International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 2 (7), pp. 110-116, October, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

In vivo antidiarrheal and ex-vivo spasmolytic activities of the aqueous extract of the roots of Echinops kebericho Mesfin (Asteraceae) in rodents and isolated guinea-pig ileum

Fisseha Shiferie[1] and Workineh Shibeshi[2]

[1]School of Pharmacy, College of Health Sciences, Mekelle University P.O. Box, 1871, Ethiopia.
[2]School of Pharmacy, College of Health Sciences, Addis Ababa University,
P.O. Box, 1176 Addis Ababa, Ethiopia.

Accepted 20 August, 2013

The root extract of *Echinops kebericho* is used traditionally for the treatment of diarrhea. This study describes the anti-diarrheal and anti-spasmodic activities of the aqueous root extract of *E. kebericho.* The effect of extract in rodents on castor oil-induced intestinal transit, diarrhea and enteropooling was evaluated at doses of 100, 200 and 400 mg/kg body weight. The effects of extract on acetylcholine-induced guinea pig ileum contractions were also evaluated. In the castor oil induced intestinal transit test, *E. kebericho* produced a dose dependent decrease in intestinal propulsion with peristaltic index values of 45.05±3.3, 42.71±2.25 and 33.17±3.3%, respectively at doses of 100, 200 and 400 mg/kg compared with control (63.43±7.3%). In the castor oil-induced diarrhea test, the mean defecation was reduced from 1.81±0.18 to 0.99 ± 0.21 compared with 2.59 ±0.81 for control. The same parameter for 400 mg/kg of extract (0.99±0.21) was comparable to loperamide (0.97 ± 0.21). The extract significantly decreased the volume of intestinal fluid secretion induced by castor oil (2.31±0.1 to 2.01±0.2) compared to control (3.28±0.3). The root extract of *E. kebericho* exhibited a dose dependent spasmolytic effect in acetylcholine-induced guinea pig ileum contraction. In conclusion, the data obtained in this study give scientific support for traditional use of *E. kebericho* as an anti-diarrheal agent.

Key words: Antidiarrheal, *E. kebericho*, enteropooling, spasmplytic, rodent.

INTRODUCTION

Diarrhea is a common gastrointestinal disorder characterized by an increase in stool frequency and a change in stool consistency. It is one of the major health threats to populations in tropical and subtropical poor countries, responsible for about 5 million deaths annually, of which 2.5 million are children of less than 5 years (Prashant 2012, Adeyemi and Akindele, 2008). The epidemiological and experimental studies indicate that acute diarrheal diseases are one of the principal causes of death in the infants, particularly in malnourished and which is of critical importance in developing countries. It thus becomes important to identify and evaluate commonly available natural drugs as alternative to currently used anti-diarrheal drugs which are not completely free from adverse effects (Hajhashemi et al., 2000).

Several medicinal plants are proven to have anti-diarrheal activity (Akindele and Adeyemi, 2006; Adeyemi et al., 2009; Uddin et al., 2005; Kumar et al., 2001; Thakurta et al., 2007; Gilani et al., 2005; Wang et al., 2006; Adzu et al., 2004; Barbosa et al., 2007). The genus Echinops is reported to comprise over 120 species, of which four (*Echinops kebericho* Mesfin*, E. buhaitensis* Mesfin*, E. ellenbeckii* O. Hoffm *and E. longisetus* A. Rich are confined to the Ethiopian highlands specially Showa and Gojjam. "In Ethiopia, E. kebericho known by common name "Kebercho " in Amharic language,

*Corresponding author. E-mail: shibeshiworkineh@gmail.com

is traditionally used for the treatment of diarrhea, stomach ache, typhus, fever and other ailments (Teklehaymanot and Giday, 2007; Bekalo et al., 2007; Wondimu et al., 2007). However, there is no any published report on the anti-diarrheal activity of the roots of Echinops kebericho. Therefore, this study was aimed to evaluate the anti-diarrheal activity of the aqueous root extract of the plant using in vivo and ex vivo experimental models.

MATERIALS AND METHODS

Drugs and Chemicals

Several chemicals used for preparation of Tyrode's solution were obtained from Sigma-Aldrich Inc., Germany. Loperamide and castor oil were obtained from a local retail outlet in Addis Ababa and Acetylcholine was donated by the Ethiopian Health and Nutrition Research Institute. Stock solutions of all the chemicals were made in distilled water and the dilutions were made fresh in normal saline on the day of the experiment.

