and drug products from the body. Kidney damage could arise due to the administration of plant extracts, but there is paucity of scientific information because the incidences of toxicity in local settings are hardly reported or documented. The present work aims to evaluate the subchronic toxicity of *A. boonei* De Wild. stem bark aqueous extract in rats.
2. Materials and methods

2.1. Collection of plant material

Fresh stem bark of *A. boonei* was collected during November 2012 from Ombessa, Center Region of Cameroon. The plant was identified by Dr. B. Kengeu and a voucher specimen (N° 43368HNHC) has been deposited at the National Herbarium of Cameroon (Nkono et al. 2014). After collection, fresh stem bark was washed thoroughly with water in order to remove any type of contamination, and then dried at room temperature. The dried stem bark was milled and preserved in airtight glass container at room temperature (25°C).

2.2. Extraction

A ratio of three hundred grams (300g) of *A. boonei* stem bark powder was weighed and extracted in 3000mL of distilled water by decoction for 30 min. The aqueous extract obtained by filtration (2500mL) was lyophilized and thereafter the paste (18.40g, yield: 9.46%, w/w) preserved at room temperature for further uses.

2.3. Experimental animals

Wistar rats weighted between 87-93g of either sex were used for subchronic oral toxicity study. Animals were maintained under standard husbandry conditions (temperature 25±2°C; 12h light: 12h dark cycle) and fed with standard pellet diet (Kamgang et al. 2005) and water ad-libitum. All animal experiments were handled according to the Cameroon National Ethics Committee (Ref. N° FWIRB 0001954) and all experiments have been examined and approved.

2.4. Subchronic oral toxicity

Toxicity studies were performed according to the OCDE guideline (OCDE 1995). Animals were divided into 8 groups of 5 animals each with 4 male groups (1, 3, 4, 5), and 4 female groups (2, 6, 7, 8). Vehicle and *A. boonei* were orally administered, daily for 4 weeks as shown below:

- Groups 1 & 2: Control: received distilled water (10ml/kg b.w)
- Groups 3 & 6: received *A. boonei* (200mg/kg b.w)
- Groups 4 & 7: received *A. boonei* (500mg/kg b.w)
- Groups 5 & 8: received *A. boonei* (1000mg/kg b.w)

The animals were sacrificed by cervical dislocation one week respectively after vehicle and aqueous extract administration, and arteriovenous blood was collected to the biochemical and haematological studies, while liver, kidneys and lungs were collected to histopathological studies. The relative weight of internal organs was calculated as follow:

\[
\text{Relativerweight of organ} = \frac{\text{Organweight}}{\text{Bodyweight}} \times 100
\]  

(1)

2.5. Determination of biochemical and haematological parameters

Lipid profile (Total cholesterol and triglyceride), urea and total protein were estimated using diagnostic kits, Fortress-UK. transaminases activities (ALT and AST) were determined using the method of Reitman and Frankel (Reitman & Frankel 1957). The hematological studies (total WBC count, total RBC count, Hb, %HCT, MCV and MCHC) were conducted according to the method of Ghai (Ghai 2004).

2.6. Histopathological studies

Histopathological studies of the liver, kidneys and lungs were carried out on control and experimental groups. Biopsies of each organ were fixed in 10% formalin, embedded in paraffin, sliced 4 μm thick and stained with hematoxylin and eosin (H&E), and then assessed for pathological changes (Kasote et al. 2012).

2.7. Statistical analysis

The values were expressed as means ± standard errors (S.E.M). Statistical analysis were calculated using “Graph Pad, Prism” software. One-way analysis of variance (ANOVA) followed by post-hoc test (Student-Newman-Keuls) were used to analyze the data. The criterion for statistical significance was P<0.05, and extreme significance was P<0.001.

3. Results

3.1. Variation of body weight

On male rats, after the first treatment week, *A. boonei* induced a significant (P<0.001) dose dependent decrease of the body weight gain (vs. control group) at all doses. From week2 to week4, *A. boonei* significantly P<0.001 and P<0.01 increased the body weight gain respectively at doses 200mg/kg (6.19%) and 500mg/kg (5.87%) (Vs. control group). The dose of 1000mg/kg showed a significant (P<0.05) increase, only at week 3 (vs. control group) (Fig. 1A).

On female rats, *A. boonei*, at all doses, showed a significant (P<0.001) body weight gain decrease (vs. control group) during the 4 treatment weeks. The decreases were up to -9.47%, -10.52% and -14.73%, respectively for the doses 200, 500 and 1000mg/kg b.w. at the end of experimentation (Fig.1B).

![Fig. 1: Subchronic Effect of the Aqueous Extract of *A. boonei* on Body Weight in Male (A) and Female (B) Rats. Test Drugs: Significant from Normal Control, *P*<0.05; *P*<0.01; *P*<0.001. Significant From the Initial Value *P*<0.01; *P*<0.001. Significant from the Week 4 Value *P*<0.001. Mean ± S.E.M = Mean Values ± Standard Error of Means of Five Experiments.](image-url)
P<0.001 and the heart P<0.01 (200 and 500mg/kg b.w.) and P<0.05 (1000mg/kg b.w.) (Fig. 2A).

