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Raphia australis: Acute toxicity, antioxidant capacity and antihypertensive effect in spontaneously hypertensive rats

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ABSTRACT

Background & Aim: The fruits of *R. australis* are eaten as snacks in some West and Central African countries. This study was aimed at evaluating the safety, antioxidant capacity and antihypertensive effects of these fruits.
Experimental: The acute toxicity of the extract of *R. australis* extract (RAE) was evaluated through single and short term oral exposure of mice to RAE. Total phenols and flavonoid contents were quantified followed by 1,1‑diphenyl‑2‑picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Spontaneously hypertensive rats (SHR) were treated with RAE and blood pressure (BP) was measured after once off treatment (phase I) and in the course of 4 weeks treatment (phase II).
Results: The LD_{50} of RAE was greater than 5000 mg/kg. RAE had mild antioxidant capacity and significantly ($P<0.001$) decreased systolic and diastolic BP in both once off treatment and 4 weeks treatment while decreasing urine output and increasing feces output.
Recommended applications/industries: These results indicate that *Raphia australis* is not toxic and thus is safe for consumption. Pharmaceutically, it has mild antioxidant and significant antihypertensive properties. It also has the ability to improve appetite, increase bulk thus aiding in peristalsis and waste removal.

1. Introduction

Hypertension is a serious medical condition affecting about 1.13 billion people globally; mostof whom are living in low and middle income countries (WHO, 2019). Although available therapies have proven to be efficient, attempts at achieving blood pressure (BP) targets in hypertensive patients are still below standard. One of the main reasons for this phenomenon is low adherence to treatment (Manolis *et al.*, 2015). In addition, the management of hypertension is fraught with challenges such as accessibility and high cost of drugs as well as poor adherence to treatment regimen due to unpleasant side effects. It is very likely this lack of compliance may result in the emergence of drug resistance after a period of use, uncontrolled BP and consequently disease progression to complications; therefore the search for alternative therapeutics is essential (Adidja *et al.*, 2018).

Medicinal plants are vital sources of alternative therapeutics. In this study, we evaluate the acute toxicity, antioxidant capacity and antihypertensive effects of the fruits of *R. australis* extract which is a snack in some West and Central African countries.

2. Materials and Methods

2.1. Extract preparation

Fresh fruits of *R. australis* were supplied by Mr Bruce Hooper of Toad Tree Lodge, KwaZulu-Natal, South Africa. The fruits were identified by Dr KL Immelman of Kei Herbarium, Walter Sisulu University. The scaly outer covering of the fruits were manually separated from the pulp and both were air-dried at room temperature and crushed to fine pieces using a mortar and pestle. The pieces were macerated in 70 % ethanol for 72 hours after which the extract was separated from the rest of the plant materials. The ethanol was recovered using a rotary evaporator (Laborator 4000, Germany) and the extract dried in a fan oven at 35^{o}C.

2.2. Animal handling

Mice (20-30 g) and rats (150-260 g) were procured from the South African Vaccine Producers (Johannesburg, South Africa) and housed in cages in the animal holding facilities at Walter Sisulu University. The animal rooms were maintained at 24^{o}C and lighting was provided exclusively by day light. The animals had free access to feed and water and were treated following National Research Council Guide for the use of Laboratory animals. Ethical clearance (approval # 51/15) for the study was obtained from the Faculty of Health Sciences Research and Ethics Committee of Walter Sisulu University, South Africa.

2.3. Acute toxicity of RAE

Acute toxicity of RAE was evaluated through single dose and short term exposure (oral treatment) of mice to RAE.Toxicity due to single dose exposure was done following Lorke's method with modifications as described by (Tata *et al.*, 2018). Briefly, three groups of three mice each were treated with 10, 100 and 1000 mg/kg of *R. australis* extract (RAE) respectively in phaseI. In phase II, three groups of one mouse each were treated with 1600, 2900 and 5000 mg/kg of RAE respectively. For toxicity due to short term exposure to RAE, four groups of six mice each were treated with distilled water (control), 200, 400 and 800 mg/kg RAE respectively for four weeks. Body weights of animals were recorded at the start of experimentation and then weekly thereafter. Food and water consumed and feces released were recorded daily. At the end of the four weeks of treatment, the mice were terminated and organs (brain, heart, kidneys, liver, testis and spleen) were harvested and weighed.

