Original Article

Anti-Diarrhoeal Properties of Stem Bark of *Cassia Abbreviata* against Castor Oil-Induced Diarrhoea in Swiss Albino Mice

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ABSTRACT

Background: *Cassia abbreviata* oliv is a plant which is commonly used in traditional medicine for treating diarrhoea in many parts of Zambia. However the use of the stem bark extracts has not been established scientifically using animal model. This study aimed at investigating anti-diarrhoeal properties of *Cassia abbreviata (Fabaceae)* stem bark crude extracts.

Study Design: This study was an experimental study where 30 Swiss albino mice were randomly divided into five groups of six mice each and treated with different doses of normal saline (10ml/kg), loperamide (5mg/kg) and plant extracts (100, 200 and 400mg/kg).

Methods*:* The antidiarrhoeal activities of methanol, ethanol and aqueous extracts from *Cassia abbreviata* stem bark were studied using mice as animal model. Diarrhoea was induced by using castor-oil. The effect of methanol extract on gastrointestinal motility and intestinal transit time was also evaluated in mice.

Results**:** The four extracts from *Cassia abbreviata* stem bark reduced the severity of diarrhoea significantly at the doses of 400mg/kg ($p<0.001$). Methanol extract had a higher percentage inhibition of diarrhoea (64.5%) when compared with the other extracts except Loperamide (67.7%). The extracts also reduced frequency of Diarrhoeal episodes and stool outputs when compared to the control group ($p<0.001$). In addition, the extracts significantly delayed the onset of castor-oil induced diarrhoea. Methanol extract significantly ($p<0.001$) reduced intestinal transit (53.3%) and delayed gastric emptying at 400mg/kg when compared to Loperamide (65.5%).

Conclusion*:* It was concluded that all the four extracts from the stem bark of *Cassia abbreviata* scientifically displayed anti-Diarrhoeal properties and this justified the use of the plant. However, this may require further scientific studies leading to identification of the actual compounds responsible for its anti-Diarrhoeal properties.

INTRODUCTION

Diarrhoea is characterized by the passage of 3 or more loose stools per day, or more frequently than is normal for the individual.[1] Diarrhoea affects all age groups and it causes around 525,000 deaths every year in children below the age of 5 years .[2]Diarrhoea is usually a symptom of gastrointestinal infection, which can be caused by a variety of pathogens. About 70% of people use plants as a source of medicine when treating different conditions.[3] Plants provide natural sources of medicines and have been used to treat different ailments for a long time.[4, 5]

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Key Words: *Anti-diarrhoea, Anti-motility, Cassia abbreviata, Castor oil, Loperamide*

Cassia abbreviata oliv is a small to medium-sized umbrella-shaped deciduous tree belonging to the family *Fabaceae*. The plant is commonly found in the tropical regions of Africa such as East Africa - Somalia, Kenya, Tanzania, Southeast DR Congo, Zambia, Malawi, Mozambique, Zimbabwe, Botswana, Swaziland, and South Africa. In Zambia, *Cassia abbreviata* is locally known as Mululwe and Munsokansoka.[6]

Parts of *Cassia abbreviata* have long been used as traditional medicine elsewhere and in Zambia for hematuria, abscesses, diarrhoea, malaria, diabetes, gonorrhea and as a purgative.[7-10] In Zambia the endogenous population commonly uses traditional herbs for various ailments. Cassia abbreviate is one such plant that is commonly used to treat diarrhoea.[7, 11] In their study Lavanya et al., [12] found that the species of *Cassia abbreviate* have the following phytochemicals as their constituents anthraquinones derivatives, guibourtinidiol, alkaloids, tannins, crude proteins, flavonoids, and sterols.

The bioactive substances in the stem-bark of *Cassia abbreviate* that may be attributed to its anti-diarrhoeal properties include phytochemicals such as alkaloids, tannins, flavonoids and sterols.[13]

In Zambia, there is little or no data to validate the anti-diarrhoeal properties of this plant. This study attempted to investigate the potential antidiarrhoeal effects of extracts of stem bark of the plant.

