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ORIGINAL ARTICLES

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2. Anti-diarrheal activity of leaf extract of *Juniperus procera* and its effect on intestinal motility in albino mice .

ORIGINAL ARTICLE

Management of complex ankle fracture: A Ghanaian experience

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Ankle fractures are among the most common conditions for surgical emergencies in most developing countries including Ghana. Despite the fact that many ankle fractures are uncomplicated, a high proportion may require surgical intervention. Decision-making depends on recognition of the fracture pattern, availability of surgical implants and anaesthetic materials. In resource-limited settings where patients are unable to afford the cost of surgical implants and anaesthetic materials associated with ankle fractures, suggested modification of the open reduction and internal fixation (ORIF) technique have proven to yield satisfactory results. This study retrospectively assessed the effectiveness of the modified ORIF method among Ghanaians living within the Tamale metropolis, a resource-limited setting located in the Northern Region of Ghana. The study reviewed 70 cases of bimalleolus fractures which were either treated using the ORIF based on the Association for the Study of Internal Fixation (ASIF) protocol or a modified version of the ORIF which involves internal fixation of the malleolus without screws. The findings indicate that the modified method is as good as ORIF (based on ASIF protocol) with added benefits such as shorter operation time, reduced risk of anaesthetic complications and cost of operation (anaesthetic agents and orthopaedic implant cost) as well as reduced number of foreign bodies (implants) leading to a lower risk of wound infections. The use of this method however demands that foot and ankle joint must be handled with extreme care so as not to dislocate the tibia malleolus post-operatively.

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Keywords: Bimalleolus, surgical, open reduction, internal fixation, bone fracture, Ghana

INTRODUCTION

As a weight-bearing joint, the ankle can absorb great amount of shock and pressure up to about 6 times the individual's body weight (Carr *et al.*, 2003). Excessive amount of energy across the ankle joint can lead to fracture. Ankle fractures are considered the most common of all fractures treated in hospitals (Yang *et al.*, 2011). In the United States of America (USA), the incidence per year of ankle, tibia and fibula fractures is about 492,000. (Praemer *et al.*, 1992; Weening and Bhandari, 2005) and in the United Kingdom (UK), documented incidence rate of fractures is 14.8% per 10,000 persons per year (Van Staa *et al.*, 2001). In Africa however, the incidence is expected to be higher due to additional

high incidence of road traffic accidents (Tiwagirayezu *et al.*, 2008). Road traffic accidents are said to account for about 46.3% of ankle fractures in Nigeria (Ifesanya and Alonge, (2012) and about 71.5% of lower limb fractures in Rwanda (Tiwagirayezu *et al.*, 2008)

The management of ankle fracture in the general populace has been documented to range from non-operative restriction to open reduction and internal fixation (ORIF). Irrespective of management method, anatomic alignment of the ankle joint and complete healing are major factors which can ensure long-term treatment success (Dahners, 1990; Egol *et al.*, 2000) and to prevent arthritis due to abnormal pressure distribution because of malunion of the ankle fracture (Ramsey and Hamilton, 1976). The quality of bone and related cartilage injury, age and alignment of the joint surface (Walheim and Akerman, 1936; Klossner, 1962) as

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well as the type of fracture (Lindsjo, 1985) are other factors to consider during management. However, ORIF is said to offer a potential for more rapid recovery than non-operative treatment (Lindsjo, 1985; Porter *et al.*, 2008). Congruent joint, fracture union, functional motion, normal strength and optimal recovery time are the main objectives of any management method of ankle fracture (Jelinek and Porter, 2009). In a resource-limited setting where availability and accessibility to surgical implants and anaesthetic materials is a major problem coupled with the fact that the community dwellers may not be able to afford the cost of surgical implants and anaesthetic materials, a modification of the method may be necessary. This study retrospectively assessed the effectiveness of the modified ORIF method among Ghanaians living within the Tamale metropolis and its surrounding environs, a resource-limited setting located in the Northern Region of Ghana.

