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Evaluation of the Haemostatic Activities of *Sida corymbosa* in Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author LBJA designed the study, wrote the protocol, management of laboratory analysis of the study, interpreted, performed the statistical analysis and wrote the manuscript. Author MA managed the literature searches and participated in the management of the laboratory analysis of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the anti-haemorrhagic activity of the leaf extract of *Sida corymbosa* in Wistar albino rats, a plant used to arrest bleeding in ethnomedical practices.
Methods: The acute toxicity test was carried out in rats. The haemostatic activities of the extract were investigated using the tail bleeding time and amount of bleeding in rats; effects on haematological parameters were also evaluated in Wistar rats. Preliminary phytochemical analysis was conducted to detect the phytoconstituents of the extract of *Sida corymbosa*.
Results: In this study, the oral LD_{50} of the extract was found to be greater than 5 g/kg. Administration of the extract to rats for 14 days produced a dose-dependent and significant ($P \leq 0.05$) decrease in bleeding time and quantity of blood loss in pre-treated rats. On oral administration of the extract, the effects of the treatment on haematological parameters – White blood cells, Red blood cell, haemoglobin concentration were not significantly different from control.
Conclusion: This study has shown that *Sida corymbosa* has constituents with anti-haemorrhagic properties in rats thereby providing scientific validation for the ethnomedical use of the plant in bleeding control.

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Keywords: Sida corymbosa; haemostasis; bleeding time; amount of bleeding; tannins; flavonoids.

1. INTRODUCTION

The use of plants by man as treatment for illnesses dates back to the Stone Age [1] and modern medicine currently employs several plant derived medicaments which have been processed to make them suitable for use [2]. Despite the huge advances achieved in present day medical practices with about 25% of drugs prescribed worldwide being of plant origin, plants in their natural state are still being highly employed as cures for illnesses and are important components of complementary and alternative medicine [3].

Bleeding incidences have caused anaemia, inflammation, shock, ischaemia and even death: Bleeding may cause increase in heart rate, increase in stroke volume, decrease in blood haemoglobin levels, and activation of platelets resulting in increased myocardial oxygen demand thereby precipitating ischaemia, thus the need for interventions in the management of bleeding situations is of great importance and a reduction in bleeding events have yielded better survival rates [4,5].

Many plants have been reported to possess anti-haemorrhagic property and are used in traditional medicines as aids to stop blood flow or control blood loss from the site of an injury [6,7]. *Sida corymbosa* R. E. Fries commonly known as 'miyar tsanya' or 'karkashin kwado' in Northern Nigeria is a weed of cultivated fields, waste areas, road sides and open areas. The plant is an erect, basally woody perennial shrub with hairy stem up to 2 m high and occurs widely in Nigeria [8]. The plant has been reported to possess uterotonic property [9]. An earlier study reported the anti-ulcerogenic and wound healing properties of the plant extract [10]; conditions that are associated with bleeding [11]. In Northern Nigeria, the plant is used in wound care and to reduce bleeding from an injury site (personal communication – Mal Ibrahim Muazzam 08/08/2013). This present study was designed to investigate the haemostatic effects of *Sida corymbosa* in rats.

2. MATERIAL AND METHODS

2.1 Plant Material

The plant was collected around Idu, in Abuja FCT by Mal I. Muazzam. The plant material was authenticated by Mrs Grace Ugbabe, of the herbarium Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. Voucher specimen (NIPRD/H/6602) was deposited there for future reference.

2.2 Preparation of Plant Material

The leaves were collected, air-dried in the shade and pulverized using a mortar and pestle to obtain a coarse powder. 100 g of the powdered leaves were subjected to cold maceration extraction in 1.0 L of 70% methanol with occasional shaking for 24 h. The extract was filtered using Whatman filter paper No. 1. The filtrate was concentrated using a rotary evaporator and the filtrate evaporated to dryness to give a semi-solid (the extract). The percentage yield was 8.28±1.24% w/w. The extract was kept in a desiccator until use.

2.3 Phytochemical Screening

The extract was screened for the presence of tannins, saponins, alkaloids, flavonoids, carbohydrates, terpenes and sterols according to standard procedures [12].

2.4 Animals

Adult Wistar rats maintained at the Animal Facility Centre of NIPRD were used in this study. They were fed with mouse cubes (CAPS Plc. Nigeria Ltd) and had access to water *ad libitum*. They were housed under standard conditions; constant temperature, humidity and 12 h light/dark cycle were maintained. All experiments were conducted according to National Institute for Health guide and care for the use of laboratory animals as contained in NIPRD's Standard Operating Procedures for laboratory use of animals.