MATERIALS AND METHODS

Plant Material Collection and Identification

Fresh stem bark of *Cassia abbreviata* was collected from Kapete Ward near Mulola Basic School with coordinates of 15⁰ 28'S and 28⁰ 81'E in Chongwe District. The specimen was later identified and deposited at UNZA herbarium with accession number UZL 22210.

Extraction of Crude Extracts from the plant

The freshly collected Stem bark was immediately washed and chopped into small pieces, shade dried at room temperature for 14 days and after which it was ground using an electric blender and sieved to obtain a homogeneous powder. In this study, soxhlet and maceration techniques were used for extracting.

From the powder obtained above, 100g of dry powder was subjected to successful soxhlet extraction for methanol and ethanol solvents. Both extracts were then filtered using Whatman filter paper and concentrated using evaporator under reduced pressure set at 40 °C and this was followed by drying in the oven at room temperature. Re-extraction was repeated for both solvents as described above using 50g of the dried marc (obtained in the first extraction process from the thimble) and the filtrates dried at room temperature. This was done in order to maximize the yield of the extract from the plant. The powder (filtrate) collected was then ground to a fine powder using mortar and pestle, and then weighed and the percentage yield calculated using the formula shown below.

200g of dry powder was extracted using maceration method for both hot and cold water solvents. The residual from the dried marc was re-macerated. The powder (filtrate) collected was dried as described above and the percentage yield was calculated.

$$\% \text{ yield} = \frac{\text{Weight of the extract}}{\text{Weight of plant material}} \times 100\%$$

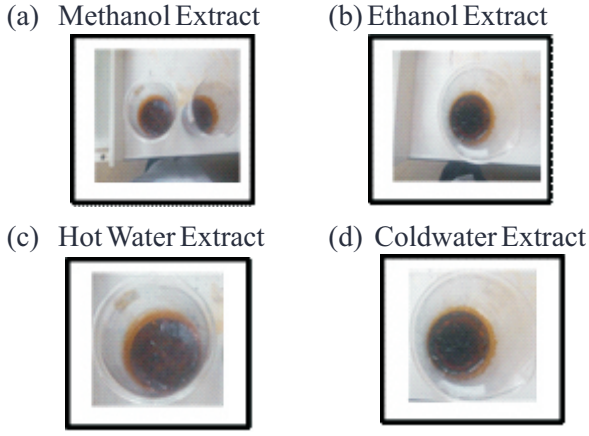

Figure 1:- Physical appearance of the 4 extracts methanol and ethanol extracts looked reddish-brownish for both extracts while hot-water and cold-water extracts looked darkish brownish residue.

Experimental Animals

Swiss albino mice of both sex weighing between 20–30 g and aged 20–24 weeks were used for the experiment. The mice were acquired from the animal house of the Department of Physiological Sciences, University of Zambia. The animals were kept in plastic cages with access to pellet food and water ad libitum. They were also acclimatized to laboratory conditions for one week before experiments. The handling and care were done according to the guidelines for the use and maintenance of experimental animals.[14]

Grouping and Dosing

The albino mice were randomly selected by generating random numbers using a graph pad and assigned into five groups of six animals each for all the four solvents. All the groups received treatment by oral gavage. The first group assigned as negative control for each extract received distilled water at 10 ml/kg. The second group assigned as positive control received Loperamide (5 mg/kg). For Methanol extract (MF), Ethanol extract (EF), Hot water (HF), and Cold water (CF) the dose levels were determined by calculating dose-volume calculations based on the weight of each mouse (100 mg/kg, 200 mg/kg, and 400 mg/kg). These were assigned to groups 3, 4 and 5 respectively.