PATIENTS AND METHODS

Study site and participants

A total of 70 patients who received complex ankle fracture or bimalleolus fracture management at the Tania Specialist Hospital between September 2005 and September 2010 were included in this retrospective study. Patient characteristics (age and gender), fracture type, mechanism of injury and treatment type were retrospectively reviewed. After review of patient data, the subjects were grouped into two based on method of treatment. Group one (n = 35) was treated with open reduction and internal fixation (ORIF) using principles of the Arbeitsgemeinschaft Osteosynthesefragen (AO/ASIF) group. The second group (n = 35) was treated with modified ORIF without syndesmosis and medial malleolus lag screws. All surgical cases were performed by a consultant orthopaedic surgeon and were usually done after initial physical and photographic assessment of the patient to confirm the position and 'personality' of the fracture. Radiographs taken at 2 and 6 weeks of treatment in both methods were also reviewed.

Treatment Procedure

All patients treated by ORIF with AO/ASIF principles followed the under listed protocol;

- a. Open reduction of laterally dislocated tibia malle-

- olus and stabilization with lag screw(s)
- b. Open reduction of fibular fracture with unstable syndesmosis
- c. Reduction of lateral dislocated Talus
- d. Fixation of syndesmosis with screw(s)
- e. Application of below knee splint for 7-10 days
- f. Application of knee circular POP after 7-10 for six weeks
- g. Removal of POP and start of physiotherapy and partial body weight bearing (15 kg) for start increasing over six more weeks

The major outcome is the anatomic fixation of the ankle joint which allows for early return to functional range of motion.

The rest of the patients who were treated with modified ORIF without bimalleolus lag screws followed the under listed procedure;

- a. Open reduction of laterally dislocated tibial malleolus and stabilization with Vincryl-2 suture (first as pair-string and fortified with Z-shape suture) over deltoid ligament without lag screws.
- b. Reduction of laterally dislocated Talus
- c. Fixation of syndesmosis with screw(s)
- d. Application of below knee splint with extreme care for 10 days
- e. Application of knee circular POP for six weeks
- f. Removal of POP and start of physiotherapy and partial weight bearing of 15 kg body weight for the start, increasing over six more weeks.

Statistical Analysis

All categorical variables were expressed as proportions and were compared using Fisher's exact test. In all statistical tests, a value of $P < 0.05$ was considered significant. All analysis was performed using GraphPad Prism 5.10 for windows (Graphpad software, San Diago, CA. USA).

RESULTS

From this study, plate 1A shows a radiograph of pre-operative fracture (*in the direction of the black arrow*). Plate 1B shows a radiograph of the ankle joint after stabilization using the modified ORIF method (*in the direction of the white arrow*) compared to Plate 1C which is a radiograph of the fracture after heal-

ing following the ORIF (AO/ASIF) (indicated with a white arrow).

From the retrospective review of available data, the general cause of injury was road traffic accident which accounts for 88.6% (62/70) of all recorded ankle fracture cases followed by direct blow (10.0%) and falls from heights (1.4%) as shown in Table 1.

Table 1: Aetiology of fractures

Aetiology	No. (%)
Road traffic accident	62 (88.6)
Direct blow	7 (10.0)
Fall from a height	1 (1.4)
Total	70 (100)

