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Haematinic Activities of the Aqueous Extract of *Kalanchoe pinnata* Leaves in Hyperlipidaemic Female Rat Models

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ABSTRACT

Background and Objective: *Kalanchoe pinnata* has been widely used traditionally as a blood boosting extract in the Niger Delta Region of Nigeria. However, there has been no scientifically published report on this claim, thus, this study evaluated the haematinic activities of the aqueous extract of *Kalanchoe pinnata* leaves in female hyperlipidemic rat models. **Materials and Methods:** The female albino rats were induced with hyperlipidaemia using a high-fat diet mixture. Thirty female albino rats were grouped into 5 groups, with 6 rats in each group. Group 1 served as normal control, group 2 was positive control, and group 3 was negative control, while groups 4 and 5 were administered 200 and 400 mg/kg aqueous *K. pinnata* extract for 21 days. The rats were sacrificed, and blood samples were collected to investigate the levels of erythrocytes, Packed Cell Volume (PCV), Haemoglobin (Hb), and platelets using standard laboratory methods. The IBM SPSS v25 was employed in the evaluation of the percentage of body weight difference (BWD), while GraphPad Prism10.2 was used for the statistical analysis of the haematological indices. **Results:** An increase in the erythrocytes, PCV, and haemoglobin levels of the experimental rats administered with the aqueous extract of *Kalanchoe pinnata* when compared to the negative control group. The greatest increase was observed with the 400 mg/kg extract group. A concurrent decrease in the platelet levels was also observed in the experimental rats on administration of the extract. **Conclusion:** The aqueous extract of *Kalanchoe pinnata* leaves has good haematinic properties and thus provides its pharmacological basis for use in the boosting of blood levels traditionally.

KEYWORDS

Kalanchoe pinnata, red blood cell, packed cell volume, hemoglobin, platelets, hyperlipidemia

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INTRODUCTION

Medicinal plants are those that have therapeutic properties or provide beneficial pharmacological effects on humans or animals. They are crucial as sources of drug lead compounds. Early humans, guided by instinct, taste, and experience, used plants to treat their ailments, making the history of medicinal plants as old as humanity itself[1]. They have been fundamental to the treatment of various diseases in African traditional medicine, as well as in other treatment practices, particularly in Africa and developing countries. They have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments[2]. Most potent medicinal plants have minimal toxic or adverse effects when used by humans, although some can be highly toxic to both humans and animals, potentially harming certain organs in the body[3].

The integrity of the blood is vital for maintaining homeostasis and overall health, especially in relation to the immune system. Blood, composed of plasma, red blood cells, white blood cells, and platelets, serves as a medium for transporting nutrients, oxygen, waste products, and immune cells throughout the body[4]. A well-functioning circulatory system ensures that immune cells can reach infection sites rapidly and efficiently, highlighting the crucial role of blood in innate and adaptive immune responses[5].

When blood integrity is compromised due to toxin exposure, oxidative stress, infections, or chronic inflammation, immune function can deteriorate. This may lead to increased susceptibility to diseases, impaired wound healing, and a weakened ability to fight infections. One promising avenue for preserving and enhancing blood integrity lies in the use of plant extracts with bioactive compounds known for their antioxidant, anti-inflammatory, and immunomodulatory properties[6,7].

Plant extracts such as those from *Ricinodendron heudelotii*, *Phoenix dactylifera*, *Cyperus esculentus*. *Cocos nucifera*, *Moringa oleifera*, *Curcuma longa* (turmeric), *Azadirachta indica* (neem), and *Allium sativum* (garlic) have shown the ability to support blood health[8,9]. These botanicals contain flavonoids, alkaloids, and phenolic compounds that help scavenge free radicals, reduce oxidative damage to blood cells, and modulate cytokine production. By supporting antioxidant defenses and reducing systemic inflammation, plant extracts help maintain the functional capacity of blood components, ensuring that immune cells remain viable and effective. The integration of these natural compounds into dietary or therapeutic regimes presents a complementary strategy to bolster immune resilience and promote long-term health[10].

