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Research Article

The Toxicity and Abortifacient Studies of *Commelina benghalensis* Leaves and Stalk in Wistar Rats

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Abstract

Scope of this paper is of Pharmacological relevance.

Introduction: The use of plants as a natural medicine has been attached to the culture of different ethnic groups and is practiced worldwide. Thus called traditional medicine, plays a vital role in today's health-care services. These plants of medicinal value, could be cultivated, wild, weeds and vegetables.

Commelina benghalensis Linn is a common invasive weed found around homes, roadside, gardens and farmlands. It is a perennial plant with upward growing stalk, commonly used for self-induced abortion amongst other numerous ethnopharmacological uses in parts of Uganda and the world at large.

Abortion is common among females despite the fact that it is illegal and immoral. Self- induced abortions could lead to mortalities because of inadequacies of self-medication whether of traditional or conventional drugs. Medicinal plants have been used as abortifacients with some cases multiple organ system failures.

Objectives: This research, studied the acute-toxicity and the potential abortifacient activities of extracts of *Commelina benghalensis* Linn. leaves and stalk in female Wistar rats. Literature reveals that there has been no research done on the plants abortifacient claims.

Materials and Methods: The plant was collected, authenticated, dried, powdered and extracted with *n*-hexane, ethyl acetate, ethanol and water. The acute oral toxicity test was carried out on female Wistar rats, then median lethal dose (LD_{50}) of the extract from each plant part were determined *in vivo* using OECD (Organization for Economic Co-operation and Development) guidelines with help of AOT (Acute Oral Toxicity), 425 software. The extracts were thereafter concentrated *in vacuo* and tested in the animals at 100, 200, and 400 mg/kg doses in the pregnant rats

Results: The acute toxicity test showed LD_{50} greater than 5000 mg/kg. There was no observable toxicity symptom including respiratory distress, salivation, weight loss, dull eyes, diarrhoea and change in the appearance of fur in rats. Extracts of *C. benghalensis* showed abortifacient activity (33.33-100.00%) at 100 - 400 mg/kg with a significant reduction in the number of live foetus compared to the normal group.

Conclusion: There was no observation of any adverse effect or clinical toxicity symptoms in the treated Wistar rats. At the dose of 100 mg/kg body weight of rat, the stalk decoction extract of *C. benghalensis* Linn was found to have the highest abortifacient activity (100% efficacy) when compared with the leaves extracts.

Keywords: *Commelina benghalensis*; Abortifacient; Toxicity; Traditional Medicine

Abbreviations

HIV/AIDS; OECD; AOT; LD_{50}; UNCST; FDA

Background

The use of medicinal plants as preventives, supplements or curative, has been a long time practice and the trend in the world, Africa and Uganda [1]. Many have turned to traditional medicine due to affordability, accessibility, availability and inherent trust in this method [2]. They serve as alternative, reachable, low-cost treatment that is considered to be safe and without harmful effects due to its 'natural' nature [3]. Modern medicines seem not to have given all the answers to prevailing health challenges such as HIV/AIDS, cancer, allergies, pains, flu and unwanted pregnancies [4]. A sizable portion of the population in developing countries, particularly those living in rural and forested regions, relies on traditional medicine for basic health care [5]. Thus, the use of indigenous herbal medicine is imbedded in their culture and is the predominant form of treatment, as cited by JU., *et al*. 2019;18:1-21.

Abortion is a wide practice in society, urban and rural areas where access to hospitals is limited and Uganda has a high demand, despite its being illegal and immoral [6]. Therefore, self-induced abortions are practiced and lead to the death of some women with untreated complications [7]. Some cases of ingested abortifacient herbs have been reported for multiple organ system failures [8]. Numerous natural products have been used as an abortifacient, each with its efficacy and adverse effects [9,10]. In reproductive health, plant toxicity affects libido, fertilization, placentation, embryo-foetal survival [11].

