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# Effect of Oral Administration of Aqueous Extract of *Khaya senegalensis* Stem Bark on Phenylhydrazine-induced Anaemia in Rats

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**Abstract:** The anti-anaemic effect of *Khaya senegalensis* stem bark on phenylhydrazine-induced anaemia was carried out in rats. Induction of anaemia was conducted using phenylhydrazine hydrochloride at a dose of 750 mg $kg^{-1}$ body weight. *In vivo* investigation showed that oral daily dose of 300 mg $kg^{-1}$ body weight of the bark aqueous extract of *K. senegalensis* in rats administered simultaneously with the phenylhydrazine produced significant ($p<0.05$) anti-anaemic effect probably by protecting the red blood cells against destruction by phenylhydrazine. However, the same oral dose did not show any significant curative effect ($p>0.05$) after the induction of anaemia using phyenylhdrazine hydrochloride. The phytochemical screening of the aqueous extract of the stem bark of *K. senegalensis* indicate the presence of carbohydrate, saponin, glycosides and tannins. Also present are important mineral elements necessary for the development and functioning of the body. These include calcium, magnesium, zinc, iron, copper, potassium, and sodium. This study showed that *Khaya senegalensis* has an anti-anaemic effect.

**Key words:** Anaemia, phenylhydrazine hydrochloride, *Khaya senegalensis*

## INTRODUCTION

The incidence of anaemia is higher in the third world than in developed countries due to the presence of many aggravating factors such as poor nutrition, low socio-economic status, high prevalence of blood parasites such as *plasmodium* and *trypanosomes* and helminthic infestation. Women are known to be susceptible to anaemia during pregnancy as a result of high demand from the developing foetus[1,2]. Anaemia is among the top ten causes of death in childhood. A study in rural population of Nigeria reported that 19.7% of the children were anaemic[3].

Anaemia could also be caused by toxic chemicals. Phenylhydrazine is a toxic nitrogen-based compound $C_6H_5NH$. $NH_2$ produced by reduction of benzene diazonium chloride. It is used as a reagent for sugar, aldehyde, ketone and related compounds. Phenylhydrazine and its hydrochloride derivatives have been extensively used experimentally to study oxidative haemolysis. Previously, these compounds were used for the treatment of Polycythemia vera[4]. It was reported that there was a rapid release of iron accompanied by methaemoglobin formation in mouse erythrocyte incubated with phenylhdrazine with subsequent haemolysis[5].

Anaemia is one of the numerous ailments claimed to have been successfully treated with plant materials by the traditional medical practitioners. In China, blood diseases such as malformation of the blood circulatory system (including blood stagnation), anaemia, varicose veins, and haemorrhages have been treated with plant materials[6]. Another report claimed the use of gum acacia for successful treatment of haemorrhage, though the treatment was found to be injurious to the liver[7]. The crude extract of *Fagara zanthoxylum* was reported to be effective in the treatment of sickle cell anaemia[8].

*Khaya senegalensis* belonging to the family Maliaceae proven scientifically to have some pharmacological properties[9-11] is used in the middle belt and Western Nigeria for treatment of anaemia, but its effectiveness as an anti-anaemic agent has not been scientifically investigated.

The present investigation was therefore, planned to investigate the anti-anaemic properties of the crude stem bark extract of the plant.

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## MATERIALS AND METHODS

**Plant collection and identification:** The stem bark of *K. senegalensis* was collected from Zaria, Nigeria between August and October, 1996. The plant was identified to be *Khaya senegalensis* by a plant taxonomist from the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

**Preparation of the extract:** After collection, the stem bark of *K. senegalensis* was thoroughly washed and sun-dried for a week. The dried bark was pounded to a coarse powder using wooden mortar and pestle and then stored in a dry container until used.

Thirty grams of the powdered sample was soaked for 5 min in 500 mL of distilled water and was later boiled for 15 min and then cooled. The supernatant was decanted and filtered through Whatman filter paper size 0.1 μm. The liquid content was stored in a screw-corked brown bottle and refrigerated until used.