Phytochemical Screening

The four solvent crude extracts were tested for the presence of alkaloids, steroids, terpenoids, tannins, flavonoids, and saponins using standard procedures adopted from Tiwari.[15]

Determination of Anti-Diarrhoeal Properties against Castor-oil Induced Diarrhoea

Umer et al., [16] methods was adopted for this study. Swiss albino mice were fasted for 18 hours before the experiment. After 1 hour of pre-treatment with crude extracts of stem bark of *Cassia abbreviata* (methanol extract, ethanol extract, hot water and cold water extract), distilled water and loperamide using oral gavage, the animals received 0.3mls of castor oil (3mg/100g). Each animal was then placed in a separate cage over a clean filter paper (weight of filter paper noted and re-weighed again with stool after 1 hour). The filter paper was changed hourly and the observations were taken every 4 hours, these included time of onset of diarrhoea (minutes), frequency of defecation (hourly), the total number of fecal outputs, and weight of stools (g) excreted (weight of filter paper plus stools minus weight of filter paper). Stools were categorized as watery, semi-solid, and normal stool (absence of diarrhoea). Mice were left to recover for seven days after one experiment before the next experiment with another extract. This was done in order to allow mice recover from dehydration. Diarrhoea inhibition and fecal output were calculated using the following formulas 1-3:

$$\% \text{ of Inhibition} = \frac{\text{Avg \# of WFC} - \text{Avg \# of WFT}}{\text{Avg \# of WFC}} \times 100 \ldots (1)$$

Where WFC= weight faeces in the control group; WFT= weight faeces in the test group

$$\% \text{ of WFO} = \frac{\text{Mean WWS of each group}}{\text{Mean WWS control}} \times 100 \ldots\ldots\ldots\ldots\ldots (2)$$

Where WFO is weight faecal output, WWS is weight of wet stool

$$\% \text{ of WTFO} = \frac{\text{Mean WTS of each group}}{\text{Mean WTS control}} \times 100 \ldots\ldots\ldots\ldots (3)$$

Where WTFO is weight of total faecal output, WTS is weight of total stool

Gastrointestinal Motility Testing

This experiment was done using charcoal as a marker. Mice of either sex were divided into five groups and then fasted for 18 hours. All the groups received castor oil 0.3mls. An hour later; Group one (control) received normal saline (10ml/kg). Group two (standard) received Loperamide (5ml/kg). Methanol extract showed highest inhibition and this was further studied. Groups 3, 4, and 5 received doses of 100, 200 and 400 mg/kg of crude methanol extract respectively. After 1 hour, 1ml of charcoal marker (in 5% distilled water) was administered through oral gavage. The animals were then sacrificed an hour later. The small intestines were

then dissected out from pylorus to caecum. The distance traveled by charcoal meal marker was measured and expressed as a percentage of distance covered using the formulas 4-5 below;

$$\text{Peristaltic Index} = \frac{\text{DT by charcoal meal}}{\text{The WL of intestines}} \times 100 \ldots\ldots\ldots (4)$$

Where DT is distance Travelled, WL is Whole length

$$\%\ \text{inhibition} = \frac{\text{PIC} - \text{PIT}}{\text{PIC}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where PIC = peristaltic index for control; PIT = peristaltic index of the test group

Figure 2:- Mouse euthanized and intestines dissected

Data Analysis

Data was expressed as mean and standard deviation and then analyzed using a one-way analysis of variance (ANOVA), Bonferroni post hoc test, and Pearson's correlation. P value of less than 0.05 or 0.001 was considered as significant.

Ethical Consideration and Clearance

The study was given ethical clearance from University of Zambia Biomedical Research Committee (UNZABREC), and National Health Research Authority. All animals were handled with care and experimental protocols for administering medications used according to guidelines.[17]

RESULTS

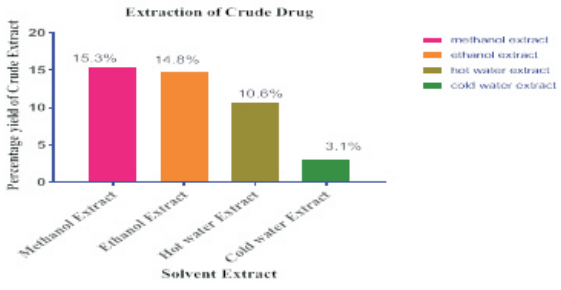

Figure 3:- Comparison of percentage yield of extracts from the four different solvents.