Experimental Animals

Wistar rats (150–200 gm), Swiss albino mice (20–25 gm) and guinea pigs (500-600 gm) of either sex, obtained from the animal houses of School of pharmacy and Department of Biology, Addis Ababa University were used. The animals were kept under controlled environmental conditions including room temperature (21-22^{o}C), equal light and dark hours (12-hour light, 12-hour dark cycle), relative humidity of 50% had free access to diet for laboratory rodents and tap water. The animals were acclimatized in the laboratory for a minimum period of 10 days prior to experimentation. All experiments were performed after fasting for 24 hours except the acute toxicity test in which the animals were fasted for 12 hours (Shoba and Thomas, 2001). The experiments were carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and this was approved by Research and Ethics committee of School of Pharmacy, Addis Ababa University.

Collection of Plant Material

The fresh roots of *E. kebericho* were collected from Chancho area, northern part of Addis Ababa, Ethiopia in January 2012. Botanical identification and authentication was done by a taxonomist and a voucher specimen, with identification number FS/01, was deposited at the National Herbarium, Department of Biology, College of Natural Sciences, Addis Ababa University.

Preparation of Plant Extract

Two hundred grams (200g) of root of the plant was washed, cleaned, shade dried for a week and was coarsely grounded with mortar and pestle. About 120g of the plant material was then boiled with 1Litre of distilled water in a conical flask for 30 minutes. The decoction was taken and allowed to cool for 30 minutes. Then it was filtered with Whatman No-1filter paper and evaporated to dryness for six hours in an air oven at 45°C (GallenKamp Oven BS Size 1). 42.3g of a dried light yellowish aqueous extract corresponding to an extraction yield of 35.25% was obtained. The extract was reconstituted in distilled water just before it is used on each day of the experiment (Adeyemi et al., 2009).

Acute Toxicity Test

Five female mice fasted (with free access to water) for 12 hours prior to the test were given different doses of *E. kebericho* orally using the limit test dose of 2000 mg/kg body weight. Each animal was then observed individually for any immediate signs of toxicity and mortality for 24 h, with special emphasis given during the first 4 hour and daily thereafter, for a total of 14 days (OECD, 2001).

Grouping and Dosing of Animals

Animals were divided into negative control, positive control and test groups of six animals each. The negative control received distilled water and the positive control received loperamide and the test groups received orally different doses of the aqueous root extract of E. *kebericho* (100, 200 and 400 mg/kg body weight). These doses were selected according to the result obtained from the acute toxicity test study. The extract was found to be safe in mice up to 2000 mg/kg body weight.

Antidiarrheal Activity Testing

The mice were fasted for 24 hours before the test, with free access to water. Then they were divided into five groups of six animals each. Diarrhea was induced by oral administration of 0.5 ml of castor oil. Group I received 10 ml/kg body weight distilled water, group II received loperamide (5 mg/kg body weight), groups III, IV and V received the extract orally (100 mg/kg, 200 mg/kg and 400 mg/kg body weight, respectively) 1hour before administration of 10 mL/kg body weight of castor oil orally. Immediately after castor oil administration, each mouse was kept under a cage, with floor lined with tissue paper, and observed for 4 hours. Then, the onset of diarrhea, consistency of the fecal matter and total weight of diarrheal droppings were measured for a period of 4hours (Yasmeen et al., 2010). The percent inhibition (PI) of the extract against the castor oil-induced diarrhea

Table 1. Effect of the aqueous extract of the roots of *E. kebericho* on castor oil- induced diarrhea in mice.

Dose	Consistency of fecal matter	Onset of Diarrhea (min)	Mean defecation in 4 h (gm)	% reduction
Normal saline (10 ml/kg)	Watery (not formed)	64.67 ± 4.82	2.59 ± 0.81	-
Loperamide (5 mg/kg)	Normal stool	187.33 ± 11.89 [a2]	0.97 ± 0.21 [a2]	62.55
EK100 mg/kg	semisolid	130.5 ± 4.35 [a1]	1.81 ± 0.18 [a1,d1]	30.11
EK200 mg/kg	semisolid	176.67 ± 30.84 [a2]	1.76 ± 0.13 [a1,d1]	32.05
EK400 mg/kg	Normal stool	182.17 ± 10.75 [a2]	0.99±0.21 [a2,b1,c1]	61.78

Values are expressed as mean ± SEM, n=6. [a] = when compared with the negative control, [b] = when compared with EK100, [c] = when compared with EK200, [d] = when compared with loperamide. [1] $p < 0.05$, [2] $p < 0.001$. EK100, EK200, and EK400: Doses of aqueous root extract of *E. kebericho* at 100, 200 and 400 mg/kg body weight, respectively.