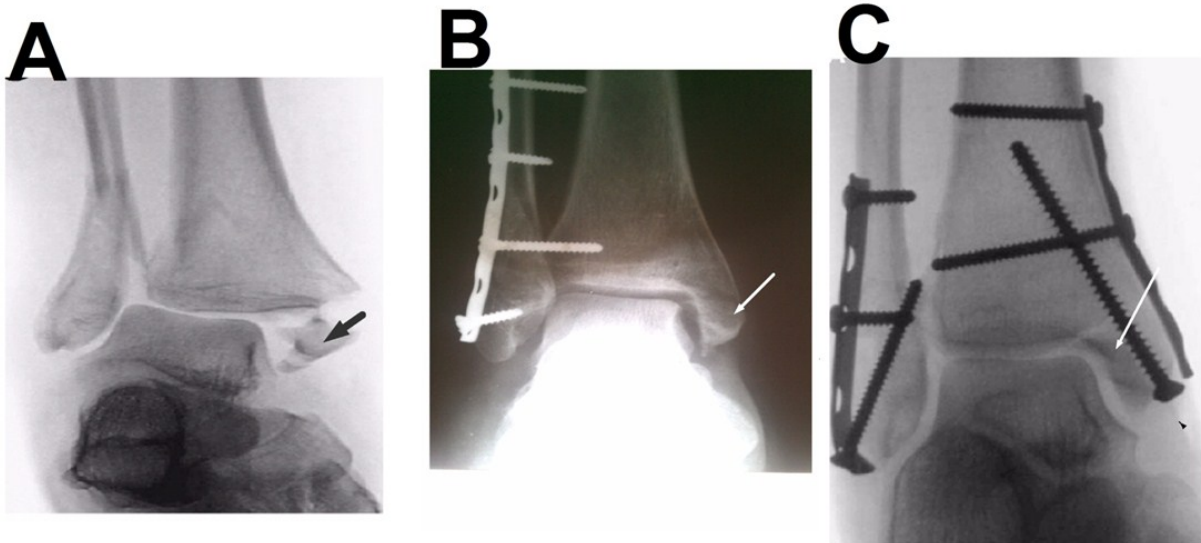


Plate 1: Radiographs of **A)** pre-operative fractures with an arrow (black) showing the medial malleolus; **B)** post-operative treatment without medial malleolus screw(s) indicated by the arrow (white); **C)** post-operative treatment with medial malleolus screw(s) indicated by arrow (white).

In this study, 85.7% (60/70) of the patients were males with only 14.3% (10/70) being females. The mean age of the patients was 36 ± 9 years with a range of 11-65 years. Majority of the patients (62.9%) were within the 31-50 year age bracket as shown in Table 2. The recovery period for the patients ranged from within 3 months to 6 months with about 67.4% (47/70) gaining full recovery within 3 months and the remaining 32.9% (23/70) recovering within 3-6 months post-operation. When the rate of recovery between the subjects treated with the AO/ASIF was compared to those treated without the malleolus lag screws, there was no significant difference ($P = 0.8075$). There was no significant difference in the recovery period between men and women as shown in Table 2. Although

younger subjects tendered to heal early there was no statistical significant difference in healing among the subjects with respect to patient age. After one year of follow up there were no post-operative complications such as deep wound infections and reflex sympathetic dystrophy in both groups.

DISCUSSION

This study reports ankle fracture incidence of 88.6% resulting from vehicular road traffic accident (RTA). This high figure could be attributed to the high number of motor cycles in the three Northern regions. According to the Regional motor traffic unit majority of users of these motor cycles are without driving licence leading to careless and reckless driving culminating in the high incidence of

Table 1: Relationship between fracture healing time and patient age, method of fixation and gender

Variable	Healing time		Total	P value
Age (Yrs)	Within 3 months	3-6 months		
10-20	2(100.0%)	0(0.0%)	2	
21-31	9(52.9%)	8(47.1%)	17	
31-40	12(57.1%)	9(42.9%)	21	
41-50	13(56.5%)	10(43.5%)	23	
51-60	3(60.0%)	2(40.0%)	5	
61-70	0(0.0%)	2(100%)	2	
Method of stabilization				
ORIF(AO/ASIF)	22(62.9%)	13(37.1%)	35	P = 0.8075
ORIF (without screws)	20(57.1%)	15(42.9%)	35	
Gender				
MALE	35(58.3%)	25(41.7%)	60	P = 1.000
FEMALE	6(60.0%)	4(40.0%)	10	

road traffic accidents (*personal communication*). The high vehicular related ankle injury reported in the present study is in conformity with results of similar studies in other parts of Africa which indicated that road traffic accidents are the leading cause of ankle fractures in Africa (T'wagirayezu *et al.*, 2008).

