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Full Length Research Paper

Haematinic activity of *Hibiscus cannabinus*

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The haematinic activity of an orally administered aqueous extract of *Hibiscus cannabinus* leaves was studied on haemolytic anaemic rats. Anaemia was induced by an oral administration of phenylhydrazine for a period of 8 days. Red blood cell count, haemoglobin concentration, and pack cell volume were analysed as indices of anaemia. The mean cell haemoglobin, mean cell volume and mean cell haemoglobin concentration were calculated accordingly. Phenylhydrazine induced a significant decrease (P<0.05) in the blood parameters indicating anaemia and also resulted to significant increase (P<0.05) in the mean cell haemoglobin, mean cell volume values, which are indicators of macrocytosis. Leaf extract of *H. cannabinus* induced a significant (P<0.05) increase in the red blood cell count, haemoglobin concentration, and pack cell volume which had been originally decreased by phenylhydrazine administration within one week of treatment. The presence of macrocytosis turn towards normal as the animals recovered from anaemic condition. The results obtained suggested that *H. cannabinus* leaves may have haematinic properties.

Key words: Haematinic activity, *Hibiscus cannabinus*, haemolytic anaemia, phenylhydrazine.

INTRODUCTION

Through the ages man has learnt to take advantage of the many resources placed at his disposal by nature to meet his essential needs in all fields. As important reserves and sources of abundance, natural resources are indispensable for socio-economic development. According to Gbile (1986), the diversity of the flora in Africa partly explains the strength of traditional medicine. This refers to the use of plants in the treatment or amelioration of diseases within an organised system.

Hibiscus cannabinus Linn. (malvoceae) also known as Kenaf is a tall annual woody herb often single stemmed or an under shrub of 1-2 m, with minute prickles on the stems and leaf stalks. It is a plant common in grassland and secondary regrowth after cultivation and along streams. In Africa It is wide spread in the tropics, found in Ethiopia, Zimbabwe, Mozambique, Uganda (Katende et al., 1999) and also widely distributed in Cameroon (Agbor et al., 2004) in such fashion that one will hardly find a village without *H. cannabinus*. The plant produces the O-hibiscanone and hydroquinone in response to infection by the wilt pathogen, *Verticillium dahlaie* (Puckhaber et al., 1998). Phytochemical screening of this plant revealed the presence of phenolics, tannin, saponin, alkaloids and steroids (Agbor et al., 2004). *H. cannabinus* is rich in nitrogen and phosphorus thus used in the treatment of polluted water with low nitrogen and phosphorus concentrations (Abe et al., 1999). The plant also has rich fibre content (Eremosele et al., 1999), which serves as a good material in the paper industry (Mohta et al., 2000).

H. cannabinus has been reported to be anodyne, aperitif, aphrodisiac, fattening, purgative, and stomachic, as well as a folk medicine for bilious conditions, bruises,

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Table 1. Effect of phenylhydrazine (10 mg/kg, o.p daily for 8 days) on some haematological parameters (T=0) (RBC, Hb, PCV and WBC).

Parameters	Group 1 (control)	Group 2 (anaemic)	Group 3 (anaemic)	Group 4 (anaemic)	Group 5 (anaemic)
Hb (g/dl)	19.09 ± 0.63	13.18 ± 0.86 ^{s}	13.77 ± 0.56 ^{s}	13.91 ± 0.97 ^{s}	13.10 ± 1.14 ^{s}
PCV (%)	58.19 ±1.80	40.72 ± 2.10 ^{s}	41.90 ± 1.26 ^{s}	41.14 ± 2.35 ^{s}	40.69 ± 2.10 ^{s}
RBC (x10^{b}/μl)	7.64 ± 0.19	4.28 ± 0.37 ^{s}	4.18 ± 0.22 ^{s}	3.98 ± 0.28 ^{s}	3. 99 ± 0.28 ^{s}
MCV (fl)	76.18 ± 2.94	95.41± 3. 20 ^{c}	102.74 ± 2.94 ^{c}	108.35 ± 2.90 ^{c}	101.08 ± 3.20 ^{c}
MCH (pg)	24.97 ± 1.42	30.14 ± 2.32 ^{c}	32.97 ± 1.08 ^{c}	35.04 ± 1.17 ^{c}	32.44 ± 0.92 ^{c}
MCHC (g/dl)	32.80 ± 0.33	30.89 ± 1.51	32.08 ± 0.53	31.81 ± 0.62	31.12 ± 0.90

Values are Mean ± Standard Deviation for 6 rats per group.
^{s} Significantly lower as compared to control (Group 1).
^{c} Significantly higher than control (Group I) $P < 0.05$.