2.5 Acute Toxicity Studies

Oral acute toxicity (LD_{50}) of the extract was determined in Wistar albino rats according to the method described by Lorke. Nine rats were randomly placed into 3 groups of 3 rats each and were administered 10, 100, and 1000 mg/kg (p.o) of extract respectively. These were observed for 24 h for signs of toxicity and mortality. Based on

the results of the first stage, another set of animals were treated with 1600, 2900 and 5000 mg/kg. Mortality within 24 h was recorded and LD_{50} determined [13].

2.6 Methodology

Adult rats were randomly divided into four groups of twelve rats each. One group which served as control was given distilled water, while the other 3 groups received extract at a dose of 250, 500 and 1000 mg/kg respectively orally, once daily for 14 days. The rats were given food and water *ad libitum*. Six rats from each group were used for bleeding time studies, another set of six rats from each group were used to determine the amount of bleeding. Evaluations were carried out 24 h after administration of the last dose.

2.7 Studies on Effect of *Sida corymbosa* (SC) Extract on Bleeding Time

Bleeding time was determined following the method described by Rajasekaran et al, 2010 with modifications [14]. The rats were placed in a standard plastic rat restraining cage with the tail hanging out freely. Bleeding time was assessed by cutting the tip of the tail of each rat with a sharp pair of surgical scissors (2 - 3 mm), and the tail was placed in an isotonic saline solution with pH 7.4 maintained at 37ºC immediately after the cut was inflicted. A stopwatch was started simultaneously with the immersion of the tail in the saline solution. Bleeding time was taken as the time taken from appearance of the first drop of blood to show to the time when the bleeding stopped completely.

2.8 Studies on Effect of *Sida corymbosa* (SC) on Amount of Bleeding

The quantity of bleeding was measured following the following the method described by Cipil et al. [15]. Rats were placed individually in a standard plastic rat restraining cage with the tail allowed to hang out freely. The tip of the tail of each rat was cut (4 – 5 mm) with a sharp pair of scissors and a stopwatch started immediately. A pre-weighed blotting paper was used to collect all drops of blood that flowed from the site of the inflicted injury. The blotting paper was re-weighed after the appearance of the last drop of blood on an electronic balance (Ohaus Analytical Balance Adventurer™ Model AR 2140, Ohaus Corp. USA). The difference in the weight of the dry and wet blotting paper was taken as the amount of bleeding [16].

2.9 Studies on Effect of *Sida corymbosa* (SC) on Haematological Parameters

After the bleeding studies were concluded, the rats were anaesthetized by ether inhalation and blood samples were obtained via cardiac puncture using a 5 ml syringe fitted with 21G needle [17]. This was immediately transferred into EDTA-containing tubes for evaluation of haematological parameters such as White blood Cells, Red Blood Cells (RBC), haemoglobin concentration (Hb), platelet count using the Automated Sysmex haematology analyzer (KX-21N, Sysmex, Japan).

2.10 Statistical Analysis

Results were expressed as mean ± SEM. Significance was determined using one-way ANOVA. Results were regarded as significant at $P \leq 0.05$.

3. RESULTS

The behavioural change of decreased activity was observed in the animals administered 500 and 1000 mg/kg of the extract. There were no observable changes in the eyes, skin and fur on oral acute administration. No tremors, convulsions, coma or other signs of acute toxicity were observed in the animals. No mortality was also recorded in any of the animals treated orally with the extract of *Sida corymbosa* at doses up to 5 g/kg; therefore the oral LD_{50} of the leaf extract of *Sida corymbosa* was estimated to be greater than 5000 mg/kg. Phytochemical analysis showed the presence of alkaloids, saponins, tannins, terpenes, sterols, flavonoids and carbohydrates.

The extract of *Sida corymbosa* significantly (*P≤0.05*) and dose-dependently decreased bleeding time when administered orally to rats at doses of 250, 500 and 1000 mg/kg. The extract produced a decrease of 16.70 %, 26.06 % and 47.14 % respectively. This effect exhibited by the extract is significant at 500 mg/kg and 1000 mg/kg when compared to the control group Table 1.

The results presented in Table 2 showed that the extract produced a dose-dependent reduction of the amount of bleeding. This reduction was significant (*P≤0.05*) at the doses (250, 500 and 1000 mg/kg) tested when compared to the control group administered distilled water thus

representing inhibition of 47.91 %, 48.89 % and 65.22 % of blood loss in pre-treated rats.