The solid content of the extract was determined as reported by a previous method[12]. In this, 20 mL of the preparation above was evaporated to dryness and then heated to a constant weight on a petri dish. The final weight was taken and solid concentration expressed in mg $mL^{-1}$ resulting to a percentage extraction of 5.6% w/w.

**Phytochemical and element analysis:** The presence of zinc, copper, magnesium and calcium in the plant was determined by atomic absorption spectrophotometry and sodium and potassium by flame photometry[13]. The phytochemical studies were performed using methods described earlier[9,13-15]

**Induction of anaemia:** Anaemia was induced in rats by subcutaneous injection of 750 mg $kg^{-1}$ body weight of 2.5% neutralized phenylhydrazine hydrochloride (British Drug House, Poole, England) as described[16]. This was followed by a maintenance dose of 350 mg $kg^{-1}$ body weight of the same chemical on alternate days for the duration of the experiment.

**Effect of plant extract on phenylhydrazine-induced anaemia:** Twenty-five healthy adult albino rats of both sexes weighing between 118 and 58 g were used for the study. They were obtained from the Institute of Agricultural Research,Ahmadu Bello University, Zaria, Nigeria. They were housed in clean plastic cages and fed commercial Sander's Feed (grower's mash) containing 3.5% oil, 15.7% protein, 8% fibre and 1% calcium. Water was provided *ad libitum*.

The rats were divided into five equal groups and treated as follows: Group 1 received 300 mg $kg^{-1}$ of the extract orally and simultaneously received 750 mg $kg^{-1}$ phenylhydrazine subcutaneously; Group 2 was treated with 300 mg $kg^{-1}$ of the extract 2 days after the onset of anaemia induced by subcutaneous administration of 750 mg $kg^{-1}$ phenylhydrazine, while Group 3 received only 750 mg $kg^{-1}$ phenyhydrazine subcutaneously. Group 4 and 5 were orally given only 300 mg $kg^{-1}$ of the extract and distilled water, respectively.

**Blood collection and analysis:** Blood was collected from the tail veins of each rat for determination of Packed Cell Volume (PCV) using microhaematocrit method[17]. The body weights of the rats were determined every two days starting from day 0.

**Statistical analysis:** The mean PCV and mean live body weight measurements were statistically analyzed using unpaired students t-test. Significant differences between data obtained for other days and that of day 0 were detected at 95% confidence limits.

## RESULTS

**Phytochemical and elemental analysis:** The result of this analysis showed that the aqueous extract of *K. senegalensis* contained glycosides, saponins, tannins and carbohydrates.

The aqueous extract of this plant contained the following elements per 100 g of the plant.
Calcium 453±3.5 mg, magnesium 230±2.6 mg, zinc 12±1.3 mg, iron 47±3.2 mg, copper 4±1.0 mg, potassium 1780±70.9 mg and sodium 90±4.2 mg.

**Effect of *Khaya senegalensis* extract on phenylhydrazine-induced anaemia:** The PCV of rats in group 1 administered the extract and phenylhydrazine simultaneously did not show significant effect compared to day 0. Also the administration of the extract alone and distilled water to rats in groups 4 and 5, respectively produced no significant effect throughout the duration of the experiment. The mean PCV of rats in groups 2 and 3 were significantly ($p<0.05$) decreased to 30.2 and 29.4, respectively by the fifth day as compare to day 0 (Table 1). No death was recorded during the course of the experiment. The weight losses in some of the experimental animals (Table 2) administered the extract were neither dose dependent nor on duration of exposure to the extract.

