Available online at www.scholarsresearchlibrary.com

Scholars research library

Archives of Applied Science Research, 2011, 3 (5):122-130
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Modulatory effect of ethanol stem bark extract of *Burkea africana* on castrol oil induced diarrhoeal on experimental animals

Tanko, Y [1*] Iliya B[1] , A, Mohammed[1], Mahdi M.A[2] and K.Y.Musa[3]

[1]*Department of Human Physiology, Ahmadu Bello University, Zaria. Nigeria*
[2]*Department of Pharmacology and therapeutics Ahmadu Bello University, Zaria, Nigeria*
[3] *Department of Pharmacognosy and drug design, Ahmadu Bello University, Zaria, Nigeria*

ABSTRACT

This study was aimed at investigating the ethanol stem bark extract of Burkea africana for antidiarrhoeal activity using castor oil induced diarrhoea in mice. The effect of the extract on perfused isolated rabbit jejunum was also evaluated. The ethanol extract produced a dependent relaxation of the rabbit jejunum. The extract protected the mice against castrol oil induced diarrheoa and it was not dose-dependent with the highest protection 60% with the two doses 20 and 40 mg/Kg body weight, while the highest dose 80 mg/kg bodyweight was 40% protection. The acute toxicity test revealed the median lethal dose LD_{50} values for the extract was found to be 2154mg/kg. Preliminary phytochemical screening of the extract revealed the presences of flavonoid cardiac glycosides, tannins and triterpenes. In conclusion the results obtained revealed that the stem back extract possess pharmacological activity against diarrhoea and may possibly explain the use of the plant in traditional medicine.

Key words: , *Burkea africana* Ethanol Diarrhoea, Rabbit jejunum, Castro oil.

INTRODUCTION

The principal cause of diarrhea stems from ingestion of unsafe drinking water (typically from admixture of raw sewage to water supplies); the occurrence is predominantly in lesser developed countries [1] Diarrhea is most commonly due to viral gastroenteritis with rotavirus, which accounts for 40% of cases in children under five [2]. In travelers however bacterial infections predominate. Various toxins such as mushroom poisoning and drugs can also cause acute diarrhea [3]. Chronic diarrhea can be the part of the presentations of a number of chronic medical

conditions affecting the intestine. Common causes include ulcerative colitis, Crohn's disease, microscopic colitis, celiac disease, irritable bowel syndrome and bile acid malabsorption. In sanitary living conditions and with ample food and water available, an otherwise healthy patient typically recovers from the common viral infections in a few days and at most a week. However, for ill or malnourished individuals diarrhea can lead to severe dehydration and can become life-threatening without treatment [4].

Burkea Africana is a deciduous, medium-sized, spreading, flat-topped tree belonging to the family *Caesalpiniaceae*. It can grow up to 20m tall and up to 80m in diameter [5]. The name *Burkea* is derived from the surname of the famous collector Joseph Burke who collected plants in the Magaliesberg area during the 1840s; *africana* refers to the continent Africa, where it is widely distributed. *Burkea* is a monotypic genus (comprises only one species[6] . The bark surface is scaly and fissured, leaves alternate and clustered near the end of twigs, bipinately compound. The fruit is an elliptical, strongly flattened pod. Seeds are ellipsoid, flattened with cavity at both sides. It is Widespread in tropical Africa, it is found in Chad, Sudan, Tanzania, Uganda, Cameroon, Central African Republic, Zaire, Benin, Burkina Faso, Côte d'Ivoire, Ghana, Guinea, Mali, Niger, Nigeria, Senegal, Togo, Angola, Malawi, Mozambique, Zambia, Zimbabwe, Botswana, Namibia, South Africa in the Transvaal. *Burkea africana* has a reputation for being difficult to cultivate, the seeds may take up to six months to germinate. It is a common and characteristic tree of sandy soils in dry deciduous bush veld and woodlands [7].

The gum from the bark is edible; it is locally considered an aphrodisiac. *Burkea africana* is planted as a roadside tree and ornamental[8]. It is host to caterpillars of Saturnid moths (*Cirina forda* and *Rohaniella pygmaea*), which are collected by people as food; after boiling and frying, these are considered a delicacy. The flowers produce nectar collected by honeybees[9]

The main aim of this research is to evaluate the modulatory effect of *Burkea africana* stem bark extract on Castrol oil induced diarrhea and isolated tissue.