Phytochemical Screening

Table 1:- Results of phytochemical screening from the different crude extracts

Phytochemical tests	Methanol fraction	Ethanol fraction	Hot water fraction	Coldwater fraction
Alkaloids (Mayer's test)	Turbidity of precipitate (+)	Turbidity of precipitate (+)	Turbidity of precipitate (+++)	Turbidity of precipitate (+)
Sterols (Liebermann-Bourchard reaction)	Red-brown ring appeared (-)	Red-brown ring appeared (-)	Red-brown ring appeared (++)	Red-brown ring appeared (++)
Tannins (Braymer's Test)	Formation of green precipitate (+++)	Formation of green precipitate (+++)	Formation of green precipitate (+++)	Formation of green precipitate (++)
Flavonoids (Shinoda reagent test)	Red colour appeared (+)	Red colour appeared (+)	Red colour appeared (++)	Red colour appeared (+)
Saponins (Frothy test)	Presence of foam (+++)	Presence of foam (+++)	Presence of foam (+++)	Presence of foam (+)
Terpenoids (Liebermann-Bourchard reaction)	Reddish brown (+++)	Reddish brown (+++)	Reddish brown (++)	Reddish brown (++)

'+' indicated the presence of tested phytochemical, '-' indicated the absence

Anti-Diarrhoeal Properties against Castor-oil Induced Diarrhoea

The four extracts of stem bark of *Cassia abbreviata* showed marked antidiarrhoeal effects as shown in Table 2. Onset of diarrhoea was significantly delayed by all the four crude extracts when compared to the control group (74.3 SD 14.4) at the dose of 400 mg/kg; MF (149.2 SD 28.2), EF (158.7 SD 30.4), HF (173.2 SD 34.1) and CF (168.3 SD 28.8) respectively. The total number of diarrhoea stools was significantly reduced by the various doses of the extracts as shown in Table 2 below. The effect on total number of stools was marked with the 400 mg/kg doses of the four extracts which were comparable to the standard group of Loperamide (3.2 SD 1.2). The percentage inhibition of diarrhoea seen with 100, 200 and 400 mg/kg of the extracts was comparable to that of Loperamide (66.7 %); MF (48.4%, 56.7% and 64.5%), HF (38.7%, 54.8% and 59.7%), EF (24.2%, 54.8% and 62.4%) and CF (44.8%, 56.9% and 60.9%) respectively. At 400 mg/kg MF had the highest diarrhoea inhibition. The percentage inhibition of diarrhoea was observed to be increasing as the dose was doubled. Whereas, the stool output (WWFO and WTFO) was observed to be low with the 400 mg/kg and highest with the 100 mg/kg doses of the four extracts.

Table 2:- Antidiarrhoeal effects of the four extracts on castor oil-induced diarrhoea in mice model

