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Stephen Ngigi Mburu
Department of Medical Laboratory Sciences, Kenyatta University, Nairobi, Kenya

Mathew Piero Ngugi
Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya

John K. Mwonjoria
Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya

Correspondence:
Dr. Stephen Ngigi Mburu
Department of Medical Laboratory Sciences, Kenyatta University, Nairobi, Kenya
Email: stevebikomb@gmail.com

Cardioprotective and anti-atherosclerotic effects of *Rhamnus prinoides* extracts in animal models

Stephen Ngigi Mburu, Mathew Piero Ngugi, John K. Mwonjoria

ABSTRACT

Rhamnus prinoides (*R. prinoides*) are small trees or shrubs which are rigid and branched. Its barks and roots are used for treatment of various ailments and diverse diseases. It is commonly distributed in rift valley and central provinces of Kenya. A little is known about its safety and antihyperlipidemic effects in management of atherosclerosis. 10% DMSO and normal saline was used to reconstitute the *R. prinoides* extracts because of its stability and reliable solvent for extraction in organic and inorganic application. Mature Albino Wistar rats three months old were fed with HCHF diet (10% egg York (5.6g/bw),10% lard (5.6g/bw),0.2% cholic acid (0.112g/bw) and 0.59% propylthiouracil (0.28g/bw), for 28 days. Onset of 28th day, the rats were euthanized and bioassays done. Both body weights and organ weights were recorded. For cardiotonic studies, 5 New Zealand male rabbits were used. They were injected with 1000 units of heparin to avoid clot formation. Chest was opened through cardiac thoracotomy and heart placed in a dish containing Tyrode solution. Langerdorff method was used using a kymograph in the study of ionotropic and chronotropic effects. In toxicity studies, male mice of age 6-7 weeks were given oral doses of plant extract inclusive of the control for 28 days of the experiment. On 29th day of the experiment, animals were sacrificed through cardiac puncture and the blood sample collected was used for hematological and biochemical assays. Mice were ruminated with rodent pellets and water without cease. OECD 407 precepts were followed when conducting toxicity studies. One way ANOVA was used in data analysis. This was followed by Tukey as post hoc and statistical significance at $p<0.05$. Extracts of *R. prinoides* showed presence of saponins, alkaloids, glycosides, terpenoids, steroids and phenolics. *R. prinoides* plant extracts exhibited positive ionotropic and negative chronotropic effects. Significance reduction on low density lipoproteins and total cholesterol was exhibited by *R. prinoides* plant extracts, following a high cholesterol high fat diet.

Keywords: Atherosclerosis, Hyperlipidemia, *Rhamnus prinoides*, LDL:HDL ratio, Cardiovascular, Coronary vascular disease.

INTRODUCTION

Plant extracts have been used in treatment and management of various ailments from time immemorial. One of the most used plants for treatment of these ailments is *Rhamnus prinoides*. Belongs to the family *Rhamnacea,* also called *Orkonyil/ Olkokola* in Maasai and *Ngukura* in Kikuyu. It is a small tree or shrubs which are rigid and branched growing to five meters in height [1]. It is used as a traditional remedy for body aches [2] inflammation of the ear, abdominal pains, angina, toothaches and dysmenorrhea. The plant is widely distributed in East, central and South African countries being native to Kenya [3]. In Kenya the plant is majorly distributed in Rift valley and Central provinces of Kenya [4]. The decoction of the roots is mixed with milk or even taken orally and have been active against pneumonia, stomachache, back pains, gonorrhea, and malnutrition [5]. *R. prinoides* have effects on anti-inflammatory, anti-oxidant wound healing, blood purifier, treatment of waterborne diseases, sexually transmitted diseases and malaria [2]. In Kenya the barks of *R. prinoides* is consumed as a folk medicine in the management of sexually transmitted infections and backaches [1]. *R. prinoides* contains phytochemicals such as anthraquinones, saponins, steroids, tannins, terpenoids, and flavonoids [2]. Study shows potential antioxidant and anti-inflammatory activities on *R. prinoides* [6]. The anti-microbial activity of *R. prinoides* against *E. coli* and *Staphylococcus spp* has also been reported. This study aimed at evaluating the cardioprotective and anti-atherosclerotic effects of *R. prinoides* extracts and their safety.

MATERIAL AND METHOD

Collection and preparation of plant materials

The roots of *Rhamnus prinoides* barks was collected in Olendeem Narok County. They were cleaned, chopped, and dried away from direct exposure of sunlight. This was done in biochemistry laboratory

of Kenyatta University for three weeks. They were then ground into powder using an electric grinding mill. A qualified taxonomist identified them and a voucher specimen number (SM2022/01TB) was allocated from the University of Nairobi Herbarium.