Table 2. Effect of aqueous extract of roots of *E. kebericho* on castor oil induced intestinal transit in mice.

Treatment	Distance travelled by charcoal	Total length of intestine	Peristaltic index (%)	% inhibition
Normal saline (10 ml/kg)	41.00 ± 4.6	64.83 ± 0.9	63.43 ± 7.3	-
Loperamide (5 mg/kg)	19.00 ± 2.5 [a3]	65.00 ± 2.1	29.63 ± 4.1 [a3]	53.29
EK100 mg/kg	27.50 ± 1.9 [a1]	61.17 ± 1.0	45.05 ± 3.3 [a1]	28.98
EK200 mg/kg	25.00 ± 1.5 [a2]	58.50 ± 1.3	42.71 ± 2.25 [a1]	32.67
EK400 mg/kg	21.17 ± 1.9 [a3]	64.00 ± 1.4	33.17 ± 3.3 [a3]	47.71

Values are expressed as mean ± SEM, n= 6; [a] = when compared with negative control.
[1] $p < 0.05$, [2] $p < 0.01$, [3] $p < 0.001$.

based on fecal output was calculated using the following formula:

$$\%\ inhibition = (PI\ negative\ control - PI\ test\ drug\ /\ PI\ negative\ control) \times 100$$

Castor oil- induced intestinal transit in mice

The mice were fasted for 24hours and were divided into five groups of six animals each. Group I received 10 ml/kg of distilled water, group II received loperamide (5 mg/kg body weight, PO) and groups III, IV and V received different doses of the plant extract orally (100 mg/kg, 200 mg/kg body weight, and 400 mg/kg body weight, respectively) 1hour before the administration of castor oil. Then one (1) ml of charcoal meal was administered orally, 1hour after the castor oil treatment. The mice were sacrificed after 1hour with an ether anesthesia and the small intestine was immediately isolated. The distance travelled by the charcoal meal from the pylorus to the caecum was measured and the peristaltic index and the percentage of inhibition were calculated by using the following equations (Yasmeen et al., 2010).

$$Peristaltic\ index = (Distance\ moved\ by\ charcoal\ /\ Length\ of\ intestine) \times 100$$

$$\%\ inhibition = (PI\ negative\ control - PI\ test\ drug\ /\ PI\ negative\ control) \times 100$$

Castor Oil- Induced Enteropooling in Rats

The rats were divided into five groups of six animals each and they were fasted for 24 hours, but were allowed free access to water. Group I (control) was orally treated with 10 ml/kg body weight distilled water and group II received loperamide (5 mg/kg body weight, PO) and used as the standard. Groups III, IV and V received the extract orally (100 mg/kg body weight, 200 mg/kg body weight and 400 mg/kg body weight, respectively. Then, 1hour later, 2 ml of castor oil was administered orally to the above groups for induction of diarrhea. Two hours later, the rats were sacrificed, their small intestines were ligated at both the pyloric sphincter and the ileocaecal junction and they were dissected. The small intestines were weighed and the intestinal contents were collected by milking into a graduated tube to measure the volume. The intestines were reweighed and the difference between the full and the empty intestines was calculated (Yasmeen et al., 2010).

Table 3. Effect of aqueous extract of the roots of *E. kebericho* on castor oil induced enteropooling in rats.

Treatment	Volume [ml]	% inhibition of intestinal volume	Weight [gm]	% inhibition of weight of intestinal content
Normal saline (10 ml/kg)	3.28 ± 0.3	-	3.61 ± 0.3	-
Loperamide (5 mg/kg)	1.87 ± 0.2 [a2]	42.99	1.67 ± 0.1[a3]	53.74
EK100 mg/kg	2.31 ± 0.1 [a1]	29.57	2.82 ± 0.5 [a1, d2]	21.88
EK200 mg/kg	2.05 ± 0.2 [a2]	37.5	2.78 ± 0.1 [a1, d2]	22.99
EK400 mg/kg	2.01 ± 0.2 [a2]	38.72	1.79 ± 0.2 [a3, b2, c2]	50.42

Values are expressed as mean ± SEM, n =6. [a] = when compared with negative control, [b] = when compared with EK100, [c] = when compared with EK200, [d] = when compared with loperamide. [1] $p < 0.05$, [2] $p < 0.01$, [3] $p < 0.001$.