Abortifacient pills like mifepristone and misoprostol are costly and have numerous side effects including heavy bleeding, vomiting, dizziness, abdominal cramps and pains [19]. In contrast, the medicinal plants used for abortion, such as; *Mormodica charantia, Anona reticulata* and *Areca catechu* are thought to cost less and have fewer side effects [12,13].

Commelina Benghalensis Linn., also known as Wandering Jew, Blue Commelina, Venus' bath, Benghal dayflower, tropical spiderworts, commeline, comme'line, [French] and Etiija in western Uganda, is a member of the Commelinaceae family that grows across tropical Africa, Asia, Madagascar, and the Mascarene islands [14]. *Commelina Benghalensis* Linn. mucilaginous's leaves, particularly the young leaves, are cooked and consumed in areas of West Africa, East Africa [Kenya, Uganda, Tanzania, Ethiopia, and Sudan], Indonesia, the Philippines, and India [15]. In Uganda, the Bagandans use it to treat cancer, the Kasese people utilize the seeds to make tea to aid in labour and postpartum management [16] and the Bushenyi people use the stem to perform abortions [17]. In some other parts of Uganda, Sudan and east Africa, pregnant goats and calves are restricted from eating *C. benghalensis* Linn to avoid undesired abortions [18].

C. benghalensis Linn has been reported for its folkloric medicinal use as an abortifacient and many other uses [19].

C. benghalensis Linn is commonly distributed along roadsides, fields, in waste grounds, and in-home gardens and is noted as a very serious weed on farms, an aggressive invasive weed, therefore having no fear for genetic erosion [20].

Abortion is a matter of concern in several societies. The global yearly rate of abortion is 35 per 1000 women for married women and 26 per 1000 women for unmarried women between 2010 and 2020 [21,22]. The sub-Saharan Africa, has also experienced a high rate of treatment for medical complications resulting from unsafe pregnancy termination [23]. The availability of mifepristone in combination with misoprostol for medical abortion has increased its usage as an alternative to surgical abortion for early pregnancy termination [24]. The Food and Drug Administration [FDA] of the United States has authorized this combination of medicines for use at home during the first ten weeks of pregnancy [25]. Most women feel medical abortion provides more autonomy than surgical abortion, is more convenient, protects privacy, and has fewer adverse effects than surgery [26]. Despite its superior efficacy and safety, prior research has indicated that the medication may still result in incomplete abortion, with a full successful expulsion rate of 92.3 percent for 64-70 days of pregnancy and 86.7 percent for 71-77 days of pregnancy [27].

Previously, several studies have separately established the toxicity effects of *C. benghalensis* L. plant in rat models at limit doses of 2000 mg/kg, *C. benghalensis* L. leaves extract was shown to be non-toxic [28]. To our knowledge, no prior ethnopharmaceutical investigation has been undertaken to confirm *C. benghalensis*'s traditional claims about its abortifacient action and toxicity at 5000 mg/kg limit dose.

Materials and Methods

The materials and equipment used for this experimental procedure included female wistar rats, *Commelina benghalensis* leaves and stalk, Misoprostol tablets (200 mcg), intragastric cannulas,distilled water, Extraction reagents of analytical grade, glassware, surgical blades, Vacuum pump filter, analytical balance, animal cages and frigde.

Aim

This research, studied the acute-toxicity and the potential abortifacient activities of extracts of *Commelina benghalensis* Linn. leaves and stalk in female Wistar rats.

Study design

The study was a Laboratory-based experimental design.

Study site the study was conducted at the pharmaceutical chemistry laboratory and animal research

Facilities of the Mbarara University of Science and Technology, situated along Kabale Road, Mbarara, Uganda.

Plant collection

Commelina benghalensis was collected in February, 2020 from an identified site, where the plant of interest grows as weeds in gardens, in Katete village (1° 41' 22.1100'' N, 31° 42' 40.5756'' E), (near Kampala settlement), Nyamitanga division, Mbarara district, Western Uganda. The *Commelina benghalensis* plant collected was identified and authenticated by Dr. Olet Eunice (taxonomist), Department of Botany, Faculty of Science, Mbarara University of Science and Technology, Mbarara, Uganda. Plant collection voucher number is Chioma N Adeyemi #001.