Plant extracts preparations

Standard weighing scale (*Scout pro SPU 402 Echas Scientific*) was used to weigh about 100g of the powdered plants. They were soaked in absolute methanol repeatedly for 72 hours. Immediately after adding the solvent agitation was done for about eight seconds this increased extraction efficiency by breaking the plant's cell wall thus resulting to enhanced release of the soluble phytochemicals. They were later decanted.

After overnight incubation, the supernatant was filtered using Whatman No 1 filtering paper. The filtered substance was then concentrated using a Rotor evaporator at reduced pressure to obtain the extracts. The final product was a solid mass devoid of the solvent used. The dried mass was then stored at room temperature in airtight bottles which were later used for desired pharmaceutical formulations and also phytochemical testing. Dimethylsulfoxide (DMSO) and normal saline in the ratio mixture of 1:10 was used to reconstitute the dried extracts.

Experimental animals

Five male White New Zealand Rabbits aged 4-5 months old weighing about 1000g, were used for cardiotropic studies. Male and female Swiss Albino mice aged 7-8 weeks weighing about 18-25g and equally distributed were used for toxicity studies. Male and female Wistar rats age 8-10 weeks weighing 40-60g were used for hyperlipidemic studies. They were placed in cages in the animal house at room temperature and allowed to acclimatized for seven days, prior to commencement of the experiment. Food and tap water were provided *ad lipitum*.

The experiment was conducted per the guidelines for laboratory animal use and care [7] the research permit was granted by the Kenyatta University Animal Care and Use Committee (KU/ACUC/CON /014) and the National Commission for Science and Technology (NACOSTI) Research License No 411801.

Standard Drugs and chemicals

The following drugs and chemicals were used on the course of the study: petroleum ether analytical grade, methanol analytical grade, normal saline, formalin, from Sigma Aldrich chemicals. Other standard drugs and chemicals used were; avastatin, Dimethyl sulfoxide analytical grade, Tyrode solution, distilled water adrenaline, acetylcholine, atropine, and propylthiouracil.

Bioassays

Cardiotonic assays

Five male rabbits aged 4-5 months weighing 1000g were used. The assay was conducted using Langendorff method [8] as follows. The animal was sacrificed and immediately the chest opened up through cardiac thoractomy. The heart together with the aorta harvested just below the aortic bifurcation. It was then transferred to warm aerated Tyrode solution and maintained at 37 ºC. The heart was then mounted on the organ bath containing Tyrode connected to a kymograph with a rotating drum and recorder. The doses administered were plant extract, acetylcholine and adrenaline at doses of 25, 50 and 100mg/kg. The contraction of the heart was recorded on the kymograph mounted on the rotating drum. The heights of the tracings (amplitude) were then quantified as the force of contraction of the isolated heart (ionotropic effect) while the frequency represented the heart rate (chronotropic effect).

Lipidemic assay

Lipidemic assays were done using methods by [9] as follows; Male and female Wistar rats aged 8-10 weeks weighing 50-60g were used for the hyperlipidemic studies. They were fed with a high cholesterol high fat diet. The high cholesterol high fat diet consisted of 10% egg York (5.6g/bw), 10% lard (5.6g/bw), 0.2% cholic acid (0.112g/bw) and 0.59% propylthiouracil (0.28g/bw). They were grouped in five groups (n=5). Each group received 25, 50 and 100mg of *Rhamnus prinoides* extract. The fourth group got 40mg/kg of avastatin which was the standard drug and the fifth received the vehicle (distilled water) orally daily for 28 days. Oral gavage was used to administer the drugs daily. Throughout the period of drug administration, animals were monitored for any behavior changes which could arise due to toxicity such as vomiting, diarrhea, ataxia or even death. Body weights were recorded weekly. On 28th day, the animals were euthanized by cotton applied with chloroform and blood sample collected through a cardiac puncture. Body weights were recorded before euthanasia. Vital organ weights of the liver, kidney, spleen, lungs, heart and brain were also recorded.

Toxicity assay

Sub-acute toxicity study involved male and female albino mice's age between 6-7 weeks with average body weight of 18-25gms. They were acclimatized in the cages at room temperature for one week before the study commenced. Standard commercial rodent pellets were provided ad libitum. After one week of acclimatization, animals were sorted into groups; 1, 2, 3 that received 100, 17, and 300 mg of *R. prinoides* extracts respectively. Group 4 was the baseline received distilled water. The determination of the doses levels was done. At the end of the experiments the animals underwent euthanasia in ajar using chloroform soaked in cotton wool and the total blood was collected through the cardiac puncture and immediately placed in vacutainer tubes containing EDTA (Ethylene Diamine Tetra Acetic Acid) anticoagulant and serum separator tubes (SST) tubes for biochemical analysis. The complete blood count was done using a Sysmex XP 300 Hematology analyzer machine while the biochemical test was done using Cobas 111 analyzer. Quality control and calibration of the analyzer was done prior to testing.