Table 2. Haematological parameter of rats after one week treatment with extract of *H. cannabinus*.

Parameters	Group 1 (control)	Group 2 (anaemic control)	Group 3 (400 mg/kg)	Group 4 (800 mg/kg)	Group 5 (1600 mg/kg)
Hb (g/dl)	19.33 ± 1.23	15.45 ± 0.81^{s}	19.26 ± 1.12 ^{d}	20.19 ± 0.95 ^{d}	20.02 ± 0.79 ^{d}
PCV (%)	59.54 ±1.11	50.48 ± 2.57 ^{s}	60.12 ± 1.26 ^{d}	60.32 ±1.42 ^{d}	61.86 ± 1.91^{d}
RBC(x 10^{6}/μl)	7.75 ± 0.21	5.08 ± 0.50	6.44 ± 0.38 ^{sd}	6.90 ± 0.31 ^{sad}	7.06 ± 0.40 ^{sad}
MCV (fl)	76.88±2.45	99.48±1.40	93.47±3.00 ^{c}	87.48±2.45 ^{c}	87.78±2.68 ^{c}
MCH (pg)	24.94±1.56	20.84 ^{c}±2.56 ^{c}	29.98±2.53 ^{c}	29.27±0.89 ^{c}	29.19±1.64 ^{c}
MCHC (g/dl)	32.56±1.39	32.98±1.68	32.04±1.67	33.46±0.87	32.36±0.61

Values are mean ± Standard Deviation for six rats in each group.
^{s} significantly lower compared with control (Group 1), ^{d} significantly higher compared with anaemic control (Group 2), ^{a} significantly different compared with group 3.
^{c} Significantly higher than control (Group I) $P < 0.05$.

Table 3. Haematological parameters of rats after 2 weeks treatment with extract of *H. cannabinus*.

Parameters	Group 1 (control, H_2O)	Group 2 (anaemic control, H_2O)	Group 3 (400 mg/kg)	Group 4 (800 mg/kg)	Group 5 (1600 mg/kg)
Hb (g/dl)	19.72 ±1.37	19.66 ± 1.12	20.15 ± 0.98 ^{c}	20.59 ± 0.68 ^{c}	21.40 ± 1.59 ^{cd}
PCV (%)	60.00 ± 1.49	58.15 ±1.80	60.63 ±1.08 ^{d}	61.21 ±1.22 ^{d}	62.39 ±1.35 ^{cad}
RBC (x 10^{6}/μl)	7.74 ± 0.33	6.11± 0.10 ^{s}	6.69 ±0.22 ^{sd}	7.70 ±0.09 ^{d}	7.67 ± 0.18 ^{ad}
MCV (fl)	78.49±2.51	94.88±2.49 ^{c}	90.73±3.06 ^{c}	79.66±1.50	81.23±2.73
MCH (pg)	26.21±2.76	32.12±1.93 ^{c}	30.15±1.91 ^{c}	26.78±0.72	27.22±1.66
MCHC (g/dl)	32.85±2.15	33.77±1.02	33.23±1.46	33.65±0.77	34.29±2.15

Values are mean ±Standard Deviation for six rats in each group.
^{s} significantly lower compared with control (Group 1), ^{c} significantly higher compared with control (Group 1),
^{d} significantly higher compared with anaemic control (Group 2)
^{a} significantly different compared with group 3, $P < 0.05$

Table 4. Haematological parameters of rats after 3 weeks treatment with extract of *H. cannabinus*.

