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Effects of bioactive principles from stem bark extract of *Quassia amara*, Quassin and 2-methoxycanthine-6-one, on haematological parameters in albino rats

Raji Yinusa
Department of Physiology, College of Medicine, University of Ibadan. Nigeria

Summary: The effect of *Quassia amara* extract and two isolated compounds from the extract, quassin and 2-methoxycathine-6-one on haematological parameters was studied in rats. All doses of the extract and those of the quassin significantly increased ($p<0.05$) red blood cell count, packed cell volume and haemoglobin concentration. However, there was no significant increase ($P>0.05$) in the total white blood cell count. There was also no significant change ($P>0.05$) in all parameters studied with 2-methoxycanthine-6-one. The results suggest that quassia extract possesses antianaemic property.
Keywords*:* Anaemia, Quassinoid, Quassin, 2-methoxycanthine-6-one, Rats

**Address for correspondence:*yinusaraji@yahoo.com, yoraji@yahoo.com; Tel: +2348023263626

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INTRODUCTION

Quassia amara L. (Simaroubacae) is a tree endemic to different regions of the tropics. It is a native of Central America which later spread into tropical countries following a lead provided in the 1947 antimalarial screening programme (Spencer *et al*., 1947). Traditionally, the tree is reputed for its good stomachic, antianaemic, antibiotics, antimalarial, antiamoebic and cytotoxic properties (Trager *et al*., 1981, & Polonsky, 1985). Its reproductive activities (Njar *et al*., 1995, Raji & Bolarinwa 1997, & Parveen *et al*., 2003) and its insecticidal, larvicidal and vermifuge activities have also been well reported (Jenson, 1978, Jenson, 1979, Park *et al*., 1987, Evans & Raji, 1991). Quassinoids are the bitter principles and the main constituents of the quassia plant (Polonsky, 1973).

Several studies on biological activity of quassia extract revealed that quassinoids possess antimalarial, antiamoebic, anti-tumour and antianaemic properties. The plant is used in traditional medicine, frequently given as aqueous extracts (i.e used in the form of a 'tea') to prevent or to ameliorate the complications of the aforementioned ailments. Interest in the quassionoids has accelerated rapidly with the finding by the American National Cancer Institute in the early 1970s that these compounds display marked antileukaemic activity. Beside the traditional uses of quassia extract cited by earlier workers, there seem to be no information in the literature on the effect of quassia extract on haematological parameters. The present study was designed to investigate the effects of stem bark extract of *Quassia amara* and the two isolated principles (quassin and 2-methoxy-canthine-6-one) on haematological indices of male albino rats.

MATERIALS AND METHODS

Extraction and purification of plant material: Stem bark of *Quassia amara* was collected at the botanical garden, University of Ibadan, Nigeria. A voucher specimen was deposited at the Forestry Research Institute of Nigeria (FRIN) herbarium, Ibadan. The stem bark was air-dried and pulverized with blender to obtain 1kg of the plant materials. This was carried out as described by (Grandolini *et al*., 1987 and Njar *et al*., 1993). The pulverized stem bark (1kg) was exhaustively extracted with methanol by means of Soxhlet apparatus and the extract evaporated *in vacuo*. Water was added to the residue and the mixture extracted with hexane and then with $CHCl_3$. The $CHCl_3$ extract was dried using (anhydrous magnesium sulphate ($MgSO_4$) and evaporated to give a residue (3.5g) called quassionoid. The residue was chromatographed on a silica gel column as previously described (Njar *et al*., 1993) to yield 2-methoxy-canthine-6-one and quassin.

