ORIGINAL ARTICLE

Effects of Aqueous *Quassia amara* L. (Korales) Leaf Extract on the Cardiovascular and Respiratory Functions of Male Sprague-Dawley Rats

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ABSTRACT

Objective. To evaluate potential effects of the aqueous extract of *Quassia amara* L. leaves on the cardiovascular and respiratory systems of adult male Sprague- Dawley rats.

Methods. The cardiovascular and respiratory effects of the *Quassia amara* L. leaf extract on adult male Sprague-Dawley rats were assessed using non-invasive blood pressure (NIBP) determination and head-out plethysmography, respectively, in a randomized, parallel group study. Mean observations of blood pressure and heart rate were recorded at different time periods after dosing. Respiratory flow and irritation effects were evaluated using mean observations of respiratory rate (RR), tidal volume (TV), mid-expiratory flow rate (EF50), time of inspiration (TI) and expiration (TE), and time of break (TB) and pause (TP).

Results. There were no significant differences among the control and the treatment groups in SBP, DBP and HR parameters. The extract showed statistically significant effect on mean RR by time period (F=2.45, p=0.0234), trends over time of TV among the dose groups (F=2.00, p=0.0202), and EF50 among dose groups ((F=3.11, p=0.0422). However, these did not correlate with the changes in the time of break (TB) and time of pause (TP) which are more sensitive and specific tests for respiratory irritation.

Conclusion. Aqueous leaf extract of *Quassia* appeared to have no significant effects on SBP, DPB, Pulse pressure, and HR. There are no conclusive dose-related respiratory flow or pulmonary irritation effects.

Key Words: Quassia amara, cardiovascular effects, respiratory effects

INTRODUCTION

There is an impetus to use cost-effective and affordable drugs in the management of common illnesses in developing nations including the Philippines. Discovery and in-depth study of easily accessible herbal medicinal plants can empower the marginalized in treating common ailments. Moreover, it may herald discovery of novel molecules that can contribute to worldwide health.

Korales (*Quassia amara*) is a small tree indigenous to South America but is presently used as an ornamental plant in the Philippines. It is traditionally used for malaria and other parasitic diseases. Different (wood, bark, sap, leaf) extracts have been subjects of preclinical studies.[1-3] A local research testing the anti-ameobic, antimalarial, antihelmintic and anti-giardia properties of *Quassia amara* has recently

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concluded. The latter is the first local study to be conducted on the species grown in the Philippines. (CC Maramba, personal communication, June 27, 2017)

Quassia amara is Generally Recognized as Safe (GRAS) by the US-FDA. Toxicity studies of bark (Brazil) ethanol extracts performed on rats and mice showed no toxicity in oral dosages up to 5 g per kg of body weight and up to 1 g/kg administered intraperitoneally. Although wood aqueous extract given intraperitoneally showed acute toxicity signs with a 24-hour recovery at 500 mg/kg; and 100% lethality to mice at 1000 mg/kg within 24 hours.[1,2] There is paucity of safety profile of the plant covering the major organ systems.

Safety pharmacology is a discipline that further probes, beyond the therapeutic targets, the important pharmacodynamic effects of substances that can help predict rare adverse effects, especially lethal events, and aid in regulation. It is an essential part of the drug development process.The cardiovascular, respiratory and nervous systems are included in the core battery of tests.[4-7] This study aims to assess potential effects of the aqueous extract *of Quassia amara L.* leaves on the cardiovascular and respiratory system using non-invasive tests on adult male Sprague- Dawley rats.

MATERIALS AND METHODS

This is a randomized, parallel group study, using 48 adult male Sprague-Dawley rats. The study was conducted at the Department of Pharmacology & Toxicology laboratory, University of the Philippines (UP) College of Medicine after approval from the UP Institutional Animal Care and Use Committee (IACUC).

A. Preparation of the Lyophilized Aqueous Extract of *Quassia amara* leaves

Quassia amara leaves were obtained at UP Los Banos. Extraction and Lyophilization was done at Department of Industrial Pharmacy, College of Pharmacy UP Manila.

The previously dried, powdered and weighed *Quassia amara* leaves were transferred into a suitable glass container. Enough distilled water was added to completely submerge the leaves. Additional distilled was added for about 1 inch from the top of the leaves. The solution was macerated for 24 hours with frequent shaking for the first five (5) hours and then allowed to stand for nineteen (19) hours. The solution was filtered rapidly using previously boiled cheesecloth, and filtrate set aside. The residue was washed with distilled water and filtered again. All the filtrate were combined and filtered again using Whatmann filter paper. The filtrate was freeze-dried, lyophilized extract weighed, and the percent yield computed. The preparation of the extract was done using autoclave, rotatory evaporator and freeze dryer.