Parameters	Group 1 (control, H_2O)	Group 2 (anaemic control, H_2O)	Group 3 (400mg/kg)	Group 4 (800mg/kg)	Group 5 (1600mg/kg)
Hb (g/dl)	19.93±1.24	18.00±1.25	20.33±0.52	19.63 ±1.48	19.97 ±1.06
PCV (%)	59.80 ±2.59	57.43 ±1.47	59.58 ±0.89	59.93±1.82	59.20±1.89
RBC (x 10^{6}/μl)	7.82±0.25	7.15±0.16 ^{s}	7.43±0.46	7.80±0.43	7.55±0.45
MCV (fl)	76.54±3.78	80.31±2.71	80.41±4.78	77.01±4.94	78.54±3.94
MCH (pg)	25.49±1.56	25.18±1.98	27.45±1.52	25.21±2.07	26.48±1.44
MCHC (g/dl)	33.42±1.28	31.34±1.60	33.89±0.44	32.73±1.73	33.71±0.78

Values are mean ± Standard Deviation for six rats in each group.
^{s} significantly lower compared with control (Group 1) $P<0.05$.

and fever (Duke, 1983). In African folk medicine, *H. cannabinus* is used as an anthelmintic (Iwu, 1993). In Northern Cameroon, the plant is used as vegetable and in Southern Cameroon as a panacea in anaemic therapy. In an earlier study, this plant was observed to be well tolerated in acute, sub-acute and sub-chronic administration in albino rats (Agbor et al., 2004). The present study is undertaken to verify the acclaimed haematinic activity.

MATERIALS AND METHODS

Plant material

Fresh leaves of *H. cannabinus* were collected from Yaounde in the month of October 2001. Identification of the plant was confirmed in the National Herbarium, Yaounde, Cameroon where voucher specimen has been deposited. The leaves were air -dried and ground to a powder. The ground material (360 g) was then transferred in to boiling water (3 litres) for 15 min while stirring. After cooling, the resulting suspension was then filtered and the residue was further extracted twice as before. The filtrate was concentrated using a rotary evaporator with the aid of a vacuum pump. The concentrate was further evaporated to dryness in an oven at 40°C to obtain a powder. The powdered extract was stored in a refrigerator until use.

Animals

Male albino rats (150-180g) were used for the study. The rats were housed in wire-mesh cages with a 12 h light/dark cycle. They had continuous access to food and water during the entire period of experimentation.

Experimental design

Six rats were kept as normal control group (Group 1 below), while 30 rats were made anaemic by oral intubations of phenylhydrazine (10 mg/kg body weight) daily for 8 days (Unami et al., 1996). Rats that developed anaemia with haemoglobin concentration <14 g/dl were recruited for the study. Anaemic rats were randomly divided into 4 groups (2 to 5 below, 6 rats per group) and treated as follows:

Group 1: received distilled water (1 ml) daily (normal control),
Group 2: received distilled water (1 ml) daily (anaemic control),
Group 3: received oral single dose (1 ml) of the extract 400 mg/kg body weight/day
Group 4: received oral single dose (1 ml) of the extract 800 mg/kg body weight/day,
Group 5: received oral single dose (1 ml) of the extract 1600 mg/kg body weight/day.

The experiment lasted for 3 weeks.

Haematological investigation

Blood collected from the caudal vein of experimental animals after an overnight fast (T=0) and after 1 2 and 3 weeks of treatment with *H. cannabinus* leaves extract, was used for the determination of red blood cell count (RBC), haemoglobin (Hb) concentration and pack cell volume (PCV) (Dacie and Lewis, 1994; Schalm et al., 1975).

The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were calculated employing the formula of Schalm et al. (1975).

Statistical analysis

Experimental data was analysed using analysis of variance (ANOVA) and Duncan's multiple range tests to determine significant differences between means. The statistical analysis system (SAS) package was used for this analysis.

RESULTS

The changes in the haematological parameters of the rats during the study are presented in Tables 1, 2, 3 and 4. The RBC, Hb, and PCV of rats administered phenylhydrazine (PHZ) decreased significantly ($P<0.05$) while the MCV and MCH increased (Table 1) giving rise to macrocytic anaemia. One week of treatment of anaemic rats (Groups 3, 4, and 5) with *H. cannabinus* extract reversed the effect of PHZ resulting to a significant ($P<0.05$) increase in RBC, Hb, and PVC (Table 2). During the experimental period, the Hb, RBC, and PCV of the untreated anaemic rats (anaemic control, Group 2) also increased but at a slow rate. The Hb and PCV only reached the normal range at the second week of the experiment (Table 3) while the RBC reached normal range at the 3rd week of experiment (Table 4). The Hb, RBC and PCV of group 3, 4 and 5 reached normal values after one week of treatment (Table 2) with maximum level of increase in the second week (Table 3). At this point, the Hb and PCV were significantly ($P<0.05$) higher in group 5 rats than in the normal control rats while no significant difference ($P>0.05$) was observed between the normal control rats and group 3 and 4 rats (Table 3). This explains that the response to treatment was dose related. After the 3rd week of experiment, the Hb, RBC and PCV return to normal with no further increases Table 4)

Figures 1-3 presents the changes in Hb, PCV and RBC per group during the experimental period. The Hb of anaemic rats increased sharply within the first week of the experiment, though the increase was higher for the groups treated with *H. cannabinus* than the anaemic control. This increase slowed down at week 2 and stabilises in week 3 (Figure 1). Similar results were obtained for PCV (Figure 2) and RBC (Figure 3).