Phytochemical screening

Qualitative Phytochemical screening was carried out by methods described by [10].

Statistical analyses

The data was expressed as means and standard error of the means and analyzed using one-way ANOVA and Tukey's *post hoc* test. The significance level was set at a value of $p < 0.05$.

RESULT

This study was conducted to evaluate effect of *R. prinoides* extract on cardioprotective and antihyperlipidemic as well as toxicity effects on repeated doses. At concentrations of 25, 50 and 100 mg/ml, *R. prinoides* extracts resulted in alterations in heart rate of isolated rabbit's heart (chronotropic). The extract at the three concentrations, including acetylcholine caused a significant decline in the heart rate from minute 0 to the 5th minute of the experiment ($p<0.5$), Table 1. The effect of *R. prinoides* extract at the three concentrations on the heart rate was not significantly different in the 0, 1st, 2nd and 3rd minutes of the experiment ($p>0.05$). However, the heart rate at the three concentrations was significantly different ($p<0.05$) in the 4th and 5th minutes of the experiment. The effect of adrenaline remained steadily significantly increased in heart from the 1st minute of the experiment.

The effect of extract of *R. prinoides* at the concentration of 25, 50 and 100mg/ml revealed a significant increase in height of force of contraction of isolated hearts (ionotropic) Table 2. Therapy with the extract at the three concentrations, as well as adrenaline resulted in a significant increase in the height of force of contraction ($p<0.05$) from minute zero to the fifth minute of the experiment. The therapeutic effect of *R. prinoides* extract at the concentrations of 25, 50 and 100mg/ml on the height of force of contraction never differed significantly in the 1st minute ($p>0.05$) of the experiment. However, the effect of the extract at the three concentrations was significantly different in the 2nd, 3rd, 4th and 5th minutes ($p<0.05$) of the experiment. Table 2.

The total percentage change in height of force of contraction of the heart perfused with adrenaline was significantly higher than those ($p<0.05$) in the other treatment groups. Figure 1. The effect of acetylcholine noted a significantly lower total percentage change in height of force of contraction compared to those seen ($p<0.05$) in the other treatment groups. The total percentage change in height of force of contraction in the heart isolated from normal control rabbits was significantly lower than those reported ($p<0.05$) in extract-treated and adrenaline-treated isolated rabbits' heart. Figure 1.

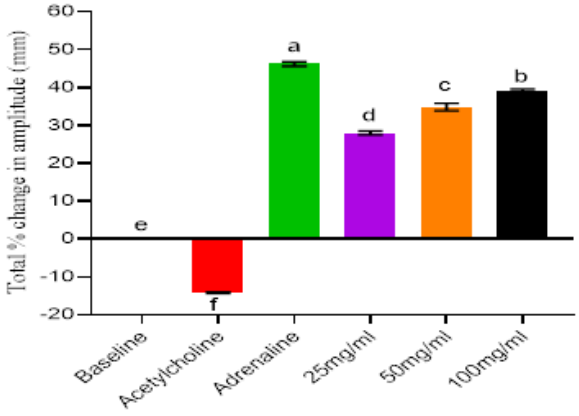

Figure 1: Effect of extracts of *Rhamnus prinoides* on total percentage change in height of force of contraction of rabbit's isolated heart

An HFD-induced hyperlipidemia, rats that were administered with *Rhamnus prinoides* extract doses of 25, 50, and100 mg/kg bw and revealed alterations in body weights throughout the experiment. Table 3. The negative control rats had significantly higher body weights compared to those ($p<0.05$) of the other treatment groups throughout the experiment. Treatment with the extract, as well as avastatin reduced the body weights of rats close to those of the normal control rats. The body weights of the rats declined in a dose-dependent manner after therapy with the extract. Table 3.

Therapy with methanol extract of *R. prinoides* doses of 25, 50 and 100mg/kg bw resulted in changes in levels of HDL, TC, TGs and LDL on HFD-induced hyperlipidemia in rats. Table 4. The effects of the extract at the three tested dosages, as well as the reference drug, avastatin increased the HDL levels and lowered the levels of TC, TGs and LDL close to those of normal control rats.