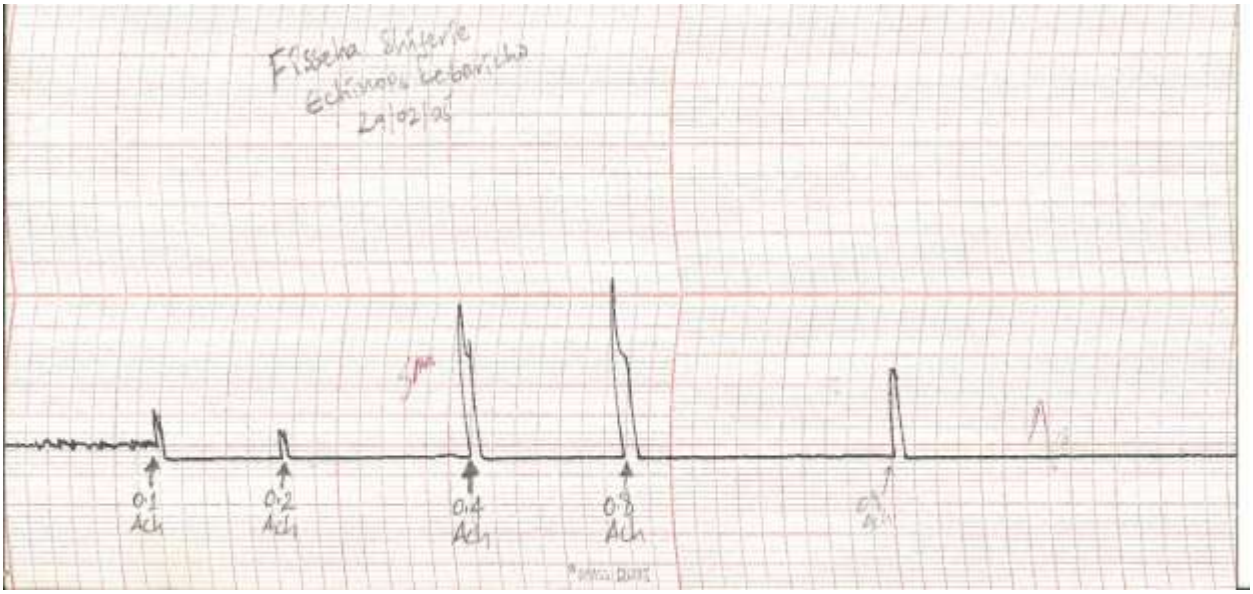

Figure 1. Spasmogenic effect of acetylcholine on isolated guinea-pig ileal preparation.

Ex-vivo Spasmolytic Activity in isolated Guinea-pig Ileum

The isolated tissue experiments were carried out as described previously (Jabeen et al., 2007, Tiwari et al., 2011) with slight modification. Briefly, guinea-pigs were starved for 24 hours with free access to water and were sacrificed using ether anesthesia. The abdomen was cut open and a piece of ileum 10-20 cm long was removed. The segment of ileum about 2 cm long was hanged in a 20 ml tissue bath containing Tyrode's solution, bubbled with a mixture of 95% oxygen and 5% carbon dioxide (carbogen gas) and maintained at 37^{o}C. The composition of Tyrode's solution, in mM, was KCl 2.7, NaCl 136.9, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, Glucose 5.6 and $CaCl_2$, 1.8 (pH 7.4). One end of the segment was attached to the metal tissue hook and the other was attached by a cotton thread to an isotonic transducer and connected to an Oscillograph. A preload of 1 g was applied to each tissue and kept constant throughout the experiment. The tissue was washed several times within 5 minutes interval and was allowed to equilibrate for 30 minutes before isotonic contractions to Ach were recorded. Different doses of acetylcholine (1 µg/ml) were tested and responses to a sub-maximal dose of acetylcholine were recorded. An agonist contact time of 30 second, was used together with a 5 minutes interval between doses. Once the tissue was stabilized with reproducible effects from the doses of the standard, test material was tested. This was done by adding, every 5 minutes, graded doses (10 mg/ml) of the aqueous extract to the tissue bath followed by fixed amount of acetylcholine (0.4 ml) from the final organ bath concentration of acetylcholine (1 µg/ml). The responses of

Table 4. Response of aqueous extract of *Echinops Kebericho (*EK, 10 mg/ml) to acetylcholine (Ach, 1 µg/ml) induced contractions.

Dose of Ach and EK	Height of response (mm)	%inhibition (relaxation)	Response
Ach (0.1 ml)	7	_	Contraction
Ach (0.2 ml)	5	_	Contraction
Ach (0.4 ml)	26	_	Contraction
Ach (0.8 ml)	30	_	Contraction
Ach (0.4 ml) + EK (0.1 ml)	22	15.38	relaxation
Ach (0.4 ml) + EK (0.2 ml)	21	19.23	relaxation
Ach (0.4 ml) + EK (0.4 ml)	20	23.07	relaxation

isolated ileum were recorded on a computerized data processing system through an isotonic force transducer (Ateufack et al., 2010).