On female groups, A. boonei showed no significant (P>0.05) effect on the liver, lung and heart, while on the kidney, the extract, at all doses, showed significant increases (vs. control group), especially at the dose 1000mg/kg, which presented the highest increase value (0.88g/100g of kidney) (P<0.001) (Fig. 2B).

The results of the effect of graded doses of aqueous extract of A. boonei stem bark on serum biochemical parameters of male and female rats in oral route are shown in Figure 3.

For other parameters, on male groups, A. boonei induced a significant (P<0.001) decrease of AST at doses 200mg/kg (134.6mg/dL) and 500mg/kg b.w. (132.8mg/dL), a significant decrease of urea at all doses (no dose-dependent effect), especially at the dose 500mg/kg b.w. (P<0.01) (49.15mg/dL), a significant decrease (P<0.01) of TP only at the dose of 1000mg/kg b.w. (vs. control group) (Fig. 3 A).

On female groups, A. boonei induced dual effects on TG and urea according to doses: at the dose 200mg/kg (b.w.), the extract increased both parameters values 145.1mg/dL and 120.1mg/dL. 3mg/dL respectively to TG (P<0.001) and urea (P<0.01) and decreased them at dose 500mg/kg b.w. 120.1mg/dL for TG (P<0.01) and 49.01mg/dL for urea (P<0.05). The extract also decreased AST significantly (P<0.05) only at the dose 200mg/kg b.w. (Fig. 3B).

Fig. 2: Effect of the Aqueous Extract of A. boonei on Relative Weight of the Internal Organ in Male (A) and Female (B) Rats.
Test Drugs: Significant from Normal Control, a P<0.05; b P<0.01; c P<0.001. Mean ± S.E.M = Mean Values ± Standard Error of Means of Five Experiments.

3.4. Haematological parameters
The results of the haematological study in male rats (Fig. 4A) indicated a significant decreases (P<0.05) in the total RBC count at the doses of 500 and 1000mg/kg b.w, a significant decrease (P<0.01) in Hb at the dose of 500mg/kg, although it showed any dose-dependent effect. In the same order, the percentage of HTC also decreased significantly but unlike the total RBC count and the Hb, it was dose-dependent. The extract also caused a significant increase in the total WBC count P<0.001 and MCHC P<0.05 only at the dose of 1000mg/kg b.w of the aqueous extract and significant decreased P<0.01 in the total WBC count only at the dose of 200mg/kg b.w (vs. control group).
The results of the haematological study of the female rats as showed in Figure 4B indicate a significant increases P<0.01 in the total WBC count at doses of 500 and 1000mg/kg b.w. (vs. control group), significant decreases the total RBC count P<0.05 and P<0.01 respectively at the doses of 200 and 1000mg/kg b.w. (vs. control group), Hb also decrease significantly but non-dose dependent unlike the HTC percentage which decreased significantly dose-dependent manner (vs. control group).
Fig. 4: Effect of the Aqueous Extract of A. boonei on Haematological Parameters in Male (A) and Female (B) Rats.
Test Drugs: Significant from Normal Control, *P<0.05; **P<0.01; ***P<0.001. Mean ± S.E.M = Mean Values ± Standard Error of Means of Five Experiments.

3.5. Histopathology of liver, kidney and lung

The results of microscopic examination of liver sections of animals from control group showed normal liver architecture (Fig. 5A). On the liver sections of male rats, only A. boonei at the dose of 1000mg/kg b.w. (Fig. 5D) exhibited dilatation of capillary caused by infiltration of macrophages. On female rats, the dose of 500mg/kg b.w. showed necrosis, congestion and dilatation, lymphocytic and granulocytic infiltration of portal area (Fig. 5F) while, the dose of 1000mg/kg b.w. showed pyknotic and nuclear degeneration characterized by lymphocytic and granulocytic infiltrations (Fig. 5G).

On the kidneys, sections treated with A. boonei 1000mg/kg b.w. induced damage only in female rats, characterized constriction of the glomerulus and increase of the Bowman space. Moreover, hyperplasia of cells around tubules appeared with hemorrhage and lymphocytic infiltration, as well as necrosis of the cells (Fig. 5G).