Kalanchoe pinnata is one of those medicinal plants known for its numerous therapeutic properties. Odinga-Israel *et al.*[11] conducted a biochemical evaluation on the reduction of oxidative stress and cardiovascular risk markers in hyperlipidemic rats treated with *Kalanchoe pinnata* aqueous extract. They concluded that *Kalanchoe pinnata* could serve as a raw material for treating cardiovascular diseases and as a source of antioxidants. Ramon *et al.*[12] researched the phytochemical and pharmacological properties of *kalanchoe pinnata* and reported that the plant contains bioactive components such as alkaloids, diterpenoidal lactones, steroids, phenolics, and aliphatic compounds. They added that it exhibits anticancer, anticonvulsant, antifungal, antimicrobial, anti-inflammatory, antidiabetic, antineoplastic, antioxidant, immunomodulation, antilipidaemic, and antiallergic properties.

Kalanchoe pinnata is a plant commonly used in traditional medicine for treating different ailments such as diarrhoea, ulcer, and asthma[13]. However, there is limited or no literature on investigating the effect of this plant on haematological indices, therefore, this study evaluated the effect of *Kalanchoe pinnata* aqueous extract on the level of the haematological indices in high-fat diet-induced hyperlipidaemic albino rats.

MATERIALS AND METHODS

Study area: This study was carried out at the Department of Biochemistry and the Department of Pharmacology of the Rivers State University from September, 2022 to December, 2022.

Experiment animals: Female albino rats weighing between 250-300 g were obtained from the Department of Pharmacology and Therapeutics, Rivers State University, Port Harcourt. A total of thirty rats were grouped into five groups, and each group consisted of six rats. The animals were acclimatized for seven days before inducement and administration procedures began. They were maintained under standard environmental conditions and were fed standard rat feed and water *ad libitum*. During the period of induction and administration, treatment was administered to the rats using an orogastric tube based on the calculated doses per weight of rats, and food was withdrawn for 24 hrs before the experiment (sacrifice) was to be carried out.

Plant collection and preparation of extract: Fresh leaves of *Kalanchoe pinnata* were harvested in Emoh Community in Abua/Odual Local Government Area, Rivers State, Nigeria, in September, 2022, and only matured leaves without signs of lesion were used. The sample authentication was adopted by Dr. M. Ajuru of the Department of Plant Science and Biotechnology, Rivers State University, Nigeria (voucher number SUK 5279). The plant sample (2 kg) was ground by means of a mechanical grinding machine and then macerated with water (3 L) for 24 hrs. After filtration and lyophilisation, 59.2 g was obtained. The solution (1 g/mL) was prepared by dissolving the above extract in distilled water freshly each time before use for administration. The extracts were stored at 4°C until used.

Preparation of standard drug (simvastatin): Simvastatin was obtained from a commercial Pharmacy in Port Harcourt. It was prepared by dissolving 20 mg into normal saline (12.5 mL) in a beaker using a spatula to dissolve the mixture to make a concentration of 1.6 mg/mL.

Administration of standard drug (simvastatin): The standard drug was administered to the experimental animals in group 3 (positive control). It was prepared daily and was administered to each rat in the group using the oral gavage tube.

Induction of hyperlipidemia: The female rats with an average body weight of 250-300 g were made hyperlipidemic by giving a high-fat diet (HFD) for 7 days. The composition of the high-fat diet was as reported by Abdul Kadir *et al.*[14]. The high-fat diet contained 414.0 kcal/100 g with 43% as carbohydrate, 17% as protein, and 40% as fat (Table 1). The diet consists of a mixture of 68% normal rat chow pellet, 20% instant milk powder (peak milk), 6% corn oil (Mazola), and 6% ghee (popularly called Manshanu in Northern Nigeria). Meanwhile, the normal rat chow diet contains 306.2 kcal/100 g with 48.8% as carbohydrate, 21% as protein, and 3% as fat. All ingredients of the high-fat diet were thoroughly mixed and baked in a Thermo Fisher Scientific Inc., Heratherm Advanced Protocol Oven, 230V 60 Hz models, at 65°C overnight.