Proponents of open reduction and internal fixation suggest that restoration of the normal anatomy will reduce the risk of subsequent osteoarthritis due to incongruence (Weber, 1966). In areas where surgical implants are not readily available or accessible, surgeons find it difficult to manage complex ankle fractures leading to complications such as arthritis and bacterial infections (Ifesanya and Alonge, 2012). According to Steiner and Kotisso (1996), it is not clear whether or not internal fixation should have a place in Africa. It is often argued that there would be too many infections and other complications because in Africa there is neither adequate training in internal fixation nor adequate infrastructure in the operating theatre (Steiner and Kotisso, 1996). This perception is however changing with the establishment of specialist hospitals around Africa. In this study internal

fixation of bimalleolus fracture without lag screw has proven successful with added benefits such as a shorter duration of surgical process, reduced risk of anaesthetic complications, reduced cost of operation (anaesthetic agents and orthopaedic implant cost) and reduced number of foreign bodies (implant) leading to a lower risk of wound infections. Clinical studies have consistently failed to show any difference in outcome between fractures treated operatively and those managed conservatively. The findings of the present study is similar to Yde and Kristensen (1980) who compared operations based on ASIF techniques with closed treatment and immobilisation in a plaster cast and found no difference in outcome at a minimum follow-up of three years. The modified ORIF procedure without lag screws also ensures early restoration of anatomical function similar to the ORIF based on ASIF principles. There were no post operative complications such as deep wound infections and reflex sympathetic dystrophy which are usually associated with the ASIF procedure as reported by Paudel (2011) giving this procedure an added advantage.

CONCLUSION

The results of this retrospective study suggest that the modified ORIF treatment protocol for complex ankle injuries is safe, satisfactory and equally effective with good functional outcome similar to the AO/ASIF ORIF treatment protocol which uses screws for internal fixation. The use of the modified ORIF protocol however demands that foot and ankle joint must be handled with extreme care so as not to dislocate the tibia malleolus post-operatively.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Carr J.B., Browner B.D., Jupiter J.B. and Levine A.M. (2003) *Malleolar fractures and soft tissue injuries of the ankle.*, 3 ed. Philadelphia: Saunders.

Dahners L.E. (1990) The pathogenesis and treatment of bimalleolar ankle fractures. *Instr Course Lect* 39, 85-94.

Egol K.A., Dolan R. and Koval K.J. (2000) Functional outcome of surgery for fractures of the ankle. A prospective, randomized comparison of management in a cast or a functional brace. *J Bone Joint Surg Br* 82, 246-249.

Ifesanya O.A. and Alonge O.T. (2012) Operative stabilization of open long bone fracture: A tropical Tertiary hospital experience *Nigeria Medical Journal* 53, 16-20.

Jelinek J.A. and Porter. D.A. (2009) Management of Unstable Ankle Fractures and Syndesmosis Injuries in Athletes. *Foot Ankle Clin N Am* 14, 277-298.

Klossner O. (1962) Late results of operative and non-operative treatment of severe ankle fractures. A clinical study. *ActaChirScandSuppl* 293, 1-93.

Lindsjo U. (1985) Operative treatment of ankle fracture-dislocations. A follow-up study of

306/321 consecutive cases. *ClinOrthopRelat Res* 199, 28-38.

Nilsson G., Jonsson K., Ekdahl C. and Eneroth M. (2007) Outcome and quality of life after surgically treated ankle fractures in patients 65 years or older. *BMC Musculoskelet Disord* 8, 127.