Preparation of solutions of quassia extracts:
10mg of methanolic extract of quassia stem bark (quassionoid) was dissolved in 1ml of 95% ethanol. This was sterilized by filtration through a millipore filter (0.20µm) attached to a syringe. Further dilutions were carried out in phosphate buffered saline. Quassin was dissolved in 95% ethanol at a concentration of 1mg/ml. A stock-solution was prepared by mixing 1ml of the ethanol solution with 9ml phosphate buffered saline. This was sterilized by filtrations through a millipore filter (0.02µm) attached to a syringe. The same procedure was repeated for 2-methoxycanthine-6-one. Appropriate dilutions were prepared individually for quassin and 2-methoxycanthine-6-one, in phosphate buffered saline before administered to the rats. The highest concentration of ethanol introduced was 1 part in 10,000. Controls with ethanol at the concentrations introduced with the drugs were prepared from a stock solution of 1ml 95% ethanol with 9ml phosphate buffered saline.

Experimental Animals: Male Wistar albino rats weighing 150 -200g were used for the study. These animals were obtained from the preclinical animal house, University of Ibadan, Nigeria. They were kept under standard laboratory conditions, fed with rat pellets and water *ad libitum*. The animals were later divided into experimental groups.

Experimental Design: The study was divided into three experiments. Each experiment was further sub-divided into four groups of five rats each. Rats in the first experiment were treated with quassinoid as follows: group I, (control) received 0.5ml of the vehicle for the stock solution of quassinoid. Groups II, III and IV rats received 100, 1000 and group 2000 mgkg $^{-1}$ b.w of the quassinoid respectively. Rats in the second experiment were treated with quassin as follows: group I, (control) received 0.5ml of the vehicle for quassin, groups II, III and IV rats received 10, 100 and 1000µgkg^{-1} bw of quassin respectively. Rats in the third experiment were treated with 2-methoxycanthine-6-one as follows: group I, (control) received 0.5ml of the vehicle for 2-methoxycanthine-6-one, groups II, III and IV rats received 10, 100 and 1000µgkg^{-1} bw of 2-methoxycanthine-6-one respectively. Administration was done daily for two weeks using oral needle, in all groups.

Haematological Analysis: Blood samples were obtained on day 15 from the tails of the animals for determination of blood parameters under investigation; packed cell volume (PCV), haemoglobin levels (Hb concentration), red blood cell counts (RBC) and white blood cell counts (WBC). Packed cell volume was measured by the microhaematocrit technique using a Hawksley microhaematocrit centrifuge and spinning for 5min at 12,000x*g* before reading with the haematocrit reader. Heparinized capillary tubes were products of the British Drug House (BDH). Haemoglobin levels were measured by the cyanomethaemoglobin method using a CE 404 colorimeter (Cecil Instruments). The red blood cell and white blood cell counts were done using the haemocytometer method.

Statistical Analysis – Data were expressed as mean±SEM and were analysed using student's't' test. Values were considered significant at $p<0.05$.

RESULTS:

Effects of quassinoid on haematological parameters
The results in table 1 show that the red cell counts, haemoglobin concentrations and PCV were significantly higher ($p<0.05$) in 100, 1000 and 2000 mgkg^{-1} b.w. quassinoid treated rats when compared with the control. The values for total white cell count in quassinoid treated rats did not differ significantly ($p>0.05$) from those recorded for normal control rats (Table 1.)

Table 1
Effect of quassinoid on blood parameters

Dose of Quassinoid (mgkg^{-1} b.w.)	R B C (10^6/ml)	P C V (%)	Hb (g%)	W B C (10^3/ml)
1	5.81 ± 0.34	42.10 ± 1.02	14.22 ± 0.41	5.30 ± 0.60
100	6.46 ± 0.41	45.1 ± 1.00	14.94 ± 0.43	5.10 ± 0.50
1000	6.52 ± 0.47	45.60 ± 0.97	15.00 ± 0.38	5.00 ± 0.50
2000	6.53 ± 0.36	45.60 ± 0.96	15.1 ± 0.37	5.00 ± 0.40
Published standard values	8.50 (5-12)	45.90(36-52)	14.20 (11-18)	-