The mice that were administered with methanol extract at the doses of 100, 174 and 300mg/kg bw showed no significant variations ($p>0.05$) in the levels of total WBC, RBCs, HGB, HCT, MCV, RDW, PLT, MPV, PDW and PCT. There were also no significant variations in levels of liver and kidney parameters. The levels of these parameters statistically matched with those observed ($p>0.05$) in the normal control mice (Table 5, Table 6, and Table 7).

The qualitative phytochemical analysis of *R. prinoides* noted the presence of saponins, alkaloids, terpenoids, cardiac glycosides, steroids, and phenolics Table 8.

Table 1: Chronotropic effects of *Rhamnus prinoides* extracts on isolated rabbit's heart

	Heart rate (percentage change in heart rate)					
Treatment group	**0 min**	**1st**	**2nd**	**3rd**	**4th**	**5th**
Baseline	210.00 ± 2.89^{aA}	210.00 ± 2.89^{bA}	208.33 ± 1.67^{bA}	208.33 ± 1.67^{bA}	210.00 ± 2.89^{bA}	211.67 ± 1.67^{bA}
	(0.00 ± 0.00)	(0.00 ± 0.00^{b})	(0.00 ± 0.00^{b})	(0.00 ± 0.00^{b})	(0.00 ± 0.00^{b})	(0.00 ± 0.00^{b})
Acetylcholine	210.00 ± 2.89^{aA}	178.33 ± 1.67^{dB}	161.67 ± 1.67^{dC}	141.67 ± 1.67^{dD}	131.67 ± 1.67^{eE}	121.67 ± 1.67^{eF}
	(0.00 ± 0.00)	(-15.79 ± 0.79^{d})	(-22.40 ± 0.80^{d})	(-32.00 ± 0.80^{d})	(-37.30 ± 0.79^{e})	(-42.52 ± 0.787^{e})
Adrenaline	211.67 ± 1.67^{aD}	230.00 ± 2.89^{aC}	245.00 ± 2.89^{aB}	250.00 ± 2.89^{aAB}	260.00 ± 2.89^{aA}	260.00 ± 2.89^{aA}
	(0.00 ± 0.00)	(9.52 ± 1.37^{a})	(17.60 ± 1.39^{a})	(20.00 ± 1.39^{a})	(23.81 ± 1.37^{a})	(22.83 ± 1.36^{a})
25mg/ml	210.00 ± 2.89^{aA}	190.33 ± 2.67^{cB}	180.00 ± 2.89^{cC}	165.00 ± 2.89^{cD}	156.67 ± 1.67^{cD}	155.00 ± 2.89^{cD}
	(0.00 ± 0.00)	(-7.94 ± 0.79^{c})	(-13.60 ± 1.39^{c})	(-20.80 ± 1.39^{c})	(-25.40 ± 0.79^{c})	(-26.77 ± 1.36^{c})
50mg/ml	211.67 ± 1.67^{aA}	186.67 ± 1.67^{cdB}	175.00 ± 2.89^{cC}	163.33 ± 1.67^{cD}	150.00 ± 2.89^{cdE}	146.67 ± 1.67^{cdE}
	(0.00 ± 0.00)	(-11.11 ± 0.79^{c})	(-16.00 ± 1.39^{c})	(-21.60 ± 0.80^{c})	(-28.57 ± 1.37^{cd})	(-30.71 ± 0.79^{cd})
100mg/ml	211.67 ± 1.67^{aA}	185.00 ± 2.89^{cdB}	171.67 ± 1.67^{cdC}	155.00 ± 2.89^{cD}	141.67 ± 1.67^{deE}	141.67 ± 1.67^{dE}
	(0.00 ± 0.00)	(-11.90 ± 1.37^{cd})	(-17.40 ± 0.80^{cd})	(-25.60 ± 1.39^{c})	(-32.54 ± 0.79^{d})	(-33.07 ± 0.79^{d})

Means of heart rate that do not share a lowercase letter along the column, as well as means of percentage change in heart rate (within parenthesis) that does not share a letter along the column are, significantly different, ($p<0.05$). Means that do not share an uppercase superscript letter along the row are significantly different ($p<0.05$).