Paudel K.P. (2011) Early weight bearing compared with non-weight bearing functional mobilization after operative treatment of an ankle fracture. *Journal of College of Medical Sciences-Nepal* 7, 40-46.

Porter D.A., May B. and Berney T. (2008) Functional outcome after operative treatment for distal fibula and tibia fractures in young athletes: a retrospective, case series. *Foot Ankle Int* 29, 887-894.

Praemer A., Furner S and Rice D.P. (1992) *Musculoskeletal Conditions in the United States.* Park Ridge, IL.: *American Academy of Orthopedic Surgeons.*

Ramsey P.L. and Hamilton W. (1976) Changes in tibiotalar area of contact caused by lateral talar shift. *J Bone Joint Surg Am* 58, 356-357.

Steiner A.K. and Kotisso B. (1996) Open fractures and internal fixation in a major African hospital. *Injury* 27, 625-630.

Tunturi T., Kemppainen K., Patiala H., Suokas M., Tamminen O. and Rokkanen P. (1983) Importance of anatomical reduction for subjective recovery after ankle fracture. *Acta Orthop Scand* 54, 641-647.

Twagirayezu E., Dushimiyimana J.M.V. and Bonane V. (2008) Open Fractures I Rwanda: The Kigali Experience. *East and Central African Journal of Surgery* 13, 77-83.

Van Schie-Van der Weert E.M., Van Lieshout E.M., De Vries M.R., Van der Elst M. and Schepers T. (2012) Determinants of outcome in operatively and non-operatively treated Weber-B ankle fractures. *Arch Orthop Trauma Surg* 132, 257-263.

Van Staa T.P., Dennison E. and Leufkens H.G.M. (2001) Epidemiology of fractures in England and Wales. *Bone* 29, 517-522.

Walheim T. and Akerman N. (1936) Intraarticular malleolar fractures. *ActaChirScand* 76, 166.

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Kunbiere et al.,

- Weber B.G. (1966) Die verletzungen des oberen sprunggelenkes. *Bern: Huber.*
- Weening B. and Bhandari M. (2005) Predictors of functional outcome following transsyndes-motic screw fixation of ankle fractures. *J Orthop Trauma* 19, 102-108.
- Yang E., Wu Y. and Dorcil J. (2011) Surgical versus nonsurgical treatment of the SE4-equivalent ankle fracture: a retrospective functional outcome study. *Orthopedics* 34.
- Yde J and Kristensen K.D. (1980) Ankle fractures: supination-eversion fractures stage II: primary and late results of operative and non-operative treatment. *Acta Orthop Scand* 51, 695-702.



ORIGINAL ARTICLE

Anti-diarrheal activity of leaf extract of *Juniperus procera* and its effect on intestinal motility in albino mice

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This study was designed to evaluate the anti-diarrheal property of *Juniperus procera* using albino mice. An aqueous extract of *J. procera* leaves was administered to albino mice at 150, 300, and 450 mg kg⁻¹ (p.o). Wet feces, intestinal accumulation (enteropooling) and intestinal motility were recorded. The aqueous extract of *J. procera* significantly (p < 0.0001) decreased the mean number of wet faeces produced by the albino mice in a dose dependent manner as well as decreasing the distance travelled by the charcoal meal (p < 0.0001) from 28.5 ± 1.1 cm when treated with 150 mg kg⁻¹ to 11.8 ± 0.5 cm when treated with 450 mg kg⁻¹ through 20.0 ± 1.0 cm when treated with 300 mg kg⁻¹. Results obtained for the extract especially the 450 mg kg⁻¹ dose was almost equivalent to diphenoxylate and atropine sulphate (the reference drugs used). In conclusion, aqueous extract of *J. procera* demonstrated anti-diarrheal activity and could be an inexpensive and readily available anti-diarrheal remedy.