Dose mg/kg	Onset of Diarrhoea (min)	Number of wet stool (4hrs)	Total number of stools (4hrs)	Average weight of wet stools (gm)	Average weight of total stools (gm)	% inhibition of Diarrhoea	% WWFO	% WTFO
D/w	74.3 (SD 14.4)	6.2 (SD 0.7)	6.6 (SD 0.9)	1.2 (SD 0.2)	1.3 (SD 0.2)			
Lop	162.7 (SD 39.6) ***	2.0 (SD 1.4) ***	3.2 (SD 1.2) ***	0.4 (SD 0.2) ***	0.4 (SD 0.2) ***	67.7	29.3	34.1
MF 100	101.2 (SD 11.6) *	3.2 (SD 0.8) ***	4.0 (SD 0.9) ***	0.9 (SD 0.3) *	0.9 (SD 0.3) *	48.4	70.7	70.6
MF 200	119.3 (SD 23.8) *	2.5 (SD 1.5) ***	3.2 (SD 0.8) ***	0.7 (SD 0.4) *	0.8 (SD 0.4) *	56.7	59.4	60.3
MF 400	149.2 (SD 28.2) ***	2.2 (SD 1.5) ***	3.0 (SD 1.1) ***	0.4 (SD 0.3) ***	0.4 (SD 0.3) ***	64.5	30.9	32.5
EF 100	124.3 (SD 14.7) **	4.7 (SD 1.2) *	5.1 (SD 1.2) *	0.9 (SD 0.5) *	0.9 (SD 0.5) *	24.2	75.6	76.2
EF 200	144.3 (SD 20.8) ***	2.8 (SD 0.9) ***	2.8 (SD 1.1) ***	0.8 (SD 0.4) *	0.7 (SD 0.4) *	54.8	58.5	58.7
EF 400	158.7 (SD 30.4) ***	1.8 (SD 0.9) ***	2.4 (SD 1.4) ***	0.5 (SD 0.2) ***	0.5 (SD 0.2) ***	62.4	36.6	37.3
HF 100	113.8 (SD 11.1) *	3.3 (SD 1.5) **	4.2 (SD 1.7) *	0.7 (SD 0.3) **	0.7 (SD 0.4) **	38.7	69.1	69.1
HF 200	156.0 (SD 15.9) ***	2.8 (SD 1.9) **	3.5 (SD 1.9) ***	0.7 (SD 0.2) **	0.7 (SD 0.3) **	54.8	55.3	57.1
HF 400	173.2 (SD 34.1) ***	1.8 (SD 1.2) ***	2.5 (SD 1.0) ***	0.5 (SD 0.2) ***	0.5 (SD 0.2) ***	59.7	39.0	40.5
CF 100	121.7 (SD 23.6) *	3.4 (SD 1.8) ***	3.9 (SD 1.8) ***	0.9 (SD 0.5) *	0.9 (SD 0.5) *	44.8	72.3	72.2
CF 200	125.7 (SD 20.9) **	2.7 (SD 0.8) ***	3.2 (SD 0.8) ***	0.7 (SD 0.4) *	0.7 (SD 0.4) *	56.9	56.1	56.3
CF 400	168.3 (SD 28.8) ***	1.7 (SD 0.5) ***	2.2 (SD 0.6) ***	0.5 (SD 0.3) ***	0.5 (SD 0.2) **	60.9	40.7	42.1

In this study, Table 2 shows values expressed as mean + SD at (n=6 per group), p>0.05*, p<0.05**, p<0.001*** when compared to control group; MF = methanol fraction; EF = ethanol fraction; HF = hot water fraction; CF = cold water fraction; WWFO = weight of wet fecal output; WTFO = weight of total fecal output.

Pearson's correlation

Results showed that there was a moderate negative correlation between time of onset of diarrhoea and the 3 variables: the total number of wet stools, had a negative correlation of 0.4948 (p-value 0.0054), the total number of stools had a negative correlation of 0.5193 (p-value 0.0033) and the total weight of wet stools had a negative correlation of 0.4634 (p-value 0.0099) respectively. In all the three variables the correlation was statistically significant. Results below showed that as the time of observing diarrhoea increase the total number of wet stools, number of total stools and total weight of wet stools reduces giving a negative slope.

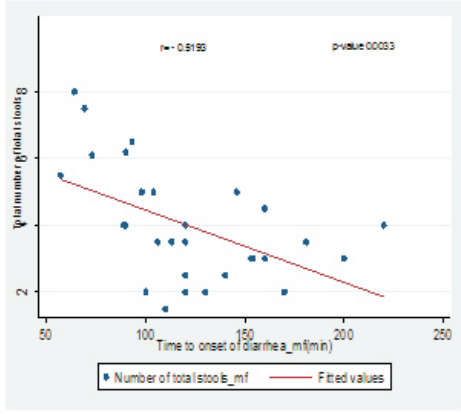

Figure 5: Scatter plot between the total number of total tools and time to onset of Diarrhoea in minutes

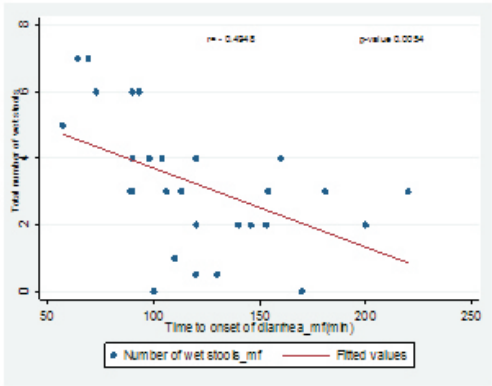

Figure 4: Scatter plot between the total number of wet stools and time to onset of Diarrhoea in minutes