MATERIALS AND METHODS

Collection and identification of plant material

The fresh stem bark of the *Burkea africana* was collected in Zaria, Kaduna state, Nigeria on the 22nd June, 2011. The plant was identified and authenticated by Mallam Musa and Umar Gallah of the herbarium section in the Department of Biological Sciences, Ahmadu Bello University Zaria, where a voucher specimen (No.1996) was deposited at the herbarium for future reference.

Animals

A rabbit (1 kg) and 37 Swiss albino mice weighed between 22- 25 g were used for the study The animals were maintained in the Animal House Facility of the Department of Human physiology , Ahmadu Bello University, Zaria, Nigeria . The animals were fed on standard small animal feeds (Excel feed, Ilorin, Nigeria) and water *ad libitum*. This research was carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally.

Drugs
All chemicals and drugs used were of analytical grade.

Preliminary phytochemical screening
The extract was subjected to preliminary phytochemical analysis using standard protocol [10].

Acute toxicity study
The method described by [11] was adopted. Briefly, 12 mice were used for each extract. In the first phase, three doses of the methanol leaves extract (10, 100 and 1000 mg/kg were administered to three groups each containing three mice). In the second phase, more specific doses 1600, 2900 and 5000mg/kg were administered to four groups each containing one mouse. The median Lethal dose (LD50) was determined as the geometric mean of the highest non lethal dose and the lowest lethal dose of which there is 1/1 and 0/1 survival.

Experimental design
Effects on isolated rabbit jejunum
The rabbit was sacrificed by cervical dislocation. Segments of the jejunums, about 3.0 cm long were removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode solution of the following compositions in millimoles (mM): NaCl, 136.8; KCl, 2.7; CaCl, 1.3; NaHCO3, 12.0; MgCl, 0.5; NaPO4, 0.14; glucose, 5.5. The tissue was mounted in a 25 ml organ bath containing Tyrode solution maintained at 35oC and aerated with air. An initial tension of 0.5 g was applied to the segments and 60 min equilibration period was allowed while the physiological solution was changed every 15 min. At the end of the equilibration period, the effect of acetylcholine, adrenaline and methanol extract of leaves was investigated. The contact time for each concentration was 1 min which was followed by washing three times. The tissue was allowed a resting period of 15 min before the next addition.

Effects of castor oil-induced diarrhoea on mice
The mice were fasted for 12 h prior to the commencement of the study and were randomly divided into five groups each containing five mice. The grouping is as follows:
Group 1-Treated with normal saline 10ml/kg bodyweight
Group 2 Treated with Loperamide 5mg/kg bodyweight
Group 3 Treated with 20mg/kg bodyweight of extract
Group 4 Treated with 40mg/kg bodyweight of extract
Group 5 Treated with 80mg/kg bodyweight of extract. After 30 min post treatment, castor oil (0.2 ml / mouse) was administered to all the groups. All treatments were given intragastrically.

The animals were then placed in individual cages on a clean Whatmann filter paper. Four hours after the castor oil administration, the cages were inspected for the presence of characteristic diarrhoeal droppings; absence of which was regarded as protection [12].

Statistical analysis
The results was analyzed by determine the percentage protection against diarrhea versus the control.

RESULTS

Acute toxicity studies

The acute toxicity studies, phase 1 shows no lethal dose for 10, 100, and 1000mg/kg of stem bark extract of *Burkea africana*. While in the phase 2, 1600, 2900 and 5000mg/kg were administered. 1600mg/kg had no lethal dose but 2900 and 5000mg/kg bodyweight both had lethal doses where the mice in each group died. The sign of toxicity were first noticed after 8-10 hours of extract administration. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake, and prostration after 18 hours of extract administration. The lowest lethal dose is 2900mg/kg and the highest non-lethal dose is 1600mg/kg, thus the median lethal dose (LD_{50}) in mice was calculated to be 2,154 mg/kg body weight.