Table 2: Ionotropic effects of *Rhamnus prinoides* extracts on isolated rabbit's heart

	Height of force of contraction (mm)					
Treatment group	**0 min**	**1st**	**2nd**	**3rd**	**4th**	**5th**
Baseline	2.93±0.07^{aA}	3.00±0.12^{dA}	3.00±0.00^{dA}	3.00±0.12^{cA}	2.87±0.13^{dA}	2.93±0.07^{dA}
Acetylcholine	2.93±0.07^{aA}	2.73±0.03^{dAB}	2.65±0.03^{eB}	2.53±0.03^{dBC}	2.40±0.03^{eC}	2.38±0.04^{eC}
Adrenaline	2.93±0.03^{aD}	3.97±0.03^{aC}	4.13±0.03^{aBC}	4.30±0.03^{aB}	4.51±0.04^{aA}	4.70±0.06^{aA}
25mg	2.97±0.03^{aE}	3.32±0.04^{bcD}	3.58±0.03^{cC}	3.92±0.04^{bB}	3.95±0.03^{cB}	4.15±0.03^{cA}
50mg	2.93±0.07^{aD}	3.57±0.03^{bcC}	3.75±0.02^{bC}	4.05±0.05^{abB}	4.15±0.03^{bcB}	4.42±0.03^{bA}
100mg	2.90±0.06^{aB}	3.72±0.04^{abB}	3.85±0.02^{bB}	4.20±0.03^{aB}	4.30±0.03^{abB}	4.50±0.03^{abA}

Means and SEM that do not share a lowercase superscript letter along the column are significantly different using one- way ANOVA and Tukey's post hoc test ($p<0.05$). Means and SEM that do not share an uppercase superscript letter are significantly different using repeated measures ANOVA and Bonferroni corrections ($p<0.05$).

Table 3: Effect of *Rhamnus prinoides* extract on body weight following high-fat-diet-induced rats

	Percentage change in the body weight (g)				
Treatment	**7th day**	**14th day**	**21st day**	**28th day**	**Total change**
Baseline	11.32±1.23^{cD}	30.27±0.59^{dC}	54.65±1.06^{dB}	68.85±1.25^{dA}	41.27±0.63^{e}
Vehicle	26.52±0.69^{aD}	52.49±0.90^{aC}	81.61±1.21^{aB}	95.34±0.88^{aA}	63.99±0.55^{a}
Avastatin	21.06±0.72^{bD}	42.13±0.94^{bC}	62.31±0.88^{bB}	79.98±1.00^{bA}	51.37±0.10^{b}
25mg	22.05±0.46^{bD}	44.09±0.84^{bC}	62.02±0.58^{bB}	82.87±0.69^{bA}	52.98±0.58^{b}
50mg	20.48±0.72^{bD}	37.42±0.58^{cC}	59.20±0.62^{bcB}	79.22±0.67^{bA}	49.08±0.28^{c}
100mg	19.77±0.69^{bD}	34.08±0.82^{cC}	56.99±0.71^{cdB}	72.89±0.71^{cA}	45.93±0.35^{d}

Means and SEM that do not share a letter along the column are significantly different. Means and SEM that do not share an uppercase superscript letter are significantly different ($p<0.05$).

Table 4: Effect of *Rhamnus prinoides* extracts on lipid profiles following high-fat-diet-induced hyperlipidemia in rats

	Lipid profiles (mmol/L)			
Treatment	**HDL**	**TC**	**TRIG**	**LDL**
Baseline	1.63 ±0.12^{b}	1.57±0.11^{d}	0.71±0.70^{d}	0.41±0.07^{d}
Vehicle	0.40±0.02^{d}	11.57±0.24^{a}	4.21±0.19^{a}	9.02±0.18^{a}
HFD + Avastatin	1.11±0.05^{c}	6.83±0.22^{b}	2.21±0.23^{b}	4.98±0.28^{b}
HFD + 25mg	1.44±0.05^{bc}	6.31±0.16^{b}	1.55±0.10^{c}	4.14±0.12^{b}
HFD + 50mg	1.75±0.10^{b}	4.40±0.40^{c}	1.10±0.05^{cd}	3.04±0.43^{c}
HFD + 100mg	2.19±0.12^{a}	3.54±0.08^{c}	0.94±0.05^{d}	2.23±0.26^{c}

Means and SEM that do not share a letter along the column are significantly different ($p<0.05$).

Table 5: Effect of *Rhamnus prinoides* extracts on body weight in mice

	Percentage change in body weight (g)			
DAYS	**Baseline**	**100mg/kg**	**174mg/kg**	**300mg/kg**
7th day	7.87±0.84^{aD}	6.29±1.04^{aD}	6.26±1.09^{aD}	6.17±1.04^{aD}
14th day	14.08±1.04^{aC}	15.15±0.94^{aC}	15.22±1.13^{aC}	14.20±1.02^{aC}
21st day	28.12±1.30^{aB}	25.95±1.15^{aB}	25.02±0.92^{aB}	24.76±0.91^{aB}
28th	35.13±0.58^{aA}	33.07±1.18^{abA}	31.24±0.98^{abA}	31.85±0.46^{bA}
Total change	21.30±0.50^{a}	20.12±0.50^{a}	19.43±0.72^{a}	19.24±0.65^{a}

Means and SEM that do not share a letter along the row are significantly different. Means and SEM that do not share an uppercase superscript letter along the column are significantly different ($p<0.05$).