Data Analysis

The data were analyzed by using SPSS16.0 for Windows. Results obtained from the study were expressed as mean ± standard error of the mean (SEM). A one-way ANOVA followed by Turkey's post hoc test for multiple comparisons was used to compare results among groups. Differences were considered statistically significant if p values were less than 0.05.

RESULTS

Acute Toxicity Test

Oral administration of the aqueous root extract of *E. kebericho* produced neither significant toxic signs nor death during the observation period of 14 days after a single administration of 2000 mg/kg. The oral LD50 of *E. kebericho* could be, therefore, greater than 2000 mg/kg in mice, which is the single high dose recommended by OECD (2001) guidelines.

Effect of *E. kebericho* on Castor Oil- Induced Diarrhea in Mice

The effect of aqueous root extract of *E. kebericho* on castor oil-induced diarrhea in mice is shown in Table 1. Diarrhea was apparent in all the animals of control group, 1 hour after the administration of castor oil. This was largely eliminated by the oral administration of 5 mg/kg (62.55% protection) loperamide, a standard anti-diarrheal agent, and comparable effect was produced by the highest dose (400 mg/kg body weight) of the *E. kebericho* (61.78%). In respect of onset of copious diarrhea, aqueous root extract of *E. kebericho* produced a significant and dose dependent delay compared to the control group. Peak effect was produced by the extract at dose of 400 mg/kg. At this dose, the onset of diarrhea was increased from 64.67 ± 4.82 minutes (as observed in the control group,) to 182.17 ± 10.75 minutes, a value not significantly different from that elicited by loperamide (187.33 ± 11.89 minutes).

Aqueous root extract of *E. kebericho* also produced a dose dependent and significant reduction in the total weight of fecal droppings relative to the control group that is mice received neither extract, nor loperamide, but castor oil only. There was significant difference in the total weight of stool between the control group (2.59 ± 0.81) and the group which received 400 mg/kg body weight of extract (0.99 ± 0.21). A statistically significant difference was not observed between the loperamide treated group and the peak dose of the extract (400 mg/kg body weight. However, a statistically significant difference was observed between loperamide and the first two doses of the extract, EK100 and EK200 ($p < 0.05$) regarding the total weight of stool. The consistency of fecal matter was also changed especially in mice treated with EK 400mg/kg which was comparable to the group treated with loperamide.

Effect of *E. kebericho* on Castor Oil- Induced Intestinal Transit in Mice

As shown in Table 2, the aqueous root extract of E. kebericho decreased the propulsion of the charcoal meal through the gastrointestinal tract significantly as compared to the control group. Loperamide (5 mg/kg body weight) produced a marked decrease in the propulsive movement (29.63 ± 4.1) and the intestinal length travelled by charcoal (19.00 ± 2.5). The oral administration of the extract (100, 200 and 400 mg/kg body weight), produced a significant and dose dependent reduction in both parameters (distance moved by charcoal and peristaltic index) compared to the control. However, there was no any statistically significant reduction of both parameters among the doses of the extract. The value for % inhibition of the treatment EK400 mg/kg body weight (47.71%) was significantly higher than the treatments with EK 200 and EK100 mg/kg.