B. Test Animals

The animals used were adult male Sprague-Dawley rats, weighing 100-250 grams, procured from a government-accredited animal facility. The rats were checked for endo – and ectoparasites and treated accordingly. The animals were housed at the DPT animal laboratory, one rat per cage, and subjected to one week acclimatization. Adequate lighting (fluorescent lamps 40 watts, lighted 9-10 hours a day), and adequate ventilation (electric and exhaust fans) were provided. Food and water were provided ad libitum except for periods when specific tests were done. The feed given was Purina Chow Dry dog food composed approximately of 24% protein, 11% fat, and 57% carbohydrates plus essential vitamins and minerals. The rats were fasted overnight prior to the conduct of the tests.

C. Acute Oral LD_{50} Determination of *Quassia amara*

Before the main study was conducted, an acute oral toxicity study was performed to determine the oral LD_{50} of the lyophilized aqueous leaf extract of the test substance in Sprague-Dawley rats. The study utilized 3 dose levels: 1.63 g/kg, 3.25 g/kg and 6.5 g/kg. Results showed that *Quassia amara* has an oral LD_{50} of 2.05 g/kg, a no observable adverse effect level (NOAEL) of lower than 1.63 g/kg and the maximum tolerated dose of 1.63 g/kg. The toxidrome consisted of decreased motor activity 15 minutes after drug administration, resumption of activity 30 minutes after drug administration. Death occurred 6 hours after the drug has been administered.

D. Study Groups, Test Substance and Dose Levels

Based on the LD_{50} value, three treatment groups were used with the following dose levels: high dose (1/4 LD50)- 0.51 g/kg; middle dose (1/6 LD50)- 0.34 g/kg; and low dose (1/8 LD50)- 0.26 g/kg The percent yield from dried powdered leaves of *Quassia amara* to lyophilized extract was 10% (10g lyophilized extract/ 1000g dried powder). The test substance was prepared as a 10% solution (1 g in 10 ml NSS). Normal saline solution served as the control. The test substance and the vehicle were singly administered by oral gavage at a dosing volume of approximately 5 ml/kg on the day of the experiment

E. Conduct of Safety Studies

E1. Non-Invasive Blood Pressure (NIBP) determination in conscious rodents

The NIBP methodology consists of placing a cuff on the animal's tail to occlude the blood flow. Upon deflation, a non-invasive blood pressure sensor, placed distal to the occlusion cuff is used to monitor the blood pressure.

Before BP determinations were performed, the subject rat was introduced to an appropriately sized restrainer. Afterwards, the length of the restrainer was adjusted to comfortably restrain the rat. The tail cuff was slid over the rat tail along with the tail cuff holder until it rested closely to the tail end of the restrainer. The tail was also placed securely within the pulse transducer. Once secured, it was ensured

that the pulse transducer did not come in contact with the tail cuff because the vibration from cuff may affect the pulse signal. The animals were allowed to acclimatize for at least 30 minutes before pressure measurements began.

The cuff and pulse transducer were connected to the PowerLab Pro software which sensed, processed and transmitted physiological information from the animal. The systolic blood pressure (SBP) and pulse were simultaneously recorded. Heart rate (HR) and diastolic blood pressure (DBP) were computed based on the pulse transduced, using the software program. As soon as received, signals were transferred to a data acquisition computer for processing.

After dosing, mean observations were recorded at the following time periods: 0, 4, 8, 12, 16, 20 and 24 hours; 4 rats of 1 group at a time, repeating the NIBP monitoring procedure each period, for each rat. Observed and recorded parameters were BP (systolic, diastolic; mmHg), pulse pressure (mmHg), HR (bpm).[7-11]

E2. Non-invasive head-out plethysmography in conscious rodents

This procedure is a well-established and widely accepted technique in assessing the effects of test substances on the breathing pattern and detecting sensory irritation, pulmonary irritation and airflow limitation. It has been found to be a reliable method to evaluate pulmonary function.[12,13] The set-up of Emka Technologies was used in this experiment.