Table 1 : Phytochemical constituents of the ethanol stem bark extract of *Burkea Africana*

Phytochemical constituents	stem-bark
Flavonoids	+
Saponins	+
Carbohydrate	_
Cardiac glycosides	+
Tannins	+
Triterpenes	+
Anthraguinone	_

Keys= + Presence - Absent

Table 2: Effect of ethanol stem bark extract of *Burkea africana.* on castor oil induced diarrhoea in mice

Treatment	Dose (mg/kg)	No. of mice with diarrhoea	Protection (%)
Normal saline	10	5/5	0
Loperamide	5	0/5	100
Burkea africana	20	3/5	40
Burkea africana	40	3/5	40
Burkea africana	80	2/5	60

Mice administered with 20, 40 and 80mg/kg of stem bark extract of *Burkea africana* .There was protection against diarrhoea with the three doses administered 3/5, 3/5 and 2/5 respectively that is 40, 40 and 60% protection respectively.

The mice administered with loperamide, 5mg/kg as positive control after castor oil-induced diarrhea show no sign of diarrhea (100% protection) while the negative control normal saline group had diarrhea.

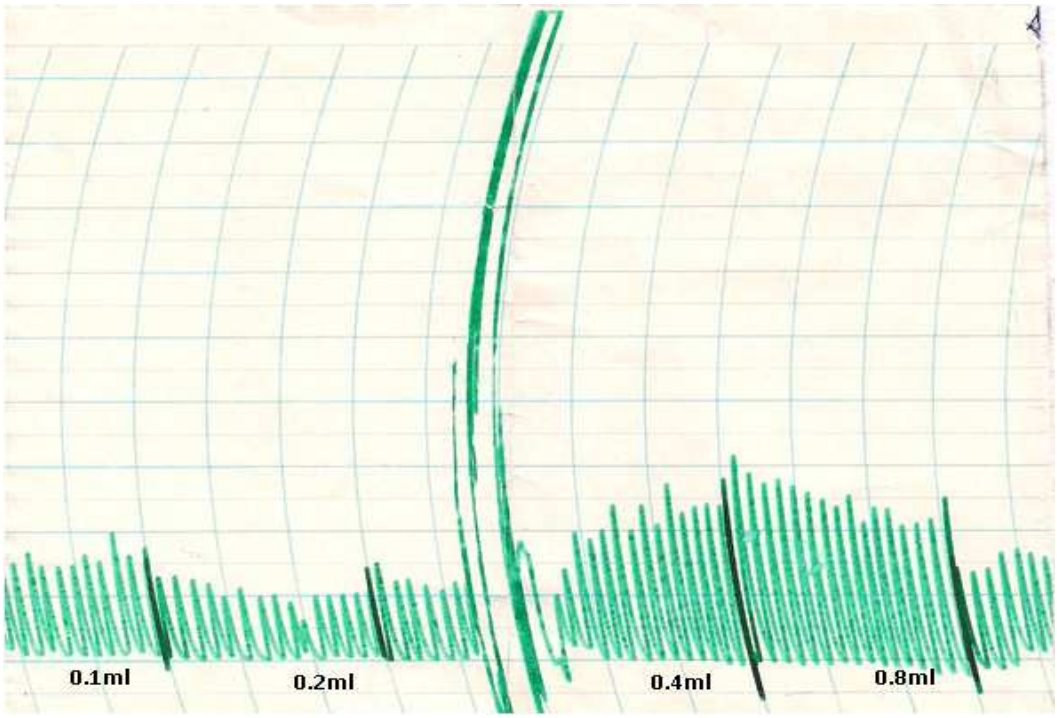
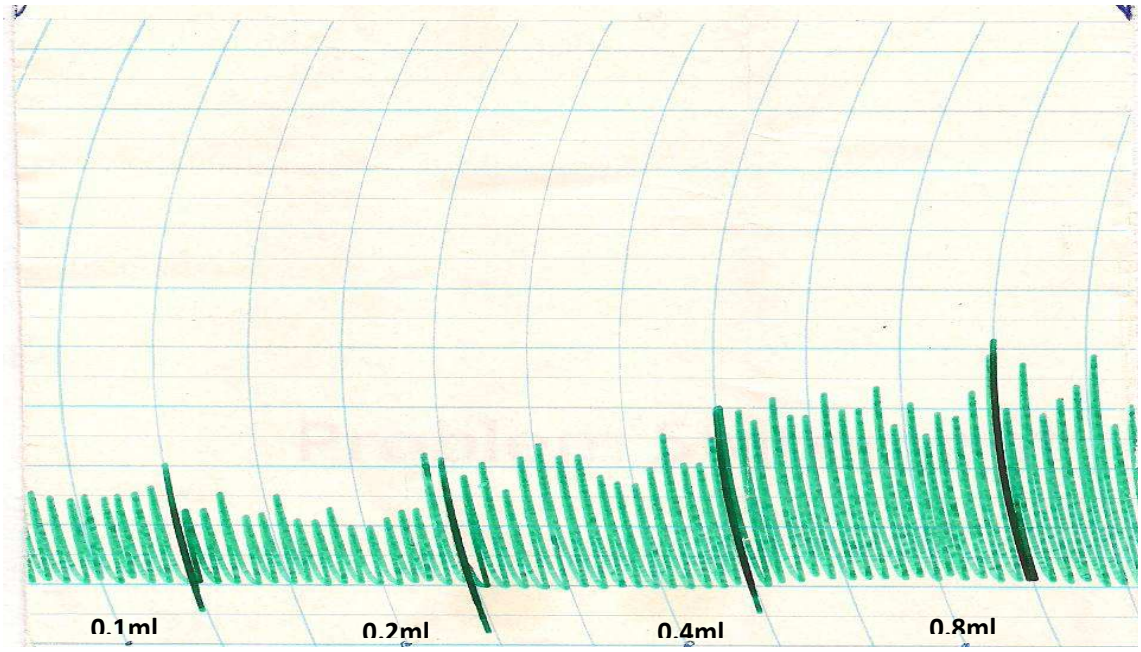