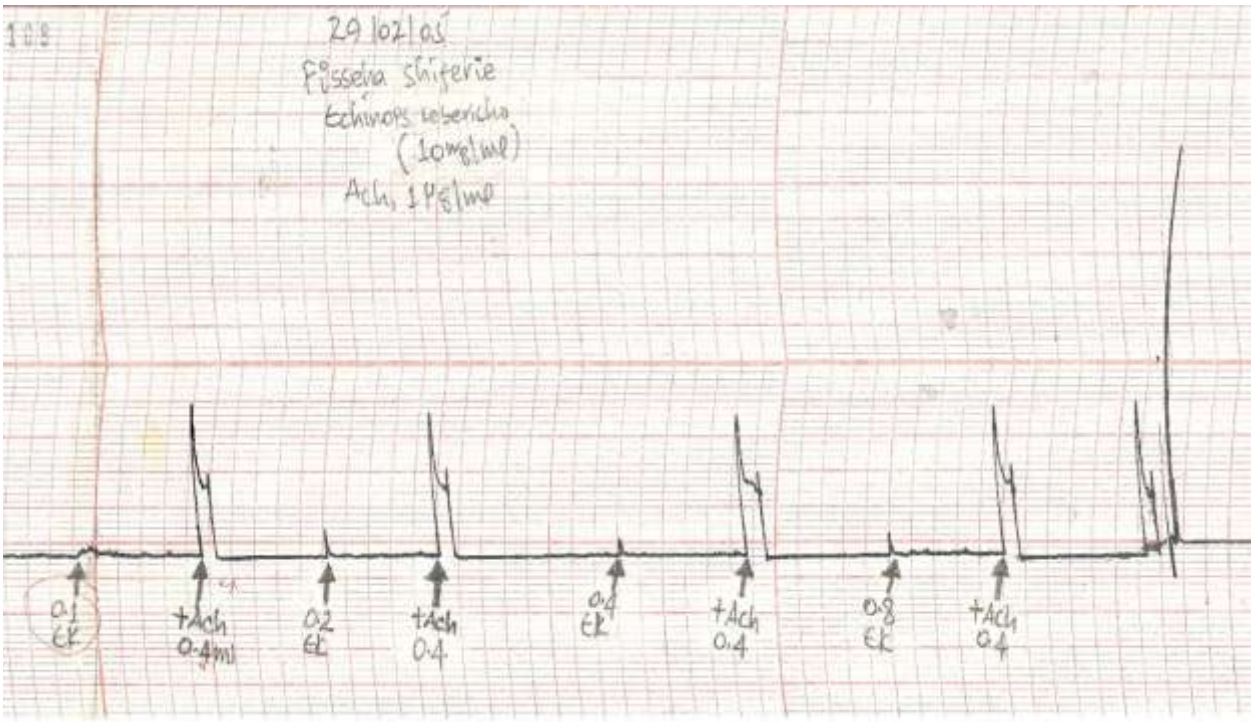

Figure 2. Spasmolytic effect of *E. kebericho* on acetylcholine -induced isolated guinea-pig ileum preparation.

Effect of *E. kebericho* on Castor Oil- Induced Enteropooling in Rats

The castor oil caused the accumulation of water and electrolytes in the intestinal loop. Treatment with the extract (100, 200 and 400 mg/kg body weight), produced a significant and dose dependent reduction in the intestinal weight and volume when compared to the control (Table 3). Significant result ($p < 0.001$) was observed with dose of EK400. The inhibition of both parameters (weight and volume) by the highest dose of the extract (EK400) was comparable to that obtained by the standard drug, loperamide. However, the inhibition of the weight of the intestinal content by loperamide showed a statistically significant ($p< 0.01$) difference from the extract doses of EK100 and EK200. Moreover, EK400 showed a statistically significant difference ($p < 0.01$) in the reduction of the weight of intestinal content from the remaining doses of the extract.

Effect of *E. kebericho* on Acetylcholine-Induced Contraction of Isolated Guinea-pig Ileum

Acetylcholine at micromolar concentration caused a concentration-dependent contraction of tissue, reaching its maximum within 30 s of contact (Figure 1). When tested on a guinea-pig ileum, *E. kebericho* exhibited a spasmolytic effect at the dose range of 0.1 ml to 0.4 ml and with declining effect at higher dose (Table 4, Figure 2).

DISCUSSION

The present study was aimed to provide the pharmacological basis for the medicinal use of root extract of *E. kebericho* in diarrhea using *in vivo* and ex vivo assays. Castor oil, a very effective laxative, is hydrolyzed in the upper small intestine to ricinoleic acid which can stimulate fluid secretion, inhibit water and electrolyte absorption, reduce active Na^+ and K^+ absorption, and decrease Na^+, K^+-ATPase in the small intestine and colon. This is brought about by the irritant effect of ricinoleic acid liberated by pancreatic lipases, which hydrolyse the oil derived from the seeds of *ricinus communis* (Mbagwu and Adeyemi, 2008).

Furthermore, ricinoleic acid can also lead to the release of endogenous prostaglandins which play an important role in the modulation of gastrointestinal tract, stimulate motility and secretion, and cause diarrhea (Hu et al., 2009).

Prostaglandins are implicated in the pathophysiology of diarrhea (Agunu et al., 2005). The molecular mechanism by which ricinoleic acid acts is linked to EP(3) prostanoid receptor as recently revealed (Tunaru et al., 2012). The EP(3) prostanoid receptor is specifically activated by ricinoleic acid which mediates the pharmacological effects of castor oil. The castor oil metabolite ricinoleic acid activates intestinal and uterine smooth-muscle cells via EP(3) prostanoid receptors. These findings identify the cellular and molecular mechanism underlying the

pharmacological effects of castor oil and indicate a role of the EP(3) receptor as a target to induce laxative effect.