Table 1: Composition of high-fat diet and normal rat chow diet[14]

	High-fat diet
Nutrients	%/100 g
Carbohydrate	43
Protein	17
Fat	40
Ingredients	g/100 g
Powdered rat feed	68.0
Maize oil	6.0
Ghee	6.0
Milk powder	20.0
Total energy (kcal/100 g)	414.0

Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins in the rats.

Experimental design: The animals were weighed before the commencement of the experiment, and they animals were observed for physical symptoms and recorded. They were allowed to acclimate to the environment for 7 days. All animals were allowed to feed *ad libitum*.

Thirty albino rats of weight 250-300 g were divided into 5 groups with 6 rats in each group.

Group 1: Normal control group (feed+water)
Group 2: Negative control group (HFD+feed+water)
Group 3: Positive control group (HFD+simvastatin+feed+water)
Group 4: 200 mg/kg *K. pinnata* extract (HFD+200 mg/kg *K. pinnata*+feed+water)
Group 5: 400 mg/kg *K. pinnata* extract (HFD+400 mg/kg *K. pinnata*+feed+water)

Administration of extracts: The extracts were administered to the rats with the aid of gavage, acting as an orogastric tube. Utmost care was taken not to inflict Oesophageal injuries on the rats.

Gain in body weight: Gain in body weight of individual rat in each group was estimated on weekly basis throughout the study period to find out the effect of treatments on body weight using electronic weighing balance (KERN 440-35 N).

Sample collection for bioassay: At the end of the administration period, the animals were fasted for 24 hrs. The rats were put in a desiccator and allowed to slightly anaesthetize, and blood samples were collected by means of jugular venipuncture. The blood samples (2 mL) were collected into sterile plain sample bottles from each rat, slightly agitated, and covered properly for analysis of the hematological indices using the method as described by Odinga *et al.*[4].

Data analysis: GraphPad Prism® Software 10.2 (San Diego, California, USA) was used for the statistical analysis. The concentrations of the various markers were expressed as Mean±SD. One-way ANOVA was used to compare the means among the groups and differences with a 95% confidence interval, resulting in $p<0.05$ were considered significant using the Tukey's HSD test for multiple comparisons.

RESULTS AND DISCUSSION

The result, as presented in Table 2, on the percentage of body weight difference of the experimental rats before and after administration of the aqueous extract of *Kalanchoe pinnata*, revealed a decrease in the mean body weight (%) difference of the hyperlipidemic rats when administered various concentrations of the extract. The greatest decrease was observed in the 400 mg/kg extract when compared with the negative control. The increase in the final body weight of the rats, as shown in their final body weight, could be attributed to the high-fat diet food which was administered to the rats to induce hyperlipidemia, also their food and water intake *ad libitum* all through the experimental period. Odinga *et al.*[15] reported that the feed of rats was rich in nutrients and calories and could add to their body weight. Albeit a decrease in the mean body weight (%) difference was seen in the extract-administered groups. The greatest decrease was observed in the 400 mg/kg extract group when compared with the negative control group. This study is in consonance with the use of plants to reduce the cholesterol concentration of the blood[16].

Table 2: Mean values of initial body weight, final body weight, and body weight difference (%) of experimental rats

Group	Initial weight (g)	Final body weight (g)	Mean body weight difference (%)
Normal control	159.00±21.49	178.67±8.62	12.37
Negative control	133.67±2.67	171.67±17.17	28.43
Positive control	160.50±2.59	194.00±22.69	19.38
200 mg/kg *K. Pinnata*	153.67±3.98	196.50±31.14	27.87
400 mg/kg *K. Pinnata*	146.17±5.49	166.33±16.59	13.79