The animals were trained for 5 days in increasing time period to get accustomed to the plethysmograph. After gavage, the animals were placed inside the body of the plethysmograph with their heads protruding. After a stabilization period of 5 minutes, measurements were made. The respiratory flow was measured as the flow through a calibrated pneumotachograph connected with the plethysmograph and induced by the thoracic movements of the test animal. Mean observations were recorded at the following time periods: 0, 10, 15, 20, 30, 60, 90, 120, 180, 240 minutes.

Observation parameters to determine amount of respiratory flow and pulmonary irritation effects were determined. For respiratory flow, the following were recorded: respiratory rate (RR, frequency/minute), tidal volume (TV, mL), respiratory minute volume (mL), tidal mid- expiratory flow (EF50, mL/sec), time of inspiration and expiration (ms). For irritation effects, the following were additionally noted and recorded: Time of brake (TB) which quantified an elongation of the period from the end of the inspiration until the start of the expiration (ms); and Time of pause (TP) which quantified an elongation of the period from the end of the expiration until the start of the new inspiration (ms).

Statistical Analysis

The mean and standard deviation of the indicators of cardiovascular parameters (BP, pulse pressure and HR) and respiratory parameters (respiratory flow, TV, RR, respiratory minute volume, EF50, time of inspiration and time of expiration) were derived. To determine statistical significance of the differences in the mean values of these parameters between dose groups and time period, an analysis of variance (ANOVA) of a repeated measures design with time as the repeated measure was performed. The sources of variation on this ANOVA were dose group, time period, dose*time interaction, rat (within dose groups) and residual error. The F-test for dose group used the rat within dose group variation as denominator. For testing for significant effects of time and dose*time interaction, the F-test using the Greenhouse-Geiser adjustment for the degrees of freedom was used. All tests used α=0.05 level of significance. The statistical analysis was done using STATA Ver 10.1.

RESULTS AND DISCUSSION

Safety pharmacology studies aim to identify possible effects of a substance that is not usually detected by standardized toxicity testing.[5] Thus, it is recognized as an important element in drug discovery and development process. Guidelines such as those of ICH have defined and standardized these safety studies on the following organ systems: central nervous, cardiovascular, respiratory, gastrointestinal and renal/excretory systems. [4-7]

The possible effects on the cardiovascular and respiratory systems are among those most important to assess since they are acutely critical to life. For the cardiovascular and respiratory systems, the regulatory guideline ICH S7A enumerated parameters for measurement such as blood pressure, heart rate, electrocardiogram and methods of repolarization and conductance for cardiovascular function; and respiratory rate and other measures of respiratory function including tidal volume and hemoglobin oxygen saturation for the pulmonary function.[7]

The extract did not show significant effect on the systolic and diastolic blood pressures, and the heart rate (Figure 1). The mean diastolic blood pressure was highest in the low dose group with $\bar{x}$=142.3 while it was lowest among the high dose group with $\bar{x}$=120.4. However, the differences in mean diastolic blood pressure were not statistically significant, owing to the large variations in the individual values. Time trends of the mean systolic and diastolic blood pressures were likewise not found to significantly vary among dose groups.

Quassia amara L extracts were mostly used for anti-parasitic and gastrointestinal symptoms. In Guatemala however, both its leaf and root have been used for high blood pressure.[1] There was a trend towards decreasing diastolic blood pressure but this was not statistically significant. The possible absence of effect on the cardiovascular system of the leaf extract may point to the root as the possible source of traditional anti-hypertensive effect. The large variation in the values may be inherent to limitations of NIBP monitoring as it is subject to tethering, handling, and restraint.[10,11,14]

Heart rate and pulse pressure were likewise unaffected. Tachycardia, which can be a compensation for decrease