The antidiarrheal activity of the aqueous root extract of *E. kebericho* may be due to various mechanisms: The liberation of ricinoleic acid by castor oil results in the irritation and the inflammation of the intestinal mucosa, thus leading to the release of prostaglandins. The extract may reduce prostaglandin secretion. Extracts of plants that contain flavonoids and alkaloids are known to modify the production of cyclo-oxygenase

1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) thereby inhibiting prostaglandin and autacoids production (Agunu et al., 2005; Yasmeen et al., 2010). Flavonoids are a large group of polyphenolic compounds and have been reported to exhibit a wide variety of biological effects such as antioxidant, anti-inflammatory, antispasmodic, and antidiarrheal activities (Hu et al ., 2009). The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions (Meite et al., 2009).

Hypermotility characterizes forms of diarrhea where the secretory component is not the causative factor. Pretreatment with the aqueous root extract of *E. kebericho* suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which clearly indicates that the root extract may be capable of reducing the frequency of stools in diarrheal conditions. Delay in gastric motility causes further absorption of water from feces and may additionally contribute to reducing its watery texture (Ezenwali et al., 2010).

In the test on enteropooling, the plant extract significantly reduced the volume of intestinal content. This effect can be attributed to either a decrease in mucosal secretion or increase in mucosal absorption of water and electrolytes (Adeyemi et al., 2009). Since electrolyte absorption determines the efficiency of nutrient absorption, it is likely that the enhanced electrolyte absorption by the extract may have encouraged the absorption of other intestinal contents. The solute absorption in any region of the intestine is a function of the rate of water uptake in that region. Thus, the extract enhanced solute absorption may have created an osmotic gradient across enterocytes which stimulated water absorption. These observations reasonably suggest that the extract inhibits gastrointestinal hyper-secretion and enteropooling by enhancing electrolytes, solutes and water absorption from the intestinal lumen (Ezenwali et al., 2010).

The protective effect of the crude extract of *E. kebericho* against the castor oil-induced diarrhea in mice, similar to loperamide, suggests that it has either an inhibitory effect on contraction or on electrolyte out flux. To see its possible inhibitory effect on gut motility, the extract was further studied in the in-vitro experiments. It is proved that *E. kebericho* blocked the spasmogenic effect of acetylcholine which might be due to its inhibitory activity at any step in the contraction cascade described elsewhere. It was previously observed that the spasmolytic constituents present in different plants usually mediate their effects through calcium channel blockade (Gilani et al., 2005). Therefore, the spasmolytic effect of aqueous root extract of *E. kebericho* with its various constituents might be ascribed to its ability to block these channels.

CONCLUSION

In conclusion, the present study supports the claims made by traditional medical practitioners about the use of the aqueous extract of *E. kebericho* in the treatment of diarrhea due to its inhibitory action on gastrointestinal propulsion, fluid secretion and its spasmolytic effect. This justifies the use of a decoction of the plant for the treatment of diarrhea as folk medicine. Present investigation suggests that *E. kebericho root* extract possesses antidiarrheal and spasmolytic activities.

ACKNOWLEDGMENT

The study was supported by Office of Vice president for Research and Technology Transfer of Addis Ababa University.

REFERENCES

Adeyemi OO, Akindele AJ (2008). Antidiarrheal activity of the ethyl acetate extract of Baphia nitida. J. Ethnopharmacol. 116: pp. 407–412.

Adeyemi OO, Akindele AJ, Ogunleye EA (2009). Evaluation of the antidiarrheal effect of Sanseviera liberica Gerome and Labroy (Agavaceae) root extract. J. Ethnopharmacol. 123: pp. 459–463.

Adzu B, Tarfa F, Amos S, Gamaniel KS (2004). The efficacy of Sphaeranthus senegalensis Vaill extract against diarrhea in rats. J. Ethnopharmacol. 95: pp.173–176.

Agunu A, Yusuf S, Andrew OG, Zezi UA, Abdurahman ME (2005). Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. J. Ethnopharmacol. 101: 27–30.

Akindele AJ, Adeyemi OO (2006). Evaluation of the antidiarrheal activity of Byrsocarpus coccineus. J. Ethnopharmacol. 108: pp. 20–25.

Ateufack G, Nguelefack BT, Tane P, Kamanyi A (2010). Spasmogenic activity of the aqueous and methanol extracts of the stem bark of anthocleista vogelii planch (Loganiaceae) in rats. Pharmacology online. 1: pp.86-101.