2.4. Antioxidant capacity of RAE

The RAE extract was evaluated for its flavonoids and phenol contents which are reported to possess antioxidant activities. Phenolic compounds were quantified by Folin Ciocalteau method with gallic acid as standard and flavonoid content was determined using Aluminium chloride method with quercetin as standard (Chandra *et al*., 2014). Antioxidant capacity of RAE was evaluated following the protocol described by (Tata *et al*., 2020). Briefly, 3 ml DPPH solution was added to 1 ml of ascorbic acid (standard) or RAE at different concentrations in a dark room and incubated for 30 minutes at room temperature. Absorbance was measured at 517 nm using UV-VIS-spectrophotometer (Phoenix-2000V, UK).The percentage DPPH radical-scavenging activity was calculated using the formula:

$$\%\text{DPPH radical scavenging} = ((A_{blank} - A_{standard/extract})/A_{blank}) \times 100$$

The percentage inhibition was plotted against concentration and IC_{50} valuewas extrapolated from plot. Freshly prepared FRAP reagent was added to 100 µl gallic acid (standard) or extract and incubated in a water bath at 40^{0}C for 4 minutes. Absorbance was measured at 593 nm. Results were extrapolated from standard curve and expressed in µg gallic acid equivalents.

2.5. Antihypertensive effects of RAE in SHR

The antihypertensive effect of RAE was evaluated in SHR (200-250 g) rats in two phases. In phase I, 18 SHR were randomly allocated to three treatment groups of six rats (n=6) each: NS - normal saline, Furo-furosemide (10 mg/kg), RAE –RAE (500 mg/kg). Each group received assigned treatment once off after measurement of baseline blood pressure (BP). Blood pressure measurement were repeated periodically as described by (Tata *et al.*, 2018). In phase II, 24 SHR ((200-250 g) were randomly allocated to four groups of six rats (n=6) each: NS - normal saline, Amlo–amlodipine (5 mg/kg), Furo-furosemide (10 mg/kg), RAE – raphia (500 mg/kg). After baseline BP was recorded, the rats were treated with assigned treatment once daily and BP was measured weekly following the protocol described by (Tata *et al.*, 2018).

2.6. Food and water consumption

Food and water consumed were monitored weekly by placing the animals individually in metabolic cages over a period of 24 hours. The animals had free access to a predetermined volume of water and food. The volume and weight of unconsumed water and food were determined respectively.

Water consumed = volume of water placed in cage – volume of water in cage after 24 hours.

Food consumed = weight of food placed in cage – food left in cage after 24 hours.

2.7. Urine and feces output

Urine and feces output were monitored weekly by placing the animals individually in metabolic cages over a period of 24 hours. The metabolic cage is built in such a way that urine is automatically separated from feces produced by the animal. The volume of collected urine and weight of feces were determined.

2.8. Termination

Treatment was stopped two days to termination. The rats were fasted for 16 hours, weighed and then terminated. The brain, heart, liver, testes and spleen were harvested and weighed after the toxicity study while the heart and kidneys were harvested and weighed after evaluation of antihypertensive effects. The percentage organ to body weight ratio were calculated:

% organ to body weight ratio = (weight of organ/body weight) *100

2.9. Statistical analysis

Statistical analyses were carried out using Graphpad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's *posthoc* test for multiple comparisons were performed to determine differences between treatment groups. A p-value less than 0.05 was considered statistically significant. Results were expressed as mean ± standard error (SEM).

3. Results and discussion

Results from this study showed that the LD_{50} of RAE was greater than 5000 mg/kg. RAE had mild antioxidant capacity and significantly (P<0.001) decreased systolic and diastolic BP in both once off treatment and 4 weeks treatment while decreasing urine output.