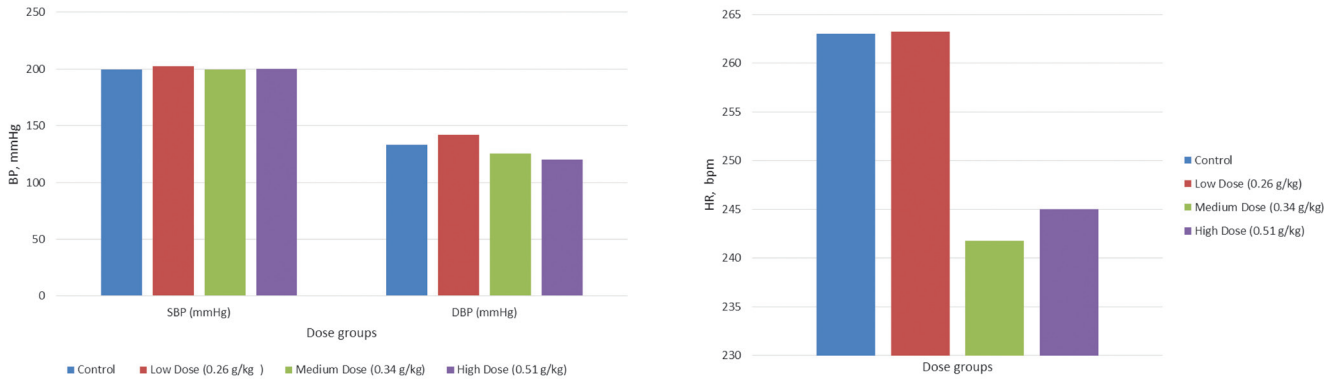

Figure 1. Effect of *Quassia* on mean SBP, DBP, HR of rats. Figure 1A shows no significant differences among the control and the treatment groups in SBP and DBP. Figure 1B shows no significant differences in heart rate among dose groups.

SBP - systolic blood pressure (mmHg), DPB - diastolic blood pressure (mmHg). HR – heart rate (bpm).

in cardiac output, vasodilation, and decreased peripheral vascular resistance (PVR) was not observed in the test animals of the treatment group. Possible side effects of hyper- or hypotension, vasodilation by effects on pulse pressure, and brady- or tachycardia were not detected using a non-invasive blood pressure monitoring in this study.

Absence of effect on rats, nevertheless, does not entirely rule out cardiovascular effects in humans. Continued vigilance of cardiovascular parameters is necessary with use of the extract. Additional tests on other cardiovascular parameters, particularly electrocardiogram in non-rodent species, as well as in vitro hERG voltage clamp assay, to address concerns on QT prolongation may be done.[10,11]

Quassia amara extract has statistically significant effect on mean respiratory rate (RR) by time period (F=2.45, p=0.0234), trends over time of tidal volume (TV) among the dose groups (F=2.00, p=0.0202), and average mean mid-expiratory flow rate (EF50) among dose groups ((F=3.11, p=0.0422). Differences in the following parameters were marginally significant: mean time of inspiration by time period, time trends of time of pause (TP) (F= 1.75, p=0.055) and time of break (TB) (F=1.68, p=0.073).

The overall mean frequency of breaths was highest during the reading at 30 minutes ($\bar{x}$=148.1) while it was lowest during the reading at 120 minutes ($\bar{x}$=101.0). Rats given a high dose of 0.51 g/kg, at the 120th minute, had a mean of 53 breaths per minute from a baseline mean of 133.2 (Figure 2). Thus this dose approximates the concentration at which a respiratory decrease of 50% is seen (RD50). A reflex decrease in the respiratory rate may be induced by sensory irritation in the upper respiratory tract of mice. In humans, this can result to a burning and painful sensation as the trigeminal nerve is stimulated. The decrease is allegedly caused by an increase in the time of break (TB).[12] The time of break is the period from the end of the inspiration until the start of the expiration. There were however no significant differences in the mean respiratory rate among groups. And although the highest mean time of brake (10.72) was from

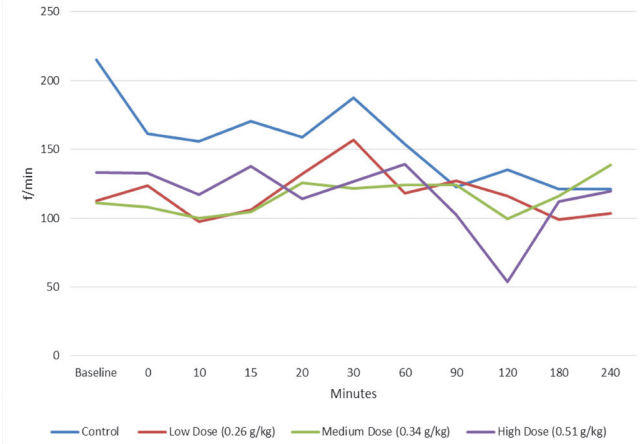

Figure 2. Effect of *Quassia amara* leaf extract on Respiratory Rate (f/min). There is a significant effect on mean RR by time period (F=2.45, p=0.0234). The high dose approximates the concentration at which a respiratory decrease of 50% is seen (RD50).

f/min - frequency of breaths per minute.

the high dose group followed by medium and low dose groups, while the control had the lowest overall mean of 7.40, these differences did not reach statistical significance.