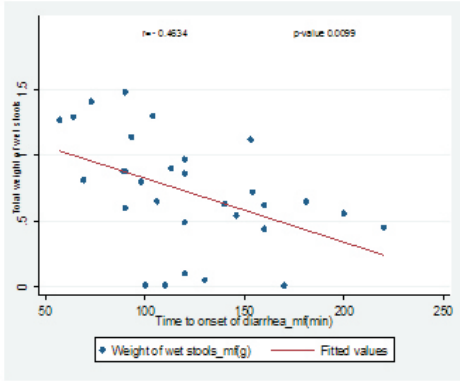

Figure 6: Scatter plot between the weight of wet stools and time to onset of Diarrhoea (min)

Gastrointestinal Motility Testing

Table 3:- Effects of methanol extract of *C. abbreviata* on intestinal motility in mice

Dose mg/kg	Length of intestines (cm)	Distance moved by charcoal maker (cm)	Peristaltic index (%)	% inhibition
Con 10ml/kg	55.3(SD 2.7)	37.2 (SD 3.5)	67.2	
Lope 5mg/kg	56.8(SD 3.1)	13.2 (SD 1.5) ***	23.2	65.5
MF 100 mg/kg	57.3(SD 2.2)	24.7 (SD 3.2) ***	43.0	35.9
MF 200 mg/kg	54.7(SD 2.9)	22.5 (SD 3.9) ***	41.2	38.8
MF 400 mg/kg	56.3(SD 2.6)	17.5 (SD 3.8) ***	31.1	53.5

Table 3 above, shows values presented as mean + SD at (n = 6 per group), $p<0.001$*** when compared to control group.

During gastrointestinal motility testing in mice model, methanol extract of *Cassia abbreviata* stem bark significantly decreased the propulsion of charcoal meal marker in the intestines at doses 100, 200 and 400mg/kg (35.9%, 38.8% and 53.5%) respectively. The maximum dose (400 mg/kg) of the extract showed comparable anti-motility effects to the standard Loperamide (65.5%, $p<0.001$).

DISCUSSION

In this study the crude extracts of the stem bark of *Cassia abbreviate* oliv were assessed for their anti-Diarrhoeal and intestinal motility effects using castor oil induced diarrhoea and charcoal meal transit time test .

Patients with diarrhoea will normally pass a lot of solutes and water in stool as there is less absorption.[18] Use of castor oil in inducing diarrhoea in experimental mice is well documented.[19] Castor oil contains over 90% of a fatty acid called Ricinoleic Acid.[20] Castor oil causes non-infective diarrhoea when taken as a laxative to treat constipation. Castor oil induces diarrhoea (1-2 hours) through Ricinoleic acid (RA). Ricinoleic acid stimulates the release of Nitric Oxide which in turn causes increased permeability of the gastrointestinal membrane to calcium and this leads to loss of calcium in the lumen which later causes diarrhoea in mice and humans.[21] RA produces local irritation and inflammation of intestinal mucosa which causes the release of prostaglandins. The prostaglandins cause increased GI motility, fluid, and electrolytes secretion into the lumen.[21] The excess fluid and electrolytes cannot be reabsorbed by the body and this result in the passing of watery stools as there is too much to reabsorb.[22] Also, RA combines with sodium and potassium to form a Ricinoleic salt in the lumen and these salts inhibit sodium-potassium ATPase. The inhibition of this pump (Na^+/K^{2+} ATPase) causes increased permeability of intestinal epithelium, leading to cytotoxic effects on absorptive cells which later results in loss of water and electrolytes in the stool.[23]

The four crude extracts of stem bark of *Cassia abbreviata* showed significant antidiarrhoeal properties when compared to the reference drug Loperamide through significantly reducing the castor oil induced diarrhoea in mice model. The greatest reduction was seen with the 400mg/kg doses implying that the antidiarrhoeal properties revolve around this dose. The four extracts were found to delay the onset of diarrhoea, reduced the total number of stools, and also reduced the severity of castor oil-induced diarrhoea in mice as shown in **Table 2.** Methanol extract had the highest inhibition of diarrhoea (64.5%) when compared to the other extracts. This made methanol extract to be close to reference drug (loperamide) which had 67.7% diarrhoeal inhibition. Determination of percentage inhibition was based on reduction of wet faecal outputs as a good marker for anti-diarrhoeal activities. Accordingly, with 400mg/kg dose of the four extracts, a dose-dependent reduction in wet faecal outputs was observed indicating the anti-Diarrhoeal potential of the extracts of stem bark of *Cassia abbreviata* (Table 2). Substances exhibiting significant antidiarrhoeal properties may have the potential to retard onset of diarrhoea as observed with 400mg/kg dose from the four extracts in the study. The crude extracts from *Cassia abbreviata* may have stimulated the re-absorption of water and electrolytes and also reduced the intestinal