Table 6: Effect of *Rhamnus prinoides* on hematological parameters in mice

Treatment	**Baseline**	**100mg/kg**	**174mg/kg**	**300mg/kg**
WBC (*10^9/L)	10.12±0.59	9.54±1.07	11.21±0.23	11.43±0.49
RBC (*10^12/L)	11.11±0.27	10.64±0.37	10.32±0.17	10.25±0.16
HGB (g/dL)	16.10±0.46	15.56±0.15	16.00±0.44	16.40±0.38
HCT (%)	60.73±1.43	59.93±2.32	57.69±1.31	58.16±0.97
MCV (fL)	54.68±0.76	56.38±0.78	55.38±0.52	56.70±0.38
RDW-CV (%)	19.74±0.48	21.27±0.33	20.32±0.93	19.52±0.92
PLT (*10^9/L)	63.58±1.21	63.30±1.9	64.36±1.02	66.00±0.94
MPV (fL)	7.56±0.26	7.34±0.23	5.21±1.79	7.44±0.29
PDW (fL)	10.30±0.41	9.64±0.40	10.35±0.32	10.46±0.19
PCT (%)	0.68±0.06	0.74±0.02	0.73±0.03	0.78±0.02

Values in the same row are not statistically significant ($p>0.05$).

Table 7: Effects of *R. prinoides* on liver function and renal function tests in mice

Treatment	**Baseline**	**100 mg/kg**	**174 mg/kg**	**300 mg/kg**
ALP (U/L)	88.00±0.83	89.20±0.37	87.40±0.92	87.20±0.58
AST (U/L)	274.80±1.30	275.80±0.58	275.60±0.81	278.80±1.16
ALT(U/L)	117.80±0.73	117.20±0.73	117.40±0.67	118.60±0.74
TP(U/L)	6.70±0.11	6.72±0.05	6.82±0.12	6.94±0.10
ALB(U/L)	3.55±0.11	3.48±0.14	3.56±0.03	3.66±0.34
TBIL(U/L)	25.40±0.51	26.20±0.37	26.80±0.49	27.20±0.66
DBIL(U/L)	10.72±0.18	10.80±0.22	11.28±0.26	11.12±0.38
CREAT (µmol/L)	64.18±0.90	64.30±0.31	64.40±0.60	65.50±0.32
Urea (µmol/L)	524.20±7.15	525.00±7.45	518.80±4.93	530.40±5.66
Na^+ (µmol/L)	144.80±1.08	144.80±0.49	143.80±0.37	144.60±0.51
K^+ (µmol/L)	5.10±0.34	5.60±0.26	4.76±0.09	5.08±0.03
Cl^-(µmol/L)	105.80±0.53	105.40±0.24	105.00±0.01	106.20±0.37
Ca (µmol/L)	2.31±0.09	2.40±0.09	2.57±0.14	2.74±0.17
P (µmol/L)	1.45±0.12	1.37±0.02	1.31±0.09	1.67±0.17

Values in the same row are not statistically significant ($p>0.05$).

Table 8: Qualitative phytochemical analysis of *Rhamnus prinoides* extracts

Secondary metabolites	*Rhamnus prinoides*
Saponins	+
Alkaloids	+
Terpenoids	+
Flavonoids	-
Cardiac glycosides	+
Steroids	+
Phenolics	+

+ = presence; - = absence

DISCUSSION

Hyperlipidemia, including hypertension and obesity, increases the risk for congestive heart failure [11]. Hyperlipidemia is a medical disorder characterized by elevated levels of blood lipids such as low-density lipoproteins, cholesterol and triglycerides, as well as reduced levels of high-density lipoproteins [12].

Several drugs with cardiotonic effects such as digitoxin, enoximone, amrinone, piroximone, and milrinone are highly prescribed to treat heart failure [13,14]. Cardiotonic medications improve the efficiency and contraction of the heart muscles, thereby increasing blood flow to all body tissues [14]. On the other hand, anti-hyperlipidemia drugs such as avastatin, fibrates, atorvastatin, Fluvastatin and lovastatin are often prescribed to treat hyperlipidemia [15,16]. Over the years, medicinal plants have served as essential bioresources for the management of many diseases and disorders [12,17]. It is therefore reasonable to refer to medicinal plants as a sleeping giant for potential development of novel therapeutic agents. The Kikuyu and Maasai communities in Kenya use *Rhamnus prinoides* in the management of both cardiovascular diseases and hyperlipidemia. However, there was a paucity of scientific evidence to validate these claims. This study aimed at determining the cardiotonic and anti-hyperlipidemia activities, as well as acute and subacute toxicity effects of methanol extracts of *Rhamnus prionides*.