DISCUSSION

Phenylhydrazine produces both aryl and hydroxyl radicals when incubated with rat liver microsomes (Gannett et al., 1997) and oxidised by hydrogen peroxide at pH 7.4 and 37^{O}C (Rehse and Shahrouri, 1998). The radicals induced oxidative stress on the red cell membrane resulting in haemolysis by lipid peroxidation (McMillan et al., 1998;

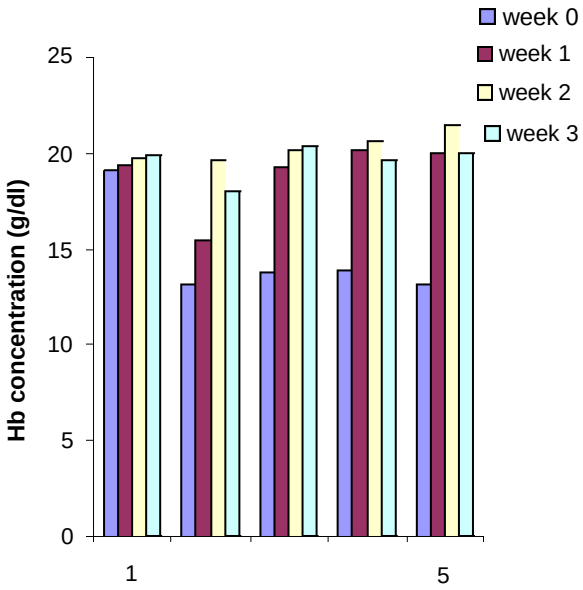

Figure 1. Changes in Hb concentration per group during experimental**Different**period. **experimental groups**

Figure 1. Changes in Hb concentration per group during experimental period.

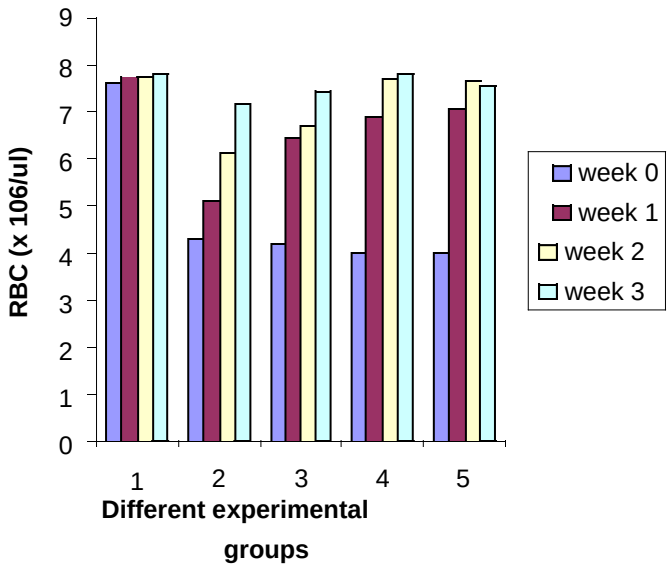

Figure 3. Changes in RBC per group during experimental period.

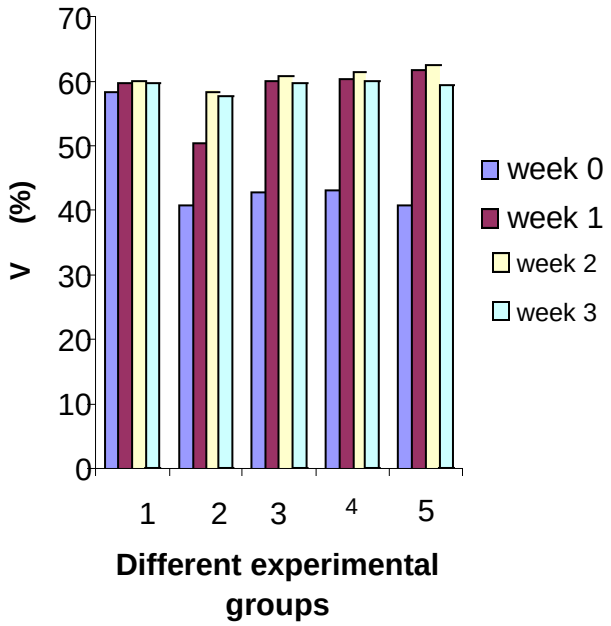

Figure 2. Changes in PCV per group during experimental period.