Figure 1: Effect of ethanol stem bark extract of *Burkea africana* (20mg/ml) on isolated rabbit jejunum

Administration of different volumes of 20 mg/ml of the extract on rabbit jejunum there was spontaneous relaxation followed by contraction of the jejunum

Figure 2: Effect of ethanol stem bark extract of *Burkea africana* (40mg/ml) on isolated rabbit jejunum



Administration of different volumes of 40 mg/ml of the extract on rabbit jejunum there was spontaneous relaxation followed by contraction of the jejunum

Figure 3: Effect of ethanol stem bark extract of *Burkea africana* (80mg/ml) on isolated rabbit jejunum

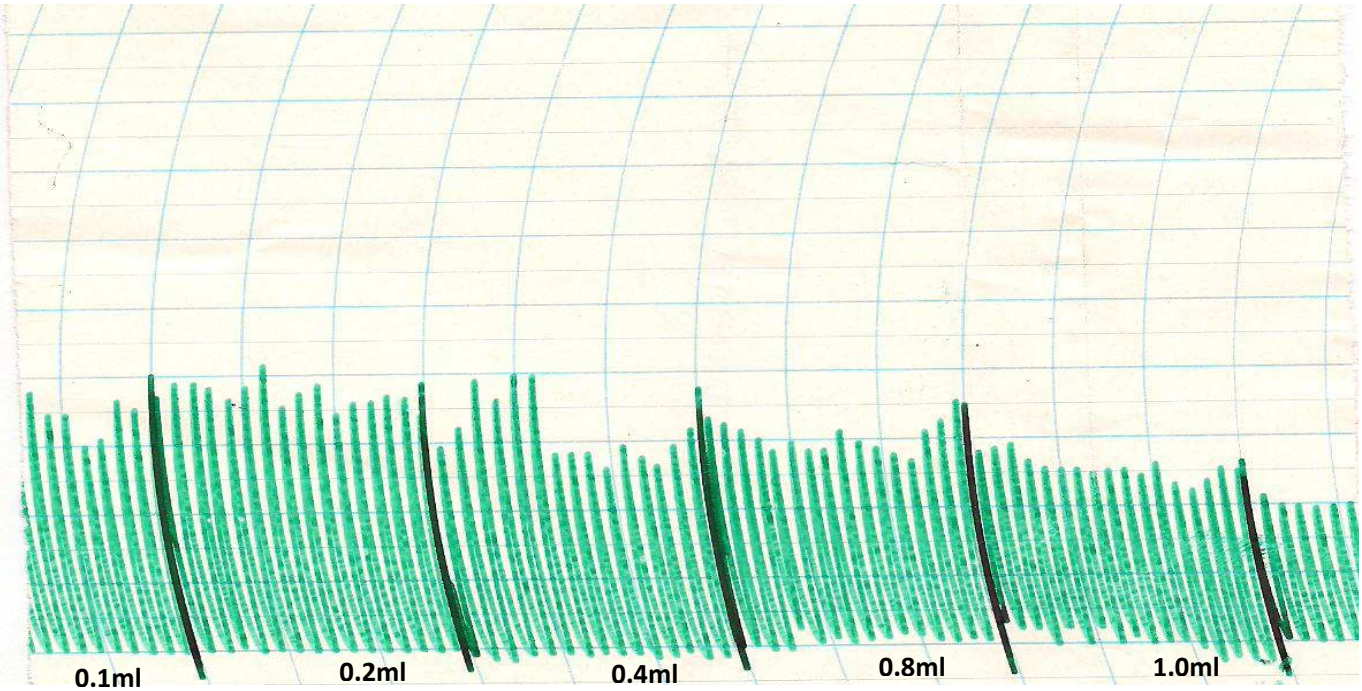
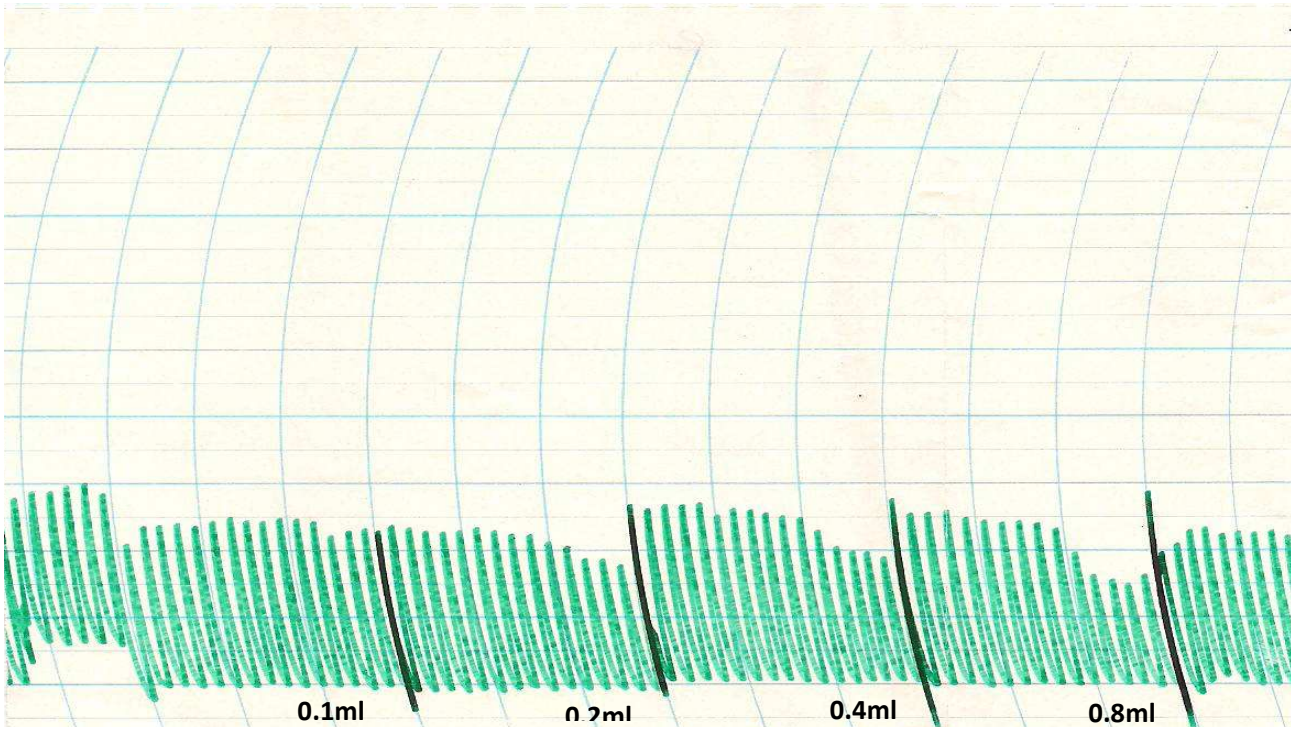