The differences in the time trends of the mean time of brake (Figure 3) of the dose groups were marginally significant (F=1.68, p=0.0737). However, no clear association of the trends with dose could be seen. In the high dose group, the mean times of brake were relatively higher at the later time periods than in the first 30 minutes. The highest TB recorded was at the 60th (21.59) followed by the 90th (15.3) minute. The respiratory rate of the high dose group started decreasing on the 90th minute but was lowest during the 120th minute. In the medium dose group, the mean time of brake was increasing from baseline to the 30th minute, then declined at the 60th and 90th minute before increasing again in the later time periods.

Airflow limitation which can be caused by bronchoconstriction, edema, or accumulation of mucus in rats and mice, can be detected by a decrease in the midexpiratory flow rate (EF50, mL/sec). Though with lower sensitivity and greater variability, there is a good correlation of EF50 with invasive measurements of lung resistance and compliance.[12] There were significant differences in mean tidal midexpiratory flow among the treatment groups (F=3.11, p=0.0422). The mean tidal midexpiratory flow in the control (NSS) group was higher than those of the groups that received treatment. Over-all mean in the NSS group was $\bar{x}$=3.10, compared to $\bar{x}$=1.67, $\bar{x}$=1.92, and $\bar{x}$=2.03, respectively for the low dose, medium dose and high dose groups, respectively (Figure 4). However, there was no dose- effect relationship with the lowest dose group having the lowest mean flow (Figure 5). There were also no differences in the means compared to baseline, or trends of increasing and waning effect of extract. Thus, hard conclusions cannot be made.

Mean respiratory minute volume was statistically different by time period with highest at the 15th minute and lowest at the last time (240 minutes) of observation (F=2.4, p=0.0416). Respiratory minute volume is a product of the respiratory rate per minute (f/min) and the tidal volume (TV, ml). However, there were no significant differences in the overall mean among groups for respiratory rate, tidal volume, and respiratory minute volume.

There were significant differences in the trends over time of tidal volume among the different groups (F=2.00, p=0.0202). This however did not show any meaningful relationship with the dose of extract given (Figure 6). The control and high dose groups had increasing tidal volume at the 15th-30th minute, and the medium dose until the 60th

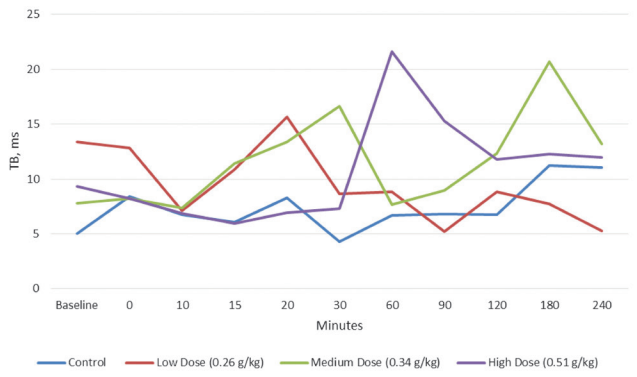

Figure 3. Effect of *Quassia amara* leaf extract on Time of Brake (TB, ms). No significant differences in the mean time of brake of the dose groups were seen.

TB, ms - time of brake in milliseconds.

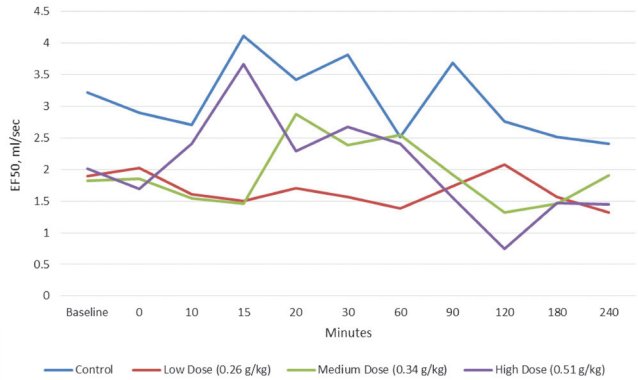

Figure 4. Effect of *Quassia amara* leaf extract on tidal midexpiratory flow over time (EF50, ml/sec). The control group has significantly higher EF50 (ml/sec) compared to the treatment groups (F=3.11, p=0.0422).