3.1. Toxicity of RAEafter single and short term exposure

Single exposure of mice to RAE showed that the extract was not toxic at all the tested doses, that is, the LD_{50} of RAE was greater than 5000 mg/kg. Pharmacological substances with LD_{50} less than 5 mg/kg are classified as highly toxic substances, those with LD50 between 5 mg/kg and 5000 mg/kg are classified as moderately toxic substances and those with LD50 greater than 5000 mg/kg are not toxic (Kennedy *et al*., 1986; Konate *et al.*, 2014). Therefore, the extract of *R. australis* is non-toxic and thus, the fruits are safe for consumption. After short term exposure of mice to RAE, there was weekly increase in body weights in all the groups and by week 4, change in body weights of the mice treated with 800 mg/kg was significantly higher compared to the control group (Table 1). This suggests that RAE enhanced appetite, which resulted in increased food intake and hence increase in body weight.

Table 1. Effect on change in body weights after short term exposure of mice to RAE.

Period	NS	200mg/kg	400mg/kg	800mg/kg
Week 2	3.13±0.2	1.07±0.3**	3.16±0.2	3.74±0.6
Week 3	5.04±0.8	3.74±0.7	4.16±0.2	7.09±0.4
Week 4	6.01±0.8	5.83±0.8	5.92±0.6	10.14±0.5**

Results are expressed as mean ± Standard error of mean. NS-normal saline control group.(n=6); **P<0.01compared to control. Change in body weight was obtained by computing (Body weight in each week – baseline body weight).

3.2. Food/water consumption and feces output after short term exposure of mice to RAE

Animals fed with the high dose of 800 mg/kg consumed significantly (P<0.001) more food compared to control groups (Table 2).

Table 2. Effect on food consumption after short term exposure of mice to RAE.

Period	NS	200mg/kg	400mg/kg	800mg/kg
Week 1	5.51±0.1	5.6±0.5	6.04±0.7	1.69±0.1***
Week 2	5.1±0.3	3.96±0.2	4.85±0.3	11.41±0.3***
Week 3	4.2±0.1	3.79±0.1	4.28±0.2	4.11±0.1
Week 4	3.32±0.1	4.73±0.1	3.52±0.1	12.44±0.3***

Results are expressed as mean ± Standard error of mean. NS-normal saline control group. (n=6); *P<0.05, ***P< 0.001compared to control. The weight of food consumed was obtained by computing (weight of food placed in cage – food left in cage after 24 hours).

In the first week of treatment, animals on RAE consumed significantly (P<0.001) higher volumes of water. In week two, there was no difference between the treatment groups, while in week 3 the groups treated with RAE had significantly lower water intake compared to the control group. In week 4, significant decrease in water intake was only observed in groups treated with 200 mg/kg (P<0.01) and 400 mg/kg (P<0.001) (Table 3).

Table 3. Effect on water consumption after short term exposure of mice to RAE.

Period	NS	200mg/kg	400mg/kg	800mg/kg
Week 1	5.2±0.7	9.8±0.1***	8.4±0.3***	12.17±0.4***
Week 2	8.5±0.6	7.67±0.4	10±0	9±0.2
Week 3	9.5±0.4	5.5±0.8***	6.5±0.7**	7.17±0.5*
Week 4	10±0	7.83±0.6**	4.6±0.1***	11±0.3

Results are expressed as mean ± Standard error of mean. NS-normal saline control group. (n=6); *P<0.05, **P<0.05, ***p<0.001compared to control. The volume of water consumed was obtained by computing (volume of water placed in cage – volume of water in cage after 24 hours).

Considering feces output, in week 1, mice treated with 200 mg/kg and 400 mg/kg produced significantly (P<0.05) higher amounts of feces compared to the control group. In week 3 all the groups treated with RAE produced significantly (P<0.001) higher amounts of feces compared to control and in week 4, the increase in feces production was sustained in the group treated with 200 mg/kg and deceased in group treated with 800 mg/kg (Table 4).

Table 4. Effect on feces output after short term exposure of mice to RAE.