Figure 4: Effect of ethanol stem bark extract of *Burkea africana* (100mg/ml) on isolated rabbit ileum



Administration of different volumes of 100 mg/ml of the extract on rabbit jejunum there was spontaneous contraction of the jejunum

Figure 5: Effect of Acetylcholine (5µg/ml) on isolated rabbit jejunum

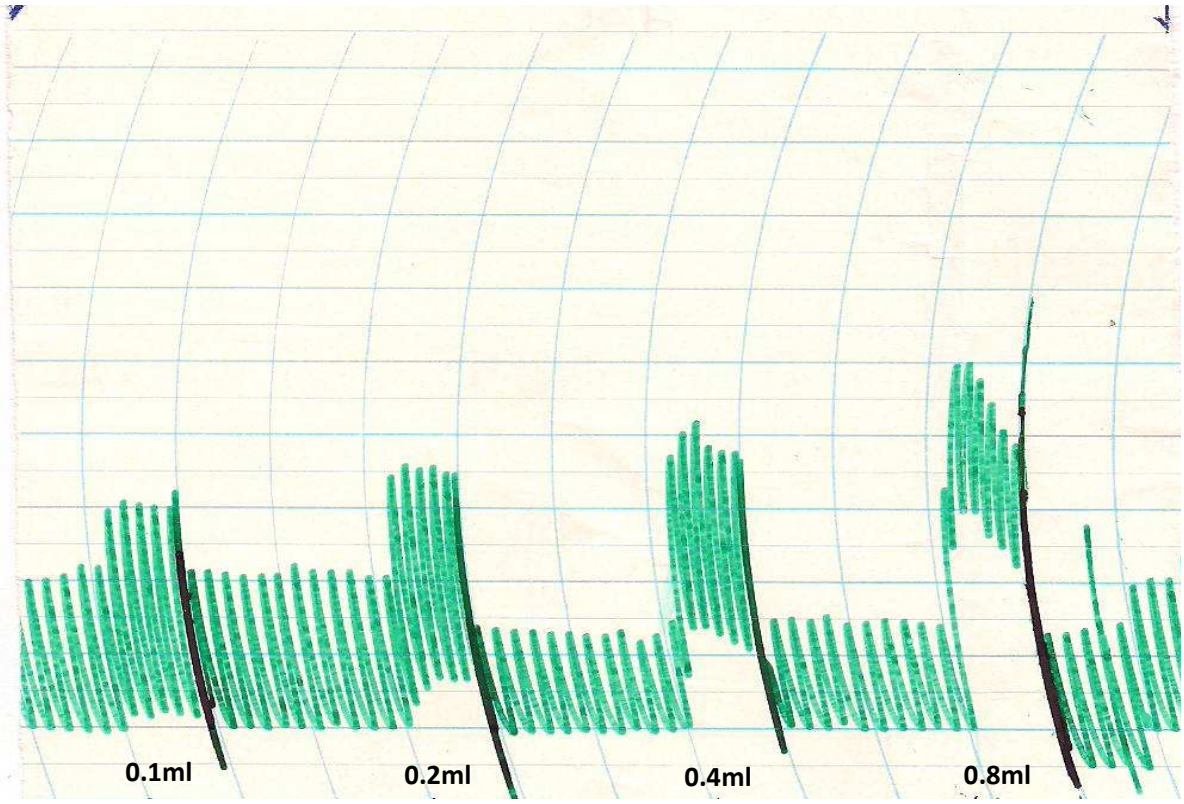
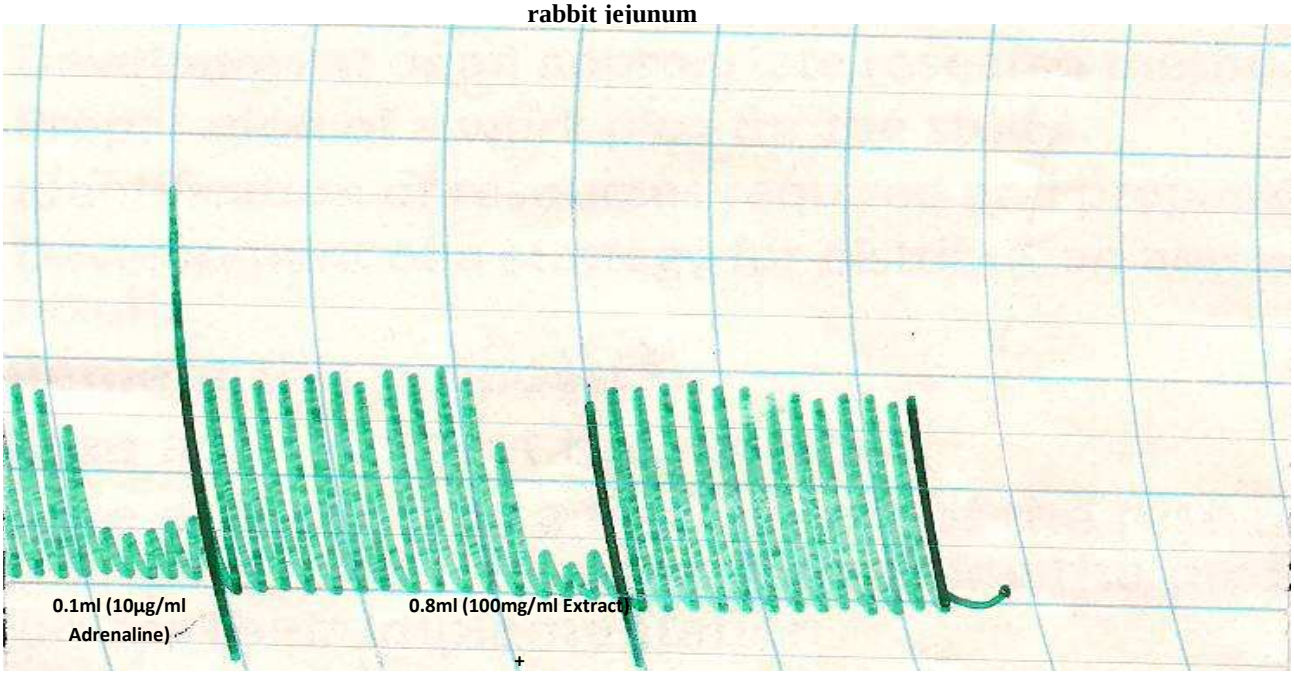