After collection, the plant was washed with running water to remove soil particles, leaves were plucked out from the stalk and dried at room temperature, powdered and then stored in glass container until used for extraction.

Chemicals and reagent

All chemicals and reagents were of analytical grade, bought from Sigma Aldrich branch office, Kampala. Halothane anaesthesia, Misoprostol tablets.

Preparation of the extract

The leaf and stalk parts were extracted using 70% ethanol respectively. The extraction was carried out at room temperature for four days with daily agitation. The homogenate was collected after being filtered using Whatman No. 1 paper; the ethanol was extracted using a rotary evaporator operating at decreased pressure, 25°C, and 115 rpm. The extract was then lyophilized and kept at -20 °C in amber bottles. The percentage yield of the extract from each of the parts was determined by measuring the recovered volume from the weight of each part.

% yield= Where x is the weight of the total extract and y is that of the plant part.

The dried ethanolic (crude) extracts of both leaves and stalk were further partition in hexane, ethyl acetate to get the polar, intermediate polar and the aqueous extracts. The infusion extraction of leaves and decoction extraction of the stalk were carried out respectively. The aqueous extracts had the highest yield of 74.68% for the stalk extract and 77.03% for the leaves extract, followed by 23.93% for the stalk decoction extract, while 0.3% for the ethyl acetate stalk extract, was the lowest yield. Percentage yield is useful for the selection of solvent that gives the highest yield of the desired extract.

Biological study

Animal care

Healthy matured White Wistar rats of both sexes weighing 100-250g were purchased age of 8 weeks, from the Animal Facility laboratory of Mbarara University of Science and Technology. The animals were housed under a 12-hour light/dark cycle with free access to water and were fed with commercial animal feed pellets and clean drinking water *ad libitum*. They were acclimatized for two weeks prior to the assay. The "Principles of laboratory animal care" [29], (National Institute of Health Publication No. 85 - 23, revised 1996; 2011) was followed and permission/approval of an Ethical Board the university was obtained.

Method for acute toxicity study

Acute oral toxicity effect of the leaves and stalk extracts of *C. benghalensis* Linn

Acute oral toxicity test was carried out on female Wistar rats, then median lethal dose (LD_{50}) of the extract from each plant part were determined *in vivo* using OECD guidelines [30] with help of AOT, 425 software, which gives the confidence intervals. One animal each was dosed with 175 mg/kg and monitored for signs of adverse effects and mortality for 30 minutes, 1, 2, 4, 24 till 48 hours

and then 14 days. In absence of toxicity signs, the dosing was increased to 550, 1750 and 5000 mg/kg as limit dose. The rats were observed for signs of adverse effects (including loss of weight, scratching, loss of appetite, etc.) and mortality for 14 days.

Investigation on the abortifacient activity of the leaves and stalk extracts of *C. benghalensis* Linn

The different doses of extract were tested in female Wistar rats for abortifacient activity using the procedure of Sandiya., *et al*. 2014 [31]. Nine (9) Male and 18 female albino rats (100-250g) were randomly selected and caged in the ratio of 1:2 in the evening of proestrus. On the next day, they were randomly examined for the presence of sperms (Vaginal plug). Pregnancy was confirmed by the presence of thick clumps of spermatozoa in the vaginal smear and the rats were separated to designate the day as day one of pregnancy. Routine affirmation of mating in the rats was done by checking for the copulatory plug or presence of spermatozoa in the vaginal lavage. The pregnant rats were divided into three groups of six animals each. The *C. benghalensis* extract-treated groups, were orally administered with the drug from the 11th to 15th day of pregnancy at doses of 100, 200 and 400 mg/kg respectively (Gestation period of Wistar rats is 19 days). Likewise, negative control group was administered with distilled water (10 ml/kg), while the positive control was administered with misoprostol (100 mg/kg) respectively. This procedure was carried out with both leaves and stalk extracts of *C. benghalensis* respectively.