Period	NS	200mg/kg	400mg/kg	800mg/kg
Week 1	0.76±0.1	1.14±0.1*	1.2±0.02*	1.07±0.1
Week 2	1.31±0.1	1.61±0.09	1.58±0.1	0.77±0.04**
Week 3	0.77±0.04	2.3±0.1***	1.16±0.05**	1.25±0.04***
Week 4	1±0.05	1.3±0.1***	1.11±0.04	0.47±0.05***

Results are expressed as mean ± Standard error of mean. NS-normal saline control group. (n=6); *P<0.05, **P<0.01, ***P<0.001compared to control. The weight of feces produced was obtained by weighing the feces that was collected for each group.

Short term exposure of mice to RAE induced increased food intake which was accompanied by increase in body weights and increase in feces output. It is likely that RAE boosted appetite and its high fibre content increased bulk of food in the gut thus ensuring better peristalsis and increased defecation (Capuano, 2017).

3.3. Organ to body weight ratio after short term exposure of mice to RAE

There was no difference in the brain, heart, kidney, liver, testes and spleen sizes of treated mice compared to control (Table 5). Its lack of toxicity was confirmed by no changes in the weights of organs of treated animals (Michael *et al.*, 2007).

Table 5. Effect on organ to body weight ratio after short term exposure of mice to RAE.

	Brain	Heart	Kidney	Liver	Testis	Spleen
NS	0.01±0.001	0.007±0.002	0.01±0.001	0.05±0.001	0.008±0.0003	0.005±0.001
200mg/kg	0.012±0.001	0.005±0.0001	0.015±0.001	0.054±0.001	0.01±0.001	0.006±0.001
400mg/kg	0.012±0.001	0.006±0.0004	0.015±0.001	0.048±0.0012	0.009±0.0003	0.004±0.0002
800mg/kg	0.012±0.0003	0.005±0.0002	0.014±0.001	0.054±0.002	0.008±0.0004	0.004±0.0001

Results are expressed as mean ± Standard error of mean. NS- normal saline control group. (n=6). Percentage organ to body weight ratio was obtained by computing (weight of organ/body weight *100).

3.4. Antioxidant capacity of RAE

RAE had very low flavonoid and total phenol contents. Results from DPPH assay showed that RAE had an IC_{50} value of 0.88 mg/ml. Results from FRAP assay showed that RAE had a reducing power of 46.95±0.3 GAE/RAE (Table 6). RAE had mild antioxidant capacity suggesting that its mechanism of action in the body may not be via combating oxidative stress. The low concentration of flavonoids and phenols explains the reason for the mild antioxidant properties since these phytochemicals are responsible for the antioxidant capacity of plants (Tungmunnithum *et al.*, 2018).

Table 6. Antioxidant capacity of RAE.

Assay	Phytochemical content and antioxidant effects of RAE
Flavonoid content	0.1±0.1 QE/mg RAE
Total phenol content	1.27±0.2 GAE/mg RAE
FRAP	46.95±0.3 GAE/mg RAE
DPPH IC_{50}	IC_{50} =0.88 mg/ml

Result of phenolic content was presented as gallic acid equivalents per milligram of RAE extract (GAE/mg RAE). Result of flavonoid content was presented as quercetin equivalents per milligram of RAE extract (QE/mg RAE).

3.5. Antihypertensive effects of RAE in SHR

Following once off treatment, RAE and furosemide caused decrease in SBP; the decrease was not significant 2H after treatment but after 4H and 6H, there was significant (P<0.001) decrease in SBP compared to NS control (Table 7).

Table 7. Change in mean systolic blood pressure upon treatment with RAE.

	NS	RAE	Furo
2H	4.2±0.3	3.74±0.3	3.88±2.1
4H	20.28±1.7	9.62±0.9***	7.17±2.1***
6H	-9.07±1.6	5.26±0.1***	3.31±1.3***

Negative values indicate increase in SBP from baseline values. Change in SBP were obtained by computing (SBP at baseline – SPB at given times after treatment). Results are presented as mean ± SEM. (n=6); NS = Control (normal saline), RAE =*Raphia australis* extract (500 mg/Kg); Furo= Furosemide (10 mg/Kg), ***P<0.001 compared to normal saline control.