movements that may contribute to the patho-physiology of diarrhoea. Many antidiarrhoeal agents act by reducing motility and gut secretions. By slowing the gastrointestinal motility the plant extracts allowed more time for the water and electrolytes to be absorbed from the intestines and colon thereby reducing the content of stool.[24] Loperamide has anti-secretory effects mediated through μ opioid receptors and non-opioid mechanisms.[25] When given at high doses Loperamide decreases motility, an effect mediated through μ opioid receptors in the myenteric plexus of the bowel. Low dosages exploit the anti-secretory and the higher dose the anti-motility effect.[25, 26] Therefore, Loperamide is used as an anti-Diarrhoeal agent in the treatment of non-infective diarrhoea. In this study, the significant antidiarrhoeal effects observed in the methanol, ethanol, hot and cold-water extracts could probably be attributed to the presence of the secondary metabolites in *Cassia abbreviata* stem bark.

During intestinal motility test with charcoal meal marker, the peristaltic index was greatest with control group (67.7%) when compared to the extracts (43.0%, 41.2% and 31.1%), and standard drug Loperamide (23.2%). In this study, methanol extract significantly inhibited gastrointestinal transit time of charcoal meal marker when compared to the control group at the dose of 400mg/kg (53.5%, $p<0.001$) while Loperamide (65.5%, $p<0.001$). This finding suggests that the extract from *C. abbreviata* stem bark has the ability to influence the peristaltic movement of intestines thereby indicating the presence of an antimotility activity. The reduction in percentage of distance travelled by charcoal marker can be attributed to the relaxation of intestinal smooth muscle. The reduction of intestinal motility is one of the mechanisms by which antidiarrhoeal drugs can act.

Preliminary phytochemical screening of the crude extracts of *Cassia abbreviata* stem bark showed the presence of alkaloids, sterols, tannins, flavonoids, saponins, and terpenoids. Some studies have shown that phytochemicals are found to have different antidiarrhoeal effects that can be achieved through different mechanisms.[18, 27] Alkaloids have an antidiarrhoeal effect through inhibition of prostaglandins release which causes an increase in fluid and electrolyte secretion.[27] Flavonoids and tannins have an antidiarrhoeal effect by increasing fluid and electrolyte reabsorption.[18] Sterols exert their antidiarrhoeal effects by increasing sodium and water reabsorption in the lumen.[27] Therefore, the antidiarrhoeal properties of the crude drug of *Cassia abbreviata* could be due to any of these phytochemical compounds that were found in the extracts.

These findings are similar to the findings found by Sisay.[28]In Zambia plants have been evaluated scientifically for their anti-Diarrhoeal and anti-diabetic properties. [29, 30]This was the first time that antiDiarrhoeal effects of a plant were studied.

CONCLUSION

The aim of the study was to investigate the antidiarrhoeal properties of the stem bark of *Cassia abbreviata* in comparison with Loperamide against castor oil induced diarrhoea in mice model. This was achieved as results showed that methanol, ethanol, hot and cold water inhibited diarrhoea by delaying the onset of diarrhoea, reducing weight of wet faecal and total faecal output in a dose dependent manner. This shows that the plant was effective in treating diarrhoea in mice model. These findings support the traditional use of the plant for treating diarrhoea. Further studies are recommended to support our findings and to assess the toxicology.

Acknowledgment

The authors are sincerely grateful to members of staff in the Department of Physiological Sciences, School of Medicine, and the University of Zambia, the staff of Pharmacology, and Chemistry Laboratories for supporting and allowing the use of the equipment during the study.

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