Values are Mean±Standard Deviation

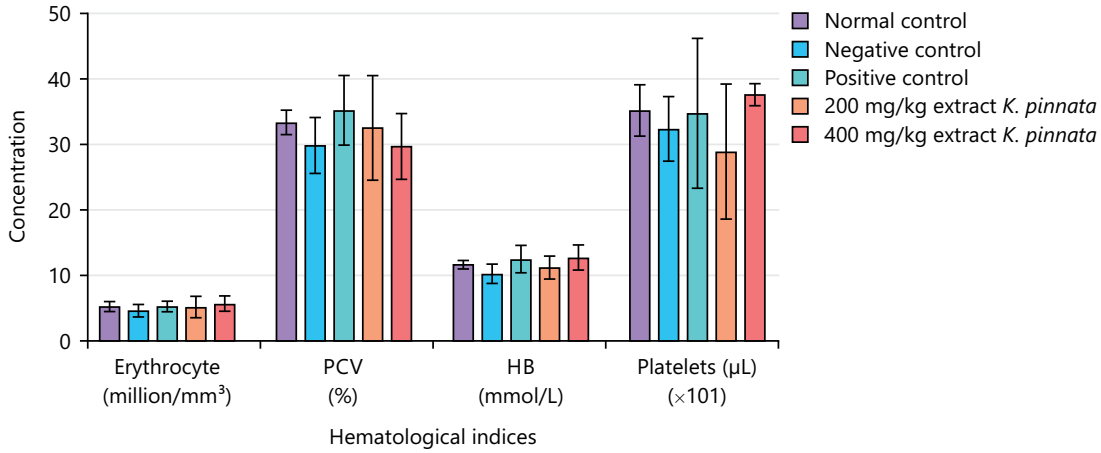

Fig. 1: Effect of *Kalanchoe pinnata* on haematological parameters in high fat-induced hyperlipidaemic albino rats

An increase in the Erythrocyte level of the blood has been reported to imply a rise in the blood oxygen-carrying capacity. Plant extracts can be employed to increase the red blood cell production due to the phytochemical content inherent in the plant. This potential has been proven to alleviate anaemia[17]. The result of the study, as shown in Fig. 1, reveals an increased erythrocyte level in the groups treated with the plant extract when compared to the negative control group. This resonates with previous studies cited above on the erythrocyte boosting capacity of plant extracts due to their phytochemical constituents. An increase in the PCV, Hb levels was also revealed in Fig. 1. However, the increase was not statistically significant. Oparaku *et al.*[18] observed that some plant extracts have shown anti-anaemic effects by their potential to increase red blood cell count and Hb.

The result, as shown in Fig. 1, also shows the effect of the extract of *K. pinnata* on the platelet levels in hyperlipidaemic Wistar rat models. A significant increase at $p = 0.05$ was seen in the platelet levels of the rats treated with 400 mg/kg extract in comparison to the negative control group. The increase observed is comparable to that of the positive control group. Jinna and Khandhar[19] in their study on Thrombocytopenia reported that a decrease in the platelet levels of the blood could be associated with increased risk of bleeding and bruising. Platelets are responsible for the formation of blood clots. Consumption of plant extract has been reported to reduce platelet activation factor-induced platelet aggregation and increase platelet activating factor catabolism[20]. Haward *et al.*[21] in their study, also reported that papaya leaf extract elevated platelet levels in individuals with Dengue fever. Platelet-activating factor, as observed by Gavriil *et al.*[20] is a potent mediator of inflammation that plays a crucial role in atherosclerosis.

CONCLUSION

This study observed that the leaf extract *Kalanchoe pinnata* caused a decrease in the hyperlipidemic condition of the rats. This was reported by a previous publication on this study and the BWD (%). The increases seen in the erythrocytes, PCV, Hb, and concurrent decrease in the platelet level of the experimental rats when treated with the leaf extract of *Kalanchoe pinnata* suggestits blood boosting integrity potential. This study, therefore, concludes that the aqueous extract of *Kalanchoe pinnata* leaves has good haematinic properties and thus provides its pharmacological basis for use in the boosting of blood levels traditionally.

SIGNIFICANCE STATEMENT

The use of *Kalanchoe pinnata* for medicinal purposes has been studied, due to limited or no literature onthe effect of this plant on haematological indices. This study evaluated the effect of *Kalanchoe pinnata* aqueous extract on the level of the haematological indices in high-fat diet-induced hyperlipidaemic albino

rats. The study therefore, concludes that the aqueous extract of *Kalanchoe pinnata* leaves has good haematinic properties and thus provides its pharmacological basis for use in the boosting of blood levels traditionally.

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