Figure 6: Effect of Adrenaline (10µg/ml) and ethanol extract of *Burkea africana* (100mg/ml) on isolated rabbit jejunum



Drug and extract interactions on the rabbit jejunum. There was relaxation of the tissue.

DISCUSSION

Castor oil stimulates peristaltic activity in the small intestine, leading to changes in the intestinal mucosa. Its action also stimulates the release of prostaglandins.

Castor oil is a triglyceride in which approximately 90% of the fatty acid chains are ricinonleic acid. Oleic and linoleic acids are the other significant components. Castor oil causes diarrhea due to its active metabolite, ribonucleic acid [13, 14] Ricinoleic acid, a monosaturated 18-carbon fatty acid is unusual in that it has a hydroxyl functional group on the 12^{th} carbon. This functional group causes ricinoleic acid (castor oil) to be unusually polar [15].

Burkea africana significantly reduced the intestinal transit time as observed by the decrease in intestinal motility of the isolated rabbit jejunum. The median lethal dose of the extract was 2154mg/kg. Phytochemical screening revealed the presence of flavonoids, tanins, saponins, cardiac glycosides .anthraquinone and triterpenes. Hence, tannins and triterpenes may be responsible for the mechanism of action of *Burkea africana* anti diarrheal activity[16,17].

The anti diarrheal activity of the extract may also be due to the presence of denatured proteins which form protein tannates. protein tannates makes the mucosa more resistant and hence, reduce secretion[18] This can be due the fact that the extract increased the reabsorption of water by decreasing intestinal motility in the isolated rabbit jejunum. Loperamide, apart from regulating the gastrointestinal tract is also reported to slow down transit in the small intestine, reduce colon flow rate and consequently any effect on colonic motility [19]. The mice in the control group were given 10ml/kg bodyweight normal saline and all the mice in the group had stool with diarrhea which indicate there was no protection. While the mice that were given 5mg/kg bodyweight loperamide as positive control after castor oil-induced diarrhea showed no sign of diarrhea indicating 100% protection against Castrol oil induced diarrhea. In relation to the extract treated groups there was 60% protection against diarrhoea in the groups treated with the doses of 20 and 40 mg/kg body weight. Also as regards to the group given the highest dose that is 80 mg/kg body weight there as 20 % protection against Castrol oil induced diarrhea.