As for DBP, only Furo significantly (P<0.01) decreased DBP 2H after treatment compared to NS control. There was decrease in DBP 4H after treatment although this decrease was not significant and 6H after treatment, RAE significantly (P<0.001) decreased DBP compared to NS control (Table 8).

Table 8. Change in meandiastolic blood pressure upon treatment with RAE

	NS	RAE	Furo
2H	-0.043±0.1	-0.24±1.62	5.57±0.5**
4H	18.05±5.83	14.38±1.4	8.98±1.7
6H	9.024±1.79	18.52±0.8***	-8.81±0.9***

Negative values indicate increase in BP from baseline values. Change in DBP were obtained by computing (DBP at baseline – DPB at given times after treatment). Results are presented as mean ± SEM. (n=6); NS = Control (normal saline), RAE =*Raphia australis* extract (500 mg/Kg); Furo= Furosemide (10 mg/Kg), **P<0.01, ***P<0.001 compared to normal saline control.

Results from phase II BP study showed that in the first week of treatment RAE and furosemide significantly (p<0.001) decreased SBP compared to NS controland amlodipine groups. After four weeks of treatment, RAE, furosemide and amlodipine significantly decreased SBP compared to NS control (Fig. 1).

The effect of treatment on DBP was similar to the effect on SBP that is, in the first week of treatment, RAE and furosemide significantly (P<0.01) decreased DBP compared to NS control and amlodipine control groups and after four weeks of treatment, RAE, furosemide and amlodipine significantly decreased DBP (Fig. 2).

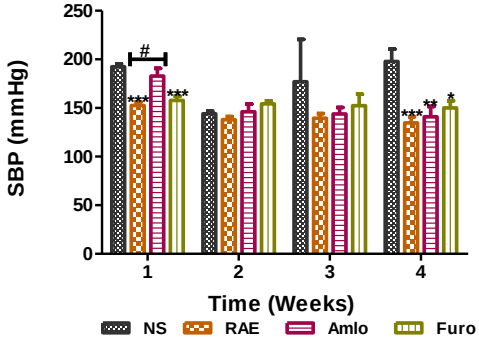

Fig. 1. Effect of RAE on systolic blood pressure. Results are presented as mean ± SEM. (n=6); NS = Control (normal saline), RAE =*Raphia australis* extract (500 mg/Kg); Furo= Furosemide (10 mg/Kg), and Amlo=Amlodipine (5mg/Kg).*P<0.05, **P<0.01, ***P<0.001 compared to normal saline group; #P<0.01 compared to standard drug group.

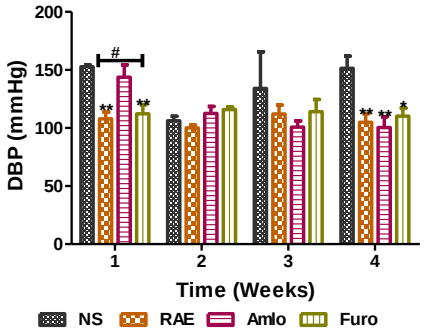

Fig. 2. Effect of RAE on diastolic blood pressure. Results are presented as mean ± SEM. (n=6); NS = Control (normal saline), RAE=*Raphia australis* extract (500 mg/Kg); Furo= Furosemide (10 mg/Kg), and Amlo=Amlodipine (5mg/Kg).*P<0.05, **P<0.01, ***P<0.001 compared to normal saline control, #P<0.01 compared to standard drug group.

Just like amlodipine and furosemide, RAE sustained a decrease in SBP throughout the treatment weeks even though the decrease was not significant in week 2 and 3. The same trend was observed for DBP through the 4 weeks of treatment. Considering the mean BP (MBP), RAE and furosemide significantly (P<0.01) decreased MBP after the first week of treatment compared to NS and amlodipine controls. Four weeks later, RAE, furosemide and amlodipine significantly (P<0.01) decreased MBP compared to NS control (Fig. 3).