Barbosa E, Calzada F, Campos R (2007). In vivo antigiardial activity of three flavonoids isolated of some medicinal plants used in Mexican traditional medicine for the treatment of diarrhea. J. Ethnopharmacol. 109:

pp.552–554.
Bekalo TH, Woodmatas SD, Woldemariam ZA (2009). An ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern nations, nationalities and peoples regional state, Ethiopia. J. Ethnobiol Ethnomed. 5: 26 doi: 10.1186/1746-4269-5-26
Ezenwali MO, Njoku OU, Okoli CO (2010). Studies on the anti-diarrheal properties of seed extract of Monodora tenuifolia. Int. J. Appl. Res. Nat. Prod. 2(4): pp.20-26.
Gilani HA, Bashir S, Janbaz HK, Khan A (2005). Pharmacological basis for the use of Fumaria indica in constipation and diarrhea. J. Ethnopharmacol. 96: pp.585–589.
Hajhashemi V, Sadraei H, Ghannadi RA, Mohseni M (2000). Antispasmodic and anti-diarrheal effect of Satureja hortensis L. essential oil. J. Ethnopharmacol. 71: pp.187–192.
Hu J, Gao W, Ling N, Liu C (2009). Antidiarrheal and intestinal modulatory activities of Wei-Chang-An-Wan extract. J. Ethnopharmacol. 125: pp.450–455.
Jabeen Q, Aziz N, Afaz Z, Gilani HA (2007). The spasmogenic and spasmolytic activities of Lavandula stoechas are mediated through muscarinic receptor stimulation and calcium channel blockade. Int. J. Pharmacol. 3 (1):pp. 61-67.
Kumar S, Dewan S, Sangraula H, Kumar VL (2001). Anti-diarrheal activity of the latex of Calotropis procera. J. Ethnopharmacol. 76: pp.115–118.
Mbagwu HC, Adeyemi OO (2008). Anti-diarrheal activity of the aqueous extract of Mezoneuron benthamianum Baill (Caesalpiniaceae). J. Ethnopharmacol.116:pp.16-20.
Meite S, N'guessan DJ, Bahi C, Yapi HF, Djaman AJ, Guina FG (2009).Antidiarrheal activity of the ethyl acetate extract of Morinda morindoides in rats. Trop. J. Pharm. Res. 8 (3): pp. 201-207.
National Research Count National Research Council (1996). Guide for the Care and Use of Laboratory animals. The National Academies Press, Washington, DC.
Organization of Economic Co-operation and Development (2001). The OECD guidelines for testing of chemical: 423 acute oral toxicity, France.
Prashant BS (2012). Significance of Black pepper in Ayurvedic antidiarrhoeal formulation. J. Chem Pharm Res. 4,(4): pp. 2162-2166.
Shoba GF, Thomas M (2001). Study of antidiarrheal activity of four medicinal plants in castor-oil induced diarrhea. J. Ethnopharmacol. 76: pp.73–76.
Teklehaymanot T, Giday M (2007). Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. J Ethnobiol Ethnomed. 3: p.12
Thakurta P, Bhowmik P, Mukherjee S, Hajra KT, Patra A, Bag KP (2007). Antibacterial, antisecretory and antihemorrhagic activity of Azadirachta indica used to treat cholera and diarrhea in India. J. Ethnopharmacol. 111: pp. 607–612.
Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H, Dayal R (2011). Spasmolytic, antidiarrheal and Intestinal modulatory activities of ethanolic extract of stem of Tinospora cordifolia on isolated rat Ileum. Pharmasciencia. 1,(1): pp.123-131.
Tunaru S, Althoff TF, Nüsing RM, Diener M, Offermanns S (2012). Castor oil induces laxation and uterus contraction via ricinoleic acid activating prostaglandin EP3 receptors. PN AS. 109(23):9179-84. doi: 10.1073/pnas.1201627109.
Uddin SJ, Shilpi JA, Alam SMS, Alamgir M, Rahman MT, Sarker SD (2005).Antidiarrheal activity of the methanol extract of the barks of Xylocarpus moluccensis. J. Ethnopharmacol. 101: pp.139–143.
Wang H, Tan C, Bai X, Du Y, Lin B (2006). Pharmacological studies of antidiarrheal activity of Gentianopsis paludosa. J. Ethnopharmacol. 105: pp.114–117.
Wondimu T, Asfaw A, Kelbessa E (2007) Ethnobotanical study of medicinal plants around 'Dheeraa' town, Arsi Zone, Etthiopia. J Ethnobiol Ethnomed. 112: pp.152-161.
Yasmeen M, Prabhu B, Agashikar NV (2010). Evaluation of the antidiarrheal activity of the leaves of Ixora coccinea Linn in rats. J. Clin Diagn Res. 4: 3298-3303

.