At the end of the experiment, the animals were laparotomised under anaesthesia using halothane on the 17th day of pregnancy for observation of the full term of the foetuses (44).

The uterine horns were subjected for the examination of the live and dead foetuses, implantation sites of resorptions.

The percentage of abortion was calculated using the formula:

$$\text{Percentage of abortion} = \frac{(\text{no. of implantation} - \text{no.of live fetuses})}{\text{no. of implantations}} * 100$$

Data management and analysis

Data was exported to Graphpad Prism software version 8.0 from Microsoft excel 2010 for analysis. All quantitative data were expressed as mean ± standard error of the mean, while variation in a set of data was analyzed through the one-way ANOVA (Analysis of variance). Analyses of variance and differences among the means were considered statistically significant at 95% confidence limit using post hoc method of Tukey HSD (honestly significant difference).

Abortifacient activities of leaves and stalk extracts of *C. benghalensis* were determined respectively by the percentage number of rats that aborted per treated group and the overall activity of the extracts compared to that of Misoprostol (positive control).

Results

Extraction

The universal solvent ethanol at a concentration of 70% was employed to make the crude extract of *C. Benghalensis* Linn because it is an effective solvent for the extraction of a large number of biologically active plant components. The stalk, in particular, has been recognized by the locals of Bushenyi natives for abortion and in some places, pregnant goats and calves are restricted from eating to prevent undesired abortions. As a result, the study compared the abortifacient activity of *C. Benghalensis* Linn stalk and leaves.

Plant part	**Stalk extracts of *C. benghalensis***					**Leaves extracts of *C. benghalensis***				
Extracts	ETOH	Hexane	Ethyl acetate	Aqueous	Decoction	ETOH	Hexane	Ethyl acetate	Aqueous	Infusion
% w/w	11.07	0.08	0.03	74.68	23.93	18.59	0.11	0.07	77.03	17.05

Table 1: Results of Percentage yield of extracts of *C. benghalensis* Linn leaf and stalk.

Table 1 showed that the aqueous extracts had the highest yield, followed by the stalk decoction, while the ethyl acetate stalk extract, had the lowest yield. Percentage yield is useful for the selection of solvent that gives the highest yield of the desired extract.

Acute oral toxicity testing results

Dose mg/kg	Hexane extract (wt of rat in g)			Ethyl ac. ext. (wt of rat in g)			Aq phase. ext. (wt of rat in g)			LEtoH (Crude) extract (wt of rat in g)			Infusion ext. (wt of rat in g)		
----	D_0	D_1	D_1-D_0	D_0	D_1	D_1-D_0	D_0	D_1	D_1-D_0	D_0	D_1	D_1-D_0	D_0	D_1	D_1-D_0
175	203	220	17	201	223	22	190	212	22	250	268	18	234	255	21
550	188	201	13	212	234	22	240	262	22	226	239	13	210	235	25
1750	203	225	22	212	235	23	248	260	12	248	264	16	250	268	18
5000	250	268	18	275	296	21	213	230	17	220	243	23	247	268	21

Table 2: Results of the effect of leaves extracts on the weight of rats after treatment.

(D_0= Original weight of rat in grams, D_1= Weight of rat in grams after 14 days, D_1-D_0= Changes in weight of rat in grams and ext. = extract).

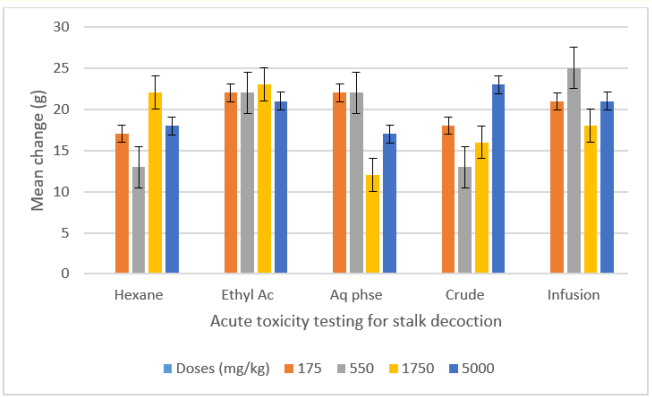

Figure 1: Mean weight changes of rats treated with *C. benghalensis* leaf extracts.