The effect of *R. prinoides* noted a significant decrease in heart rate (negative chronotropic effect) and an increase in height of force of contraction (positive ionotropic effect) on rabbit isolated heart compared to values noted in the normal control from the first minute of the experiment. According to these findings, the extract had cardiotonic effects without causing cardiac arrest even at high doses. The effect of adrenaline revealed a significant elevation of the heart rate and amplitude of contraction compared to the effect of the extracts at the three concentrations from the first minute of the experiment onwards. The effect of acetylcholine reported a significant decrease in the heart rate and the amplitude (height of force of contraction) relative to the effect of the extracts from the first minute of the study onwards.

The effects of the extracts enhanced the height of force of contraction and reduced the heart rate in a concentration-dependent manner. This implied that an increase in concentration enhanced contractility and lowered the heart rate. The positive ionotropic effects that were exhibited by the studied extracts were therefore attributed to cardiac stimulation or through increased availability of intracellular Ca^{2+} by opening membrane L-type Ca^{2+} channels or via other mechanisms such as inhibition of potassium channels and β1-adrenoceptors.

Cardiotonic agents are drugs-agents that causes an increase on the contractile strength of muscle of the heart (myocardium) and enhance its capability and efficiency. Positive inotropic and negative chronotropic effects are directly related to cardiotonic activities. The total Blood volume (volemia) exiting the left heart ventricle increases with an increase in the force of myocardial contraction. As a result, the cardiac output (the amount of blood exiting the left ventricle with each contraction) also increases, which causes an increased blood flow in all body organs [18,14]. A decrease in heart rate upon administration of the extracts could be explained by an elevation of amplitude (height of the force of contraction).

The cardiotonic activities of *R. prinoides* extracts can be attributed to the classes of phytocompounds that were identified using qualitative analysis. The extract noted the presence of glycosides, phenolics, saponins, alkaloids, terpenoids and steroids. This could explain why *R. prinoides* extract had a better cardiotonic effect. The phytochemical classes of steroids, glycosides phenolics and terpenoids have been associated with cardiotonic effects [20,21,18,14]. For instance, glycosides inhibit Na^+/K^+ ATPase resulting in increased intracellular Ca^{2+} concentrations via Na^+/Ca^{2+} exchange. This causes both the transient and slow inward Ca^{2+} influx to rise [22].

Acute toxicity study evaluates the adverse outcomes after oral single dose exposure of a test drug in a brief period. The acute toxicity effects of methanol extract of *R. prinoides* at doses of 1000 and 2000mg/kg bw never showed any toxicity signs or behaviors after a single dose exposure in mice. The extract never revealed toxicity signs such as diarrhea, altered skin and fur texture, change in breathing pattern, aggression, convulsions, or drug-related behavioral abnormalities such as agitation, paralysis, clumping together, and abnormal movement.

Since the LD_{50} of the studied extract was above 2000mg/kg bw of mice, the extract was considered relatively safe according to the classification of acute toxicity [23].

CONCLUSION

From this study it is concluded that the Methanol extracts of *R, prinoides* significantly lowered the plasma levels of low-density lipoproteins and total cholesterol while elevating high density lipoproteins in rats. It is therefore likely to pose potential anti-atherosclerotic effects. It exhibited negative chronotropic and positive ionotropic effects on the isolated rabbit heart therefore may have profound effect on cardiac output and ultimately blood pressure. *Rhamnus prinoides* extracts may not be toxic and possesses classes of phytocompounds associated with cardio-protective effects.

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Declarations

Ethical approval and consent to participate was sought from Kenyatta university ethical review board and Committee for the use of laboratory animals. The approval and documentation to carry out

research was sourced from the National Commission for Science, Technology, & Innovation (NACOSTI), permit number 411801.