EF50, ml/sec - midexpiratory flow rate in milliliter per second.

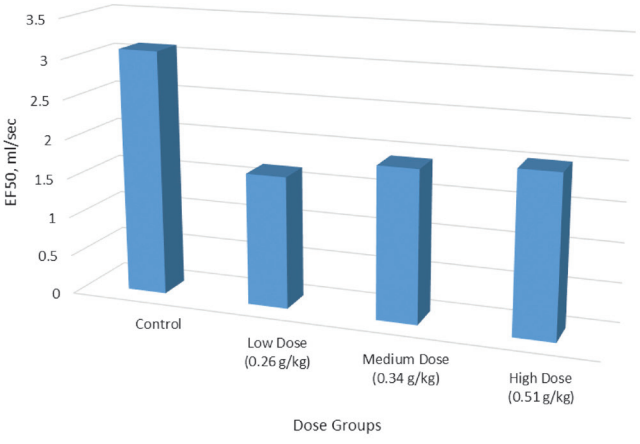

Figure 5. Figure 5. Effect of *Quassia amara* leaf extract on mean tidal midexpiratory flow over time (EF50, ml/sec). No dose-effect relationship was demonstrated with the lowest dose group having the lowest mean flow.

EF50, ml/sec - midexpiratory flow rate in milliliter per second.

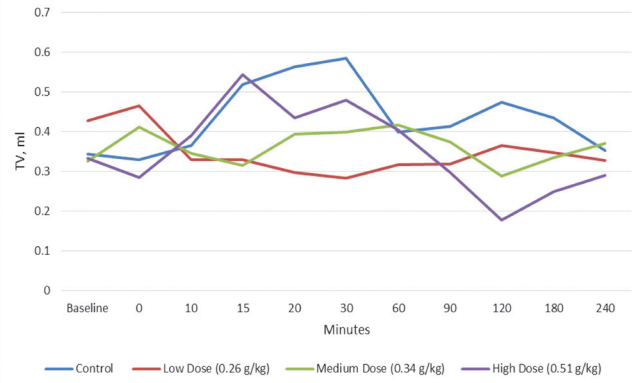

Figure 6. Effect of *Quassia amara* leaf extract on tidal volume over time (TV, ml). There were significant differences in the trends over time (F=2.00, p=0.0202) of tidal volume, but not among the dose groups.

TV, ml - tidal volume in milliliters

minute of observation; this is in contrast to the decreasing trend for the low dose group during these periods.

Mild pulmonary irritation by stimulation of the vagal nerves at the alveolar lever may result to rapid shallow breathing with increasing RR and decreasing TV.[12] Higher concentrations and effect, on the other hand, resulted to decreased RR and an increase in TP (period from expiration to following inspiration). The high dose group as previously mentioned led to a 50% decrease in the RR during the 120th minute. This however did not correlate with the trend of the more sensitive TP. The overall mean time of pause and respiratory rate did not differ significantly among the four groups. A definite pulmonary irritation effect at the alveolar level cannot be concluded.

Substances isolated from *Quassia amara* include several quassinoids, namely simalikalactone D (SkD), quassin, Quassimarin, neoquassin simalikalactone E, picrasin B, picarsin H, picrasin I, picrasin J.[15] Among these the first three is the most studied, and SkD has the most activity particularly anti-malarial, antiviral (poliomyelitis, herpes simples virus 1), and cytotoxic activities. Quassin has possible anti-fertility, antiviral, larvicidal, and anti-HIV; while Quassimarin, anti-tumor action.[1,16] The quassinoids in general were shown to have ani-ulcer activity in rats.[1,17] None of those isolated has been, to the author's knowledge, specifically tested and reported for cardiovascular or respiratory effects.

Based on the results and statistical analysis, the plant extract did not have any effect on the cardiovascular system but possible action on the respiratory system of the test animals. Translation to human effects may entail invasive tests in non-rodent species, and more so, close monitoring and pharmacovigilance once human trials have started.

CONCLUSION

The aqueous leaf extract of *Quassia amara* L. has no significant effects on blood pressure, pulse pressure, and heart rate which may translate to its cardiovascular safety. However, its possible effects on the pulmonary function (pulmonary irritation or airflow limitation) should be monitored closely.

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Statement of Authorship

All authors have approved the final version submitted.

Author Disclosure

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