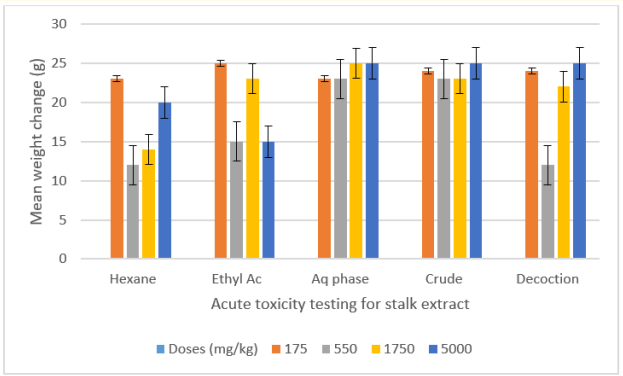

Figure 2: Mean weight changes of rats treated with *C. benghalensis* stalk extracts.

Dose mg/kg	Stalk hexane (SH) extract (wt of rat in g)			Stalk ethyl ac. (Sethyl) ext. (wt of rat in g)			Stalk Aq (SA) (wt of rat in g)			Stalk ethanolic (crude) (seth) (wt of rat in g)			Stalk decoction (SD) (wt of rat in g)		
-----	D_0	D_1	D_1-D_0	D_0	D_1	D_1-D_0	D_0	D_1	D_1-D_0	D_0	D_1	D_1-D_0	D_0	D_1	D_1-D_0
175	170	193	23	110	135	25	174	198	23	174	198	24	150	174	24
550	243	255	12	119	134	15	194	217	23	163	186	23	150	162	12
1750	234	248	14	138	161	23	165	188	25	164	190	23	169	191	22
5000	20	228	20	123	138	15	124	149	25	194	218	24	160	185	25

Table 3: Results of the effect of stalk extracts on the weight of rats after treatment.

(D_0= Weight of rat in grams, D_1= Weight of rat in grams after 14 days, D_1-D_0= Changes in weight of rat in grams and ext. = extract).

Results for the abortifacient activity of leaves and stalk extracts of *C. benghalensis* in Wistar rats

Extracts	Dose	Rat Wt	*T. foetus*	*F. remain*	*F. abort*	% Abort
Stalk Decoction (SD)	100	158.94	8.33	0	8.33	100.00 ± 0.00*** (F = 249.0; CI = -111.60 to -88.39)
	200	154.03	8.00	0.50	7.50	93.75 ± 6.25*** (F = 249.0; CI = -105.4 to -82.14)
	400	140.28	8.50	0	8.50	100.00 ± 0.00*** (F = 249.00; CI = -111.60 to -88.39)
Stalk Ethanolic (CRUDE) (Seth)	100	152.09	8.00	0.00	8.00	100.00 ± 0.00*** (F = 13.60; CI = -146.70 to -53.30)
	200	148.24	8.00	1.33	6.67	83.33 ± 16.65*** (F = 13.60; CI = -130.00 to -36.63)
	400	137.41	8.50	2.83	5.67	70.27 ± 18.81** (F = 13.60; CI = -117.00 to -23.56)
Leaves Infusion (LI)	100	159.44	7.67	5.00	2.67	33.33 ± 21.08*** (F = 9.08; CI = -146.1 to -33.05)
	200	142.90	7.67	0.83	6.83	89.58 ± 10.42* (F = 9.08; CI = 146.1 to -33.05)
	400	144.70	7.50	3.00	4.50	58.52 ± 19.30ns (F = 9.08; CI = -115.1 to -1.984)
Leaves Ethanolic (CRUDE) (Leth)	100	121.53	7.67	1.17	6.50	82.64a ± 11.61*** (F = 22.06; CI = -119.30 to -45.97)
	200	132.64	6.67	1.5	5.17	75.00a ± 15.96*** (F = 22.06; CI = -111.70 to -38.33)
	400	143.77	7.60	0	7.60	100.00a ± 0.00*** (F = 22.06; CI = -136.70 to -63.33)
Positive Control (PC)	100	137.71	8.00	0	8.00	100.00a
Negative Control (NC)	100	147.69	6.17	5.83	0	0

Table 4: Abortifacient activity of *C. benghalensis* extracts on Wistar rats.