Cighetti et al., 1999; Zimmermann et al., 1997; Nelson et al., 1997). Sub-chronic intoxication of rats with PHZ (10 mg/kg/day for 8 days) resulted in a marked haemolytic anaemia characterised by decreased RBC, Hb and PCV (Unami et al., 1996). Similar results were obtained in our study when experimental rats were administered PHZ in order to induce anaemia (Table 1). In addition, Ferrali et al. (1997) observed increased reticulocytosis, methaemoglobinemia and haemocatheresis in PHZ intoxicated rats.

The main function of the RBC is the transportation of oxygen in to the tissues of the body. At such, any pathological or physiological condition that affects the RBC alters its function and this may be detrimental to the body. In this study PHZ altered the function of RBC by haemolysis characterised by decreased levels of RBC, Hb and PCV. However, this effect was restored after one week of *H. cannabinus* treatment. The lowest administered dose of 400 mg/kg reduced the recovery time of the blood parameters from 2 weeks in the anaemic control to 1 week (Table 2). Also the recovery was progressive such that after 2 weeks of continuous treatment, the Hb concentration and PCV were higher in the treated groups than in the normal control group (Table

3). It was also observed that the recovery of the treated groups was dose related with the highest dose of 1600 mg/kg effecting the highest change. At the third week of the experiment, treatment of anaemic rats with *H. cannabinus* did not increase the RBC, Hb and PCV any further (Table 4). This shows the rats' control system over polycytaemia. Under normal condition the body can generate new RBC to replace lost once but this will take

much longer time as shown in this study. The recovery time of two weeks for untreated anaemic rats has earlier been reported when rats were bled 30% of their total blood volume to induce haemorrhagic anaemia (Agbor and Odetola, 2001).

Giving the same doses of *H cannabinus* extract to normal rats did not alter the haematological parameters (results not presented). Thus *H. cannabinus* can be consumed as vegetable or in beverages without polycytaemic risk.

A significant correlation with diagnostic values has been demonstrated between RBC, Hb, PCV and the RBC indices (MCV, MCH and MCHC) in both humans and rats (Archer, 1982; Bain, 1989). Administration of PHZ to rats also resulted in an increase ($P<0.05$) in the MCV and MCH values which are indicators of macrocytosis thus describing the anaemia as macrocytic. This condition is also common in Vit. B12 and folate deficiencies probably as a result of iron deficiency (loss of iron). Macrocytic anaemia has also been reported in rats infected with *Trypanosoma Brucei brucei* (Erah et al., 2003) and this has been linked to iron deficiency anaemia (Mwangi et al., 1995). The presence of macrocytosis reduced towards normal as the rats recovered from the anaemic condition.

Anaemia is a disease characterised by a reduction in the concentration of haemoglobin, circulating red blood cell and pack cell volume per unit of the peripheral blood below the normal for the age and sex of the patient (Aguwa, 1996; Oma, 1991). The prevalence of anaemia is high in children with a high risk of placental malaria infection (Muriel and Jean-Yves, 1998). Anaemia impairs normal development in children and it constitutes a major public health problem in young children in the developing countries with wide social and economic implications (Montalemberk and Girot, 1996). Blood parasites, bacterial infections, viral infections, drugs/chemical agents and metabolic diseases may result in destruction of red blood cells leading to haemolytic anaemia (Ramzi et al., 1994).

The speedy and progressive recovery of anaemic rats responding to treatment of *H cannabinus* may be due to increased erythropoiesis. The present results, coupled with an earlier study on the toxicity of *H. cannabinus* suggest that this plant may be used for the treatment of anaemia as claimed. However, the mechanism of action by which *H. cannabinus* produced its effect on increasing RBC, Hb and PCV in experimental animals needs to be investigated.

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