Table 1. Effect of *Sida corymbosa* (SC) extract on bleeding time in rats

Treatment	Dose (mg/kg)	Bleeding (s)
Distilled water	10 ml/kg	148.5±6.51
SC	250	123.7±8.12
SC	500	109.8±11.35*
SC	1000	78.50±10.49**

*Effect of Sida corymbosa extract on bleeding time. Values are time(s) as mean ± SEM (n=6); *P≤0.05, **P<0.01 when compared to control*

Table 2. Effect of *Sida corymbosa* (SC) on Amount of bleeding

Treatment	Dose (mg/kg)	Amount of bleeding (g)
Distilled water	10 ml/kg	0.3252±0.025
SC	250	0.1694±0.015*
SC	500	0.1662±0.017*
SC	1000	0.1131±0.015*

*Effect of Sida corymbosa extract on amount of bleeding. Values are expressed as mean ± SEM (n=6); *P≤0.05 when compared to control*

In the present study, administration of the extract at the doses tested did not demonstrate any significant difference in the values of the haematological parameters which include white blood cells (WBC), red blood cells (RBC), platelet (Plts) count, haemoglobin content (HB), mean cell volume (MCV), mean cell haemoblobin (MCH) and mean cell haemoglobin concentration (MCHC) when compared to the control group as represented in Table 3.

4. DISCUSSION

The oral LD_{50} of greater than 5000 mg/kg for the extract of *Sida corymbosa* leaves indicates that the extract is non-toxic when administered orally therefore may not possess the potential to cause serious adverse effects [18].

Irrespective of the type of wound, when an injury occurs haemostasis is one of the early steps taken in the management of all bleeding wounds [19], therefore an agent that arrests or reduces bleeding would be beneficial in the treatment regimen. Bleeding tests determine primary haemostasis which is the vessel wall response and ability of platelets to aggregate and form a platelet plug and stop bleeding immediately in the event of an injury [20]. Reduction in bleeding time represents haemostatic activity [21]. Haemostasis is the set of processes involved in the arrest of bleeding at the site of an injury while maintaining blood flow in the rest of the circulation by the formation of a haemostatic plug composed mainly of platelets and fibrin clot thereby preventing excessive blood loss; haemostasis is a function of balance between procoagulant systems which include platelets and the coagulation cascade and anticoagulation systems involving APC/protein S, fibrinolysis and serpins [22]. The reduction of bleeding time and amount of bleeding exhibited by the extract of *Sida corymbosa* leaves is an indication of haemostatic activity. Previous work has shown tannins to demonstrate haemostatic action by precipitating proteins to form vascular plugs thereby arresting bleeding [23]. Tannins and flavonoids by their astringent nature possess the ability to cause vasoconstriction thereby arresting bleeding [24]. The presence of tannins and flavonoids as shown by phytochemical analysis may play a role in the decrease in bleeding time and subsequently reduction of quantity of blood loss shown by the extract of *Sida corymbosa* leaves. However other mechanism such as promotion of blood coagulation system by modulation in production of one or more of the clotting factors [25] which were not measured in this experiment may also be involved as haemostasis is controlled by an intricate set of mechanisms.

Table 3. Effect of *Sida corymbosa* on haematological parameters

Parameters	Distilled water	SC 250 mg/kg	SC 500 mg/kg	SC 1000 mg/kg
WBC (x 10^3/µL)	14.78±0.00	14.6±0.00	14.57±0.00	14.87±0.00
RBC (x 10^6/µL)	7.37±0.25	7.18±0.20	7.26±0.19	7.19±0.34
HGB (g/dL)	12.70±0.29	12.58±0.45	12.40±0.37	12.56±0.56
MCV (fL)	57.15±0.80	55.25±0.78	55.15±0.84	57.18±0.74
MCH (pg)	17.28±0.38	17.48±0.21	17.39±0.21	17.05±0.33
MCHC (g/dL)	30.20±0.08	30.55±0.33	30.29±0.31	30.59±0.34
Plts(x 10^3/µL)	876.25±35.25	867.50±31 58	870.25±24.1	872.88±9.08

Effect of Sida corymbosa extract on haematological parameters. Values are expressed as mean ± SEM (n=12)

According to Wang et al. [26], the criteria for good haemostatic agents should include efficacy, ease of use, and safety with minimum side effects, availability and low cost. *Sida corymbosa* is a weed that is widely available thus can be considered to be cheap. The results obtained from this study have shown that *Sida corymbosa* has constituents with anti-haemorrhagic properties. The extract had no effect on haematological parameters with the high value of LD_{50} also has shown the extract to be safe and thus meets the criteria.

4. CONCLUSION

It can then be concluded from this study that the *Sida corymbosa* leaf extract possesses anti-haemorrhagic potentials, thereby validates the use of the plant in traditional medicine for the control of bleeding from wounds.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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