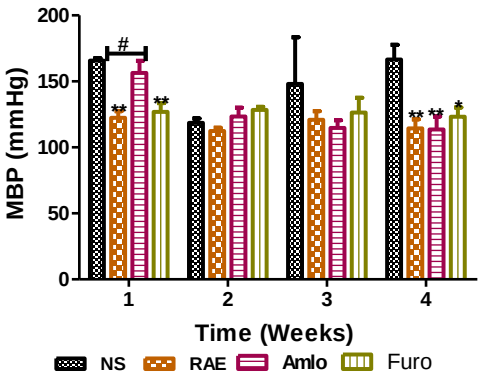

Fig. 3. Effect of RAE on mean blood pressure. Results are presented as mean ± SEM. (n=6); NS = Control (normal saline), RAE = *Raphia australis* extract (500 mg/Kg); Furo= Furosemide (10 mg/Kg), and Amlo=Amlodipine (5mg/Kg).*P<0.05, **P<0.01, ***P<0.001 compared to normal saline control, #P<0.01 compared to standard drug group.

The ability of RAE to decrease SBP and DBP after once off treatment suggested that the phytoconstituents of RAE as well as its metabolites could be effective against hypertension (Tata *et al*., 2020). The effect of RAE on BP was comparable to that of furosemide after the first week of treatment meanwhile in the fourth week of treatment its activity was comparable to that of furosemide and amlodipine. Furosemide is a loop diuretic that acts on the lumen to inhibit the Na-K-2Cl cotransporter along the thick ascending limb and macula densa. As an organic anion, it binds within the translocation pocket on the transport protein by interacting with the chloride-binding site; since it is larger than chloride, it is not transported through the pocket, and thus inhibits the transporter (Ellison, 2019). On the other hand, amlodipine is a long-acting dihydropyridine calcium channel blocker (Stepien *et al*., 2002).

3.6. Effect of RAE on organ to body weight ratios of SHR rats

There was no significant difference in the weights of hearts and kidneys from rats treated with RAE, furosemide and amlodipine compared to the normal saline control (Table 9).

Table 9. Effect of RAE on organto body weight ratios of SHR rats.

Groups	Heart	Kidney
NS	0.4 ± 0.02	0.7 ± 0.03
RAE	0.4 ±0.01	0.6 ± 0.01
Amlo	0.4 ± 0.01	0.6 ± 0.01
Furo	0.4 ± 0.02	0.6 ± 0.01

Results are expressed as mean ± Standard error of mean. NS- normal saline control group. (n=6); RAE = *Raphia australis* extract (500mg/kg); NS = normal saline control; Amlo = Amlodipine (5 mg/Kg); and Furo = Furosemide (10 mg/Kg). *p<0.05, **P<0.01, ***P<0.001 compared to normal saline control.

The fact that organ to body weight ratios were similar to those of the normal control further confirmed the safety of *R. australis* that was determined by acute toxicity testing.

3.7. Effect of RAE on urine output in SHR rats

Urine output in the RAE group tended to be lower compared to the other treatment groups though the difference was significant only in week 4 of the study (Table 10). Given the fact that RAE decreased urine output, it suggests that its mechanism of action may not be similar to that of furosemide which is a diuretic (Ellison, 2019).

Table 10. Effect of RAE on urine output in SHR rats.

Groups	1	2	3	4
NS	24.16 ± 4.65	19.16 ± 3.90	15 ± 1.24	15.6 ± 2.42
RAE	13 ±1.69	12 ± 0.68	14 ± 1.06	8.7 ± 0.3*
Amlo	16.3± 3.08	18.6 ±2.22	16.4± 1.52	14.7 ± 1.24
Furo	20 ±2.58	15.16 ±2.66	16 ±3.08	14.6 ± 1.03

Results are expressed as mean ± Standard error of mean. NS- normal saline control group. (n=6); RAE = *Raphia australis* extract (500mg/kg); NS = normal saline control; Amlo = Amlodipine (5 mg/Kg); and Furo = Furosemide (10 mg/Kg). *P<0.05= statistically different from control.

4. Conclusion

Raphia austalis is not toxic and thus safe for human consumption. It has the ability to improve appetite, increase bulk thus aiding in peristalsis and waste removal. The fruit of *R. austalis* has mild antioxidant activity and significant antihypertensive properties.

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