Histopathological results of the effects of A. boonei on male (Fig. 7B, C&D) and female groups (Fig. 7E, F&G) showed no difference in the morphology of alveoli, bronchi and bronchial wall at all doses vs. control (Fig. 7A).
effect of the ethanolic extract of A. boonei stem bark. Although in female rats there was no significant difference in the same doses. Serum biochemical markers are generally employed to assess liver function. Estimation of serum enzymes AST and ALT is the quantitative marker for the determination of type of liver and body diseases. It is established that ASAT can be found in the liver, cardiac muscle, skeletal muscle, and so forth, whereas ALAT is predominantly present in the liver (Rej 1997, Tsague et al. 2015). In the present study, the increased level of serum AST in A. boonei at 500mg/kg b.w. (P<0.001) male group indicate an increased permeability of hepatocytes. The decreased level of AST P<0.05 both in male and female rats respectively at doses of 200 and 500mg/kg b.w. of aqueous extract and non-significant difference of ALT (P>0.05) vs. control groups at all doses (male and female) suggest that A. boonei aqueous extract supports the beneficial effects of this plant on liver function (Ojo et al. 2014). The increase of total WBC count may be a consequence of inflammatory reactions produced in damaged tissues of extracts treated animals and the anemia (reduction of red blood cells and %HCT) may be due to bone marrow suppression (Mohajeri et al 2007). In our study, A. boonei aqueous extract at 1000mg/kg b.w. increased the level of total WBC count P<0.001 and decreased %HCT P<0.001 in male rats. A similar result was observed in female rats at the dose of 1000mg/kg b.w., increased level of total WBC count P<0.01 and decreased %HCT P<0.001. This result infers that, a subchronic administration of aqueous extract of A. boonei at a high dose could produce an inflammatory reactions and anemia.

4. Discussion

The present study was carried out to evaluate the subchronic toxicity of stem barks of A. boonei aqueous extract on male and female rats. The stem barks extract of A. boonei was reported to have a therapeutic importance in folk medicine. Apart from the lack of information about the adverse or toxicity effects of this plant extract, and despite its wide-spread use in folk medicinal or traditional practice, there is lack of information underlying biochemical mechanism responsible for some of the observable and reported properties of this plant.

The subchronic oral toxicity of aqueous extracts of A. boonei stem bark did not cause mortality in male and female rats. Nkono et al. (2014) reported that the acute oral toxicity of A. boonei aqueous extract at the doses of 2000mg/kg b.w., and 5000mg/kg b.w showed no toxicity signs. All animals included in the test were healthy after the 14-day observation period. The LD50 value of A. boonei was considered greater than 5000mg/kg b.w. The highest dose of the extract used for this study (1000mg/kg b.w.) was not greater than 1/5 of the highest dose (5000mg/kg b.w.) used in the acute oral toxicity study of Nkono et al. (2014). In a dose-dependent manner, the aqueous extract of A. boonei stem bark significantly increased the percentage of the body weight gain in male rats vs. control. However, significantly reduced dose dependent the percentage of the body weight gain of female rats. This result suggested that, the decrease in the body weight of female rats treated for 4 weeks with the aqueous extract could be due to the activities of the diuretics and diuretobiotics (Kucera et al. 1972, Maurica 1993). The diuretic activities of these constituents could have resulted in loss of water and electrolytes and consequently, loss in body weight in the female rats (Raji et al., 2005).

The results from this study suggest that A. boonei could cause impairment of the kidney at high doses in the male than in female rats, with more advanced sensitivity in female rats. The kidney is the primary organ for clearance and excretion of xenobiotics including drugs and drugs’ products from the body. The study of the effect of the plant extract on the kidney is essential because of the cardinal role that the organ plays in plasma clearance, some detoxification process, homeostasis and excretion of xenobiotics (Awodele et al. 2010). A. boonei stem bark (200, 500 and 1000mg/kg b.w.) significantly reduced the relative weight of heart, liver and lungs of male rats vs. control rats. This result is similar to studies of Ojo et al. (2014) who reported the hepatoprotective action of this plant on the liver of rats. This result is similar to studies of Ojo et al. (2014).

5. Conclusion

The present study concludes that the aqueous extract of A. boonei De Wild stem bark is toxic in high doses. Thus, reduction in the given doses could prevent the toxic effects produced. Therefore, aqueous extract of A. boonei stem bark appears to have no order of toxicity when ingested in therapeutic amounts. Finally, it’s suggested that the toxic chemical constituents of the aqueous extract of A. boonei stem bark has to be identified in order to study how to neutralize them.

Acknowledgement

The authors thank Professor Niemenack Nicolas, Associate Professor of the Department of Biological Sciences at the Higher Teacher-Training College, University of Yaounde 1- Cameroon, for lyophilizing the aqueous extract. The authors are also grateful to Mr. Dibari, traditional practitioner residing Ombessa (Central Region of Cameroon), for providing plant material and indicate its probable toxic effects in folk medicine.
Competing interests

Authors have declared that no competing interests exist.

Ethical approval

All authors hereby declare that “Authorization for the use of laboratory animal” was obtain from the Cameroon National Ethics Committee (Ref. N° FWIRB 00001954), all experiments have been examined and approved.

References

[16] Osu GO, Nwango HE & Onyeze GO (2007), Nephrotoxicity caused by the extract of Alstonia boonei (De wild) stem bark in Guinea pigs, The Internet Journal of Nutrition Wellness 3(2).