Data were expressed as mean ± SEM; n = 6; ***P < 0.001; **P < 0.01; *P < 0.05; ^{ns}P > 0.05 compared to negative control.

Figure 3: Effect of extracts of *C. benghalensis* Linn stalk and leaves in pregnant rats.

Where, PC = Positive control, NC = Negative control, SD = Stalk decoction, LI = Leave infusion, Leth = Leave ethanolic, Seth = Stalk ethanolic.

Discussion

The acute oral toxicity testing of leaves and stalk extract of *C. benghalensis* in Wistar rats

Graphs 1 and 2 showed random weight changes in relation to the extracts. The increase was between 12-25 g and there were no weight losses. The increase in weight was similar to the ethanolic and aqueous extracts. There was no mortality and or any sign of toxicity observed in the treated groups of rats at doses of 175, 550, 1750 and of 5000 mg/kg body weight. There were no clinical toxicity symptoms such as salivation, respiratory distress, weight loss, changes in the appearance of hair, as well as maternal mortality observed at any period of the experiment. The LD_{50} was >5000 mg/kg, which suggested that the *C. benghalensis* extracts may be non-toxic at such acute doses. Ethanolic leaves and stalk crude ex-

tracts also administered to the rats at the limit dose of 5000 mg/kg, caused no signs of toxicity.

Therefore, the herbal extract of *C. benghalensis* Linn is considered to be nontoxic even at such high doses [32].

The investigation of the abortifacient activity leaves and stalk extract of *C. benghalensis* in Wistar rats

All the experimental extracts when evaluated for their abortifacient activity, were found to exhibit pregnancy interceptive activity. The percentage aborted of the leaves extracts; infusion and ethanolic ranges from 33.33-85.19% at the dose of 100 mg/kg and 58.52-100.00% at the dose of 400 mg/kg body weight. While percentage aborted in the animals dosed with stalk extracts, decoction and ethanolic at 100 mg/kg showed 100.00%, but at 400mg/kg, Decoction extract showed 100% and higher than ethanolic extract (70.27%). The abortifacient activity results as shown in table 4 reveals that the stalk decoction extract at (lowest and highest doses had 100% activity that was significant when compared to the negative control. There was an evident increase in the number of aborted foetuses and a more potent abortifacient activity with *C. benghalensis* stalk extracts compared to that of the leaves extracts, even though leave extracts also exhibited significant p-values and percentage aborted when compared to the control. The result of stalk extracts corroborates with the findings reported by Dabhadkar and Zade (2012) using *Pityriasis rubra* pods. This is a useful information to guide researchers.

Conclusion

The LD_{50} of the extracts of *C. benghalensis* Linn leaves and stalk respectively, is more than 5000 mg/kg which showed that the extracts are acutely safe.

The increase in the number of aborted foetuses following the administration of the extracts of *C. benghalensis* Linn stalk and leaves is an indication of their possible abortifacient activity. This present work showed that the stalk had the highest abortifacient activity than the leaf.

Declarations

This research paper is part of that approved by the Uganda National Council for Science and Technology (UNCST) with the Registration number SS576ES.

This research also received ethical approval from the Research Ethical Committee of the Mbarara University of Science and Technology, Mbarara, Uganda.

Availability of Data and Materials

Not applicable.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Contributions

CNA conceptualized the study, carried out the experiments, analyzed and evaluated the results, and drafted the paper. The supervision and assistance with the whole project, from design through manuscript review was done by JO and CA. CA was involved in the study's design. Each author contributed to improve the manuscript's quality.

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