In this experiment, the ethanol extract of *Burkea africana* exhibited a significant anti diarrheal activity. Its effect depended on the dose. Based on this observation, it is plausible to suggest that the anti diarrheal effect of the extract may be due to the inhibition of prostaglandin biosynthesis. Flavonoids are known to modify the production of cyclooxygenase 1 and 2 (COX-1, COX-2) and lipooxygenase(LOX)18. Certain flavonoids inhibit inflammatory processes by inhibiting key enzymes involved in the synthesis of prostaglandins processes.

The effects of the plant extract on the rabbit jejunum were dose related. The leaves extract relaxed the spontaneous contraction of the rabbit

REFERENCES

[1] Dryden, D. The wild seringa, *Burkea africana*. Veld & Flora 1993: **1996** 107
[2] W.H.O. The World Health Organization definition, uses and popularity of traditional Medicine. **2009**
[3] Wilson, M.E. "Diarrhea in nontravelers: risk and etiology". *Clinical. Infectious. Diseases*. **2008** 41 Suppl 8 (Supplement 8): S541–6.
[4] Greenberg, H.B., Estes,M.K."Rotaviruses: from pathogenesis to vaccination". *Gastroenterology* **2009** 136 (6): 1939–51.
[5] Uhnoo, I., Svensson, L., Wadell, G. "Enteric adenoviruses". *Baillière's Clinical Gastroenterology* **1990** 4 (3): 627–42.
[6] Van Wyk, A.E.(Braam) & Van Wyk, P. *Field guide to trees of southern Africa*. **1997** Struik, Cape Town.
[7] Wilson, B.G. & Witkowski, E.T.F. Seed banks, bark thickness and change in age and size structure (1978–1999) of the African savanna tree *Burkea africana*. *Plant Ecology* **2003:**167(1): 151–162.
[8] Christopher S., William A., Dubois R.N. Prostaglandin endoperoxide synthase. Why two isomers? *American Journal of Physiology*,**1996** 270:G392-G400
[9] Dans L, Martínez E . "Amoebic dysentery". *Clinical Evidence* June **2006** (15): 1007–13..
[10] Silva GL, Lee I, Kinghorn AD . Special problems with the extraction of plants. In:
[11] Cannell RJP (Ed) Methods in Biotechnology (Natural product Isolation). Humana press, New Jersey, **1998** pp. 245-364.
[12] Lorke, D. *A New Approach to Practical Acute Toxicity Testing*. Architoxicol. **1983** Pp 54:275-287.
[13] Diurno, M.A., Izzo, A.A., Mazonni B., Bologgnese A., Capasso F. Anti diarrheal activity of the new thialidinone related to Loperamide. *Journal of pharmacy and pharmacology,***1996** 48: 760-762
[14] Ammon, P.J., Thomas, Philips, S. Effects of oleic and recinoleic acids net jejuna water and electrolyte movement. *J. Clin. Invest*. **1974** 53:374-379
[15] Watson W.C, Gordon R. Studies on the digestion, absorption and metabolism of castor oil. *Biochemical Pharmacology* **1962** 11:229-236.
[16] Knowles, C.H., Martin, J.E. Slow transit constipation: a model of human gut dysmotility. Review of possible aetiologies. *Neurogastroenterology Motility*. **2000** Apr; 12 (2):181-96.
[17] Longstreth, G.F, Thompson, W.G., Chey, W.D., Houghton, L.A., Mearin, F., Spiller, R.C. "Functional bowel disorders". *Gastroenterology,* **2006** 130 (5): 1480–91.
[18] Galvez A, Zarzuelo ME, Crespo MD, Lorente M, Ocete A, Jimenez J.
[19] Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of active flavonoid
[20] constituent. *Plant Med.* **1993** 59: 333–336.
[21] Tripathi, K.D. *Essentials of Medical Pharmacology*. Jaypee Brother Medical Publishers, New Delhi. **1994** Pp.775
[22] Theoderau, C.S., Jelacic ,S., Habeeb, R.L., Watkins, S.L., Tarr, P.I. "The risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections". *English Journal of Medicine.* June **1991** 342 (26): 1930–6.