## DISCUSSION

The decrease (62%) in the PCV of rats following the administration of phenylhydrazine subcutaneously at a dose of 750 mg $kg^{-1}$ is an indication of anaemia. Anaemia has also been induced earlier with this agent and was

Table 1: Effect of oral daily dosage (300 mg $kg^{-1}$ body weight) of the aqueous extract of the bark of *K senegalensis* on [+]mean Packed Cell Volume (PCV) % of rats treated on different days after phenylhydrazine (750 mg $kg^{-1}$ body weight) administration

| | Duration of experiment (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| Groups | 0 | 1 | 3 | 5 | 7 | 9 | 11 |
| 1 | 46.0±8 | 39.2± 0 | 39.0±4 | 39.0± 2 | 42.2±2 | 42.4±0 | 46.2±5 |
| 2 | 40.4±6 | 25.2±6* | 30.2±2* | 30.2±6* | 35.6±9 | 41.8±2 | 44.2±5 |
| 3 | 44.6±6 | 27.0±9* | 28.2±8* | 29.4±6* | 36.6±4 | 42.6±5 | 44.6±9 |
| 4 | 42.8±0 | 42.6±7 | 43.6±9 | 43.0±9 | 42.8±2 | 44.2±6 | 43.8±2 |
| 5 | 43.4±1 | 43.4±3 | 44.0±0 | 45.0±6 | 44.0±3 | 46.8±8 | 44.5±5 |

[+]Mean±SEM based on 5 observations, * Significant ($p<0.05$) decrease

Table 2: Effect of oral daily dosage (300 mg $kg^{-1}$ body weight) of the aqueous extract of the bark of *K senegalensis* on [+]mean live weight (g) of rats treated on different days after phenylhydrazine (750 mg $kg^{-1}$ body weight) administration

| | Duration of experiment (days) | | | | | | |
|---|---|---|---|---|---|---|---|
| Groups | 0 | 1 | 3 | 5 | 7 | 9 | 11 |
| 1 | 158.2± 8 | 145.0±8 | 144.2±6 | 139.1± 4 | 143.6±2 | 145.2±8 | 156.0±7 |
| 2 | 128.4±6 | 122.4±4 | 108.8±6 | 100.4±2 | 108.8±3 | 118.8±4 | 126.0±7 |
| 3 | 120.2±6 | 112.8±9 | 112.2±3 | 114.6±5 | 117.6±2 | 118.8±9 | 120.0±6 |
| 4 | 152.2±8 | 157.6±4 | 156.2±2 | 162.2±2 | 161.6±0 | 159.1±2 | 163.8±1 |
| 5 | 118.8±9 | 120.4±8 | 116.1±3 | 120.1±4 | 119.4±5 | 114.2±5 | 117.5±3 |

[+]Mean±SEM based on 5 observations

reported to produce anaemia only within six days of administration[10,17].

The simultaneous oral administration of 300 mg $kg^{-1}$ body weight of the aqueous extract and subcutaneous injection of phenylhydrazine did not produce anaemia within six days. This suggest a possible and protective effect of the extract against phenylhydrazine induced anaemia, although treatment with the extract 2 days after phenylhydrazine administration failed to protect the animals from phenylhydrazine-induced anaemi. Phenylhydrazine was reported to induce the development of Heintz bodies on RBC's after six days of exposure which confer Immunity to the cell's thereby protecting them against further destruction by the chemical[4,10]. This may explain the restoration of the PCV of the rats to normal after the 9th day. *K. senegalensis* is known to contain some important elements used for blood production and these elements may have contributed in prevention of anaemia when the extract was given simultaneously with phenylhydrazine, but failed to reverse the anaemia induced prior to its (extract) administration.

The constituents of the extract following phytochemical screening appear to agree with those of earlier studies[9,18-20].

The weight loss in some of the experimental animals could be due to interference of the extract with the feeding pattern of the rats since oral administration of high dose of saponin containing remedies could cause bloating thereby reducing the appetite of animals[21]. Saponin also has haemolytic effect when injected into the blood but not when taken orally[22]. Tannin is reputed to have a local effect (as astringent and haemostatic) and anti-inflammatory action[23]. It is believed that the anti-anaemic effect noticed was not due to these active principles which on their own could cause toxic effects.

This study showed that *K. senegalensis* contain important elements which if used in recommended doses could be of use in the body blood system[24-26].

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