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Keywords: *Juniperus procera*, diarrhea, intestinal motility, castor oil, enteropooling

INTRODUCTION

Diarrhea is a gastrointestinal tract (GIT) dysfunction, which is considered as a common symptom of infection and one of the causes of intestinal motility disorder (Maresca *et al.*, 2003). It causes loss of water and important nutrients from the GIT in addition to increasing intestinal motility (Jimba *et al.*, 2002). The rate of material movement through the intestinal lumen is directly associated with its motility. As diarrhea causes high intestinal motility the increased motility also heightens diarrheal effects through increasing the rate of movement of intestinal content (Qnais *et al.*, 2005; Hejazian *et al.*, 2007).

Diarrhea is the cause of death in about 2.2 million people each year (Guerrant *et al.*, 2001; Meite *et al.*, 2009) despite the availability of synthetic drugs. Medicinal plants have been recommended as good al-

ternatives due to their cost as well as availability. Chebula, swertia, and black pepper are some medicinal plants that are used in India and China to treat diarrheal (Das *et al.*, 2009). Many species of the Genus *Juniperus* belonging to the family *Cupressaceae* are claimed to cure diarrheal. The anti-diarrheal properties of *J. phoenicia*, *J. communis*, *J. oxycedrus* and *J. thurifera* have been validated (Cosentino *et al.*, 2003; Karaman *et al.*, 2003, Qnais *et al.*, 2005). Also, WHO has encouraged the use of traditional medicinal plants for the treatment and prevention of diarrheal since the 1980s (Syder and Merson, 1982; Park, 2000).

Castor oil is known to induce GIT enteropooling similar to that observed in diarrheal (i.e. accumulation of substances in the gut lumen) (Galvez *et al.*, 1993; Gorard *et al.*, 1994; Akomolafe *et al.*, 2003). Its effect is mediated by ricinolic acid that can induce a hypersecretory response by the gut wall leading to diarrheal (Capasso *et al.*, 1994; Chitme *et al.*, 2000; Das *et al.*, 2009). In this study *J. procera*, an evergreen indigenous gymnosperm in Ethiopia is

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tested as an antidiarrheal plant. This plant has a wide range of traditional uses including charcoal and timber productions, fire wood, fencing etc. Its leaves are smoked to deter insects (personal observation) in rural areas of the country. In the northern parts of the country people are known to use this plant to treat menorrhagia, emmenagogue, constipation, toothache, gum pain, and biliousness (Abebe and Aychu, 1993).

MATERIALS AND METHODS

Plant Material Collection

Fresh leaves of *J. procera* were collected from the campus of Natural Science College of Addis Ababa University (AAU) at an attitude around 2450 m a.s.l. in June 2010. The collection was made after identification and taxonomic authentication by the help of a botanist and sample specimen is kept in the Herbarium of Faculty of Life Science, AAU under voucher № 006. The collected leaves were allowed to dry under shade for 20 days and the air dried leaves were then ground.

Preparation of the extract

A measured amount of the ground leaves of *J. procera* was dissolved in warm distilled water in 1:10 (w/v) with continuous stirring for 30 min according to the method in Qnais *et al.*, (2005) and Oben *et al.*, (2006). The solution was filtered using cotton and filter paper. The filtrate was completely lyophilized under reduced pressure. The resultant powder was weighed and dissolved in Tyrode, a physiological salt solution. This physiological salt solution was prepared daily with the following compositions (mM): 118 NaCl, 4.7 KCl, 25 NaHCO₃, 1 NaH₂PO₄.H₂O, 0.5 Na₂HPO₄, 11.1 glucose, 2.5 MgCl₆.H₂O, and 2.5 CaCl₂.2H₂O. The pH used during this preparation was 7.4.

Experimental animals

Adult albino mice weighing between 35-45 g were used. All mice were provided with a standard pellet food and water *ad libitum*. The mice were starved for 18 h before the experiment but were provided with water.