Values in the last line of table are published standard values taken from *Canadian Council on Animal Care Guide and Use of Experimental Animal*, Vol.1, 1980 edn. National Library of Canada. n=5

Table 2: Effect of quassin on haematological parameters

Dose of quassin (μgkg^{-1} b.w)	RBC (10^6/ml)	PCV (%)	Hb (g/dl)	WBC (10^3/ml)
Control	5.21 ±0.04	42.00±0 0.37	14.18 ±0 0.07	4.91 ±0 0.07
10	6.61 ±00.04*	48.50±0 0.43*	14.93 ±0 0.08	4.90 ±0 0.11
100	6.59 ±00.04*	48.00±0 0.46*	14.86 ±0 0.08	5.01±0 0.10
1000	6.60 ±0 0.04*	48.10±0 0.39*	14.88 ±0 0.06	4.92 ±0 0.08
Published standard values	8.50 (5-12)	45.90(36-52)	14.20 (11-18)	-

Values in the last line of table are published standard values taken from *Canadian Council on Animal Care Guide and Use of Experimental Animal*, Vol.1, 1980 edn. National Library of Canada. *Significantly different from control (P<0.05)

Table 3:
Effects of 2-methoxycanthine-6-one on haematological parameters.

Dose of 2-methoxycanthine-6-one (μgkg^{-1} b.w)	**RBC (10^6/ml)**	**PCV (%)**	**Hb (g/dl)**	**WBC (10^3/ml)**
Control	5.21± 0.04	42.00 ± 0.37	14.68 ± 0.07	4.91 ± 0.07
10	5.36 ± 0.04	42.10 ± 0.35	13.81 ± 0.08	4.90 ± 0.08
100	5.41 ± 0.04	42.10 ± 0.27	14.85 ± 0.07	4.91± 0.09
1000	5.40 ± 0.04	42.12 ± 0.21	14.78 ± 0.08	4.91 ± 0.07
Published standard values	8.50 (5-12)	45.90(36-52)	14.20 (11-18)	-

*Values in the last line of table are published standard values taken from Canadian Council on Animal Care Guide and Use of Experimental Animal, Vol.1, 1980 edn. National Library of Canada. *Significantly different from control (P<0.05)*

Effects of quassin on haematological parameters
RBC count, haemoglobin concentrations and PCV levels were significantly increased ($p<0.05$) in all quassin treated rats when compared with the control values. However, there was no significant difference ($p>0.05$) in the WBC count in these rats.

Effects of 2-methocycanthine-6-one on haematological parameters
There was no significant change in RBC count, Hb concentration, PCV and WBC counts in all the 2-methoxycanthine-6-one treated rats when compared with the control (Table 3).

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DISCUSSION

In this study we present evidence to show that quassia extract and its principle- quassin caused an increase in red blood cell parameters. The values of haematological parameters in the control group were not statistically different ($p>0.05$) from those reported by the Canadian Council on Animal Care (Council on Animal Care Guide, 1980). These values also corroborate earlier ones reported in normal Wistar albino rats (Adeniyi & Olowookorun 1998, Oluwole & Bolarinwa, 1997). This finding further confirms the WHO (WHO 1968 & 1972) reports that distribution of normal haematological parameters are likely to be universally identical when allowance has been made for matching of age, sex and pregnancy. The study further showed that rats given higher doses of the extract had no preponderance increase in total WBC count, which may suggest that the extracts do not stimulate the immune function of the treated rats. The significant increase recorded in the RBC count, haemoglobin concentrations and PCV in quassin treated rats suggest a haemopoetic role of the extracts over its counterpart extract, 2-methoxycanthine-6-one. The increase in red cell parameters in this study may suggests that the extract and quassin could serve as antianaemic agents. This may be the reason for the use of *Quassia amara* extract as a general tonic and the reported antianemic properties of quassia plant (Trager & Polonsky 1981, & Polonsky, 1985). We conclude that, the haemopoeitic property of quassinoids may be associated with quassin component of the extract.

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