List of abbreviations

HCHF: High cholesterol high fat diet

LDL: Low density lipoproteins

HDL: High density lipoproteins

CVD: Cardiovascular disease

CAD: Coronary artery disease

ANOVA: Analysis of Variance

Conflict of interest

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ORCID ID

Stephen Ngigi Mburu: https://orcid.org/0000-0001-8400-5033

Mathew Piero Ngugi: https://orcid.org/0000-0002-0226-9092

Mwonjoria John Kingori Maina: https://orcid.org/0000-0002-2407-0033

REFERENCES

1. Nankaya JS. The salient traditional medicinal plants and conservation strategies of the Loita Maasai of Kenya. Proquest. 2014;3(5):165-4.
2. Nigussie G, Alemu M, Ibrahim F, Werede Y, Tegegn M, Neway S, Endale M. Phytochemicals, traditional uses and pharmacological activity of Rhamnus prinoides: A review. Int J Second Metabolite. 2021;8(2):136-151.
3. Soriano CV. Levels of essential and non-essential metals in Rhamnus prinoides (gesho) cultivated in Ethiopia. gepcomm Diagn Essay. 2021;26(3):329-342.
4. Spring B, Moller AC, Colangelo LA, Siddique J, Roehrig M, Daviglus ML, et al. Healthy lifestyle change and subclinical atherosclerosis in young adults: Coronary artery risk development in young adults (Cardia) study. Circulation. 2014;130(1):10-17.
5. Miller-Hance WC, Gertler R. Essentials of Cardiology. In: A Practice of Anesthesia for Infants and Children. 4th ed. Elsevier Inc.; 2019.
6. Nawale S, Padma Priya K, Pranusha P, Ganga Raju M. Data of antihyperlipidaemic activity for methanolic extract of Tagetes patula Linn. flower head along with piperine, as bioavailability enhancer. J Ethnopharmacol. 2018;21:587-597.
7. Cheluvappa R, Scowen P, Eri R. Ethics of animal research in human disease remediation, its institutional teaching; and alternatives to animal experimentation. Pharmacol Res Perspect. 2017;5(4):1-14.
8. Dhein S. The Langendorff heart. Practical Methods in Cardiovascular Research. 2005;155-172.
9. Cunha LF, Ongaratto MA, Endres M, Barschak AG. Modelling hypercholesterolaemia in rats using high cholesterol diet. Int J Exp Pathol. 2021;102(2):74-79.
10. Moller WC, Gertler R. Essentials of Cardiology. In: A Practice of Anesthesia for Adults. 4th ed. Elsevier Inc.; 2019.
11. Alembagheri A, Hajimehdipoor H, Choopani R, Esmaeili S. An Overview of Cardiotonic Medicinal Plants from the Perspective of Iranian Traditional Medicine. Jundishapur J Nat Pharm Prod. 2022.
12. Zhou J, Zhao L, Meng L, Liang H, Zhou T, Ye S, et al. Acupuncture treatment for carotid atherosclerotic plaques: Study protocol for a pilot randomized, single-blinded, controlled clinical trial. Trials. 2020;21-22.
13. Schure AY, DiNardo JA. Cardiac physiology and pharmacology. In: A Practice of Anesthesia for Infants and Children. 4th ed. Elsevier Inc.; 2019. p. 424-457.
14. Satria D, Harahap U, Dalimunthe A, Hertiani T, Syahputra RA, Muhammad M, et al. The Effect of Extraction Method of Vernonia amygdalina Delile. Leaves on Cardiotonic Effect. Farmacia. 2023;71(2):392-396.
15. Liu Y, Huang Y, Xiao Z, Ren X, Yang C. Effect of Ar/CCl4 combined purification on microstructure and mechanical properties of 2219 aluminum alloy ingot. Fenmo Yejin Cailiao Kexue yu Gongcheng/Materials Science and Engineering of Powder Metallurgy. 2016;21(3).
16. Muregi FW, Ishih A, Miyase T, Suzuki T, Kino H, Amano T, et al. Antimalarial activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine (CQ) against a CQ-tolerant rodent parasite, in mice. J Ethnopharmacol. 2007;4:170-175.
17. Somade PM, Atul RC, Suryakant BK, Summit DN. Cardiotonic activity of aqueous and alcoholic extracts of Leea macrophylla. Int J Pharm Res Health Sci. 2017;5(6):1945-1948.
18. Ali A, Akhtar N, Khan BA, Khan MS, Rasul A, Khalid N, et al. Acacia nilotica: A plant of multipurpose medicinal uses. 2012;6(9):1492-1496.
19. Silva-Santana G, Bax JC, Fernandes DCS, Bacellar DTL, Hooper C, Dias AASO, et al. Clinical hematological and biochemical parameters in Swiss, BALB/c, C57BL/6 and B6D2F1 Mus musculus. Anim Models Exp Med. 2020;3(4):304-315.
20. Urga K, Ababa A, Guta M. Spasmolytic Activity of the Aqueous Root Extract of Solanum incanum, Solanaceae. Ethiop J Biol Sci. 2008.
21. Spring B, Moller AC, Colangelo LA, Siddique J, Roehrig M, Daviglus ML, et al. Healthy lifestyle change and subclinical atherosclerosis in young adults: Coronary artery risk development in young adults (Cardia) study. Circulation. 2014;130(1):10-17.

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