Drugs and chemicals

A reference anti-diarrhoeal drug (diphenoxylate), castor oil (laxative agent), atropine sulphate and charcoal meal were used. All the chemicals were of pharmacological grades and obtained from BDH Merck Ltd, UK.

Experimental Procedures

Anti-diarrheal test

Five groups of mice (n=6) were set for the experiment and labeled A-E. Group A serving as a negative control was given 0.2 ml PSS. Groups B, C and D were given the extract at doses of 150, 300 and 450 mg kg⁻¹ respectively. Diphenoxylate was given to Group E, the positive control at a dose of 5 mg kg⁻¹. All administrations were by gavage. Castor oil (1 ml) was given orally to all mice an hour before the treatment (described above). Observations were made for 4 hrs and the number of both wet and dry feces was recorded. The experiment was performed in triplicate according to standard procedures. Average number of feces was taken to calculate percentage diarrheal inhibition according to the following formula (Oben *et al.*, 2006).

$$\% \text{ inhibition} = \frac{\text{No. of WFC} - \text{No. of WFT}}{\text{No. of WFC}} \times 100$$

WFC = wet feces in control and WFT = wet feces in test group

Intestinal motility test

Intestinal motility test was done according to the methods of Qnais *et al.*, (2005) and Meite *et al.*, (2009) with slight modifications. Five groups of mice (n=6) were organized and made to fast for 18 hrs. Group A served as a control and received 0.5 ml of PSS. The reference drug, atropine sulphate (5 mg kg⁻¹) was given to group E that had served as a positive control. Groups B, C and D received the extract at a dose of 150, 300 and 450 mg kg⁻¹ of body weight respectively. All administrations were made orally by gavage. Mice were given 1 ml of charcoal meal (5 g of activated charcoal suspended in 50 ml PSS) 30 min later through the same route.

After another 30 min all mice were sacrificed and their abdomen was open. The experiment was performed in triplicate according to standard procedures. The distance traveled by the charcoal meal from the pylorus to the caecum was measured and the percentage of inhibition of movement was calculated as follow (Oben *et al.*, 2006):

$$\% \text{ Inhibition} = \frac{\text{MTLI} - \text{MDCC}}{\text{MTLI}} \times 100$$

MTLI = mean total length of the intestine and MDCC = mean distance covered by the charcoal

Anti-enteropooling test

As in test for antidiarrheal and intestinal motility, triplicate experiments were conducted to test the anti-enteropooling property of the plant. Four groups of mice (n=6) were assigned as A, B, C, and D. Group A served as a control receiving PSS (0.5 ml) by oral administration. Group B, C and D respectively received *J. procera* leave extract at a dose of 150, 300 and 450 mg kg⁻¹ by the same route. Castor oil (1 ml) was given orally to the mice after an hour. Two hours later all mice were sacrificed to isolate the small intestine. Intestinal contents were collected by mixing the intestine content and the volume was measure using graduated cylinder.

Statistical analysis

Continuous variables were presented as mean ± SEM and categorical variables presented as proportion. To compare differences between groups, *one way Analysis of Variance* (ANOVA) was performed followed by Tukey test as *post hoc*. In all test p value < 0.05 was considered significant.

RESULTS

Anti-diarrheal activity

As shown in figure 1A, the aqueous extract of *J. procera* significantly (p < 0.0001) decreased the mean number of wet faeces produced by the albino mice in a dose dependent manner. Even though, the mean reduction in the number of wet faeces produced when the extract was administered at 450 mg kg⁻¹ was not as much as that produced when 5 mg kg⁻¹ of the standard drug was administered, the difference

did not reach significant level (p = 0.74). The extract also in a dose dependent manner increased the percentage inhibition of wet faeces production as the treatment dose was increased from 150 through 300 to 450 mg kg⁻¹ of *J. procera* (p < 0.001) (Figure 1B). The percentage inhibition induced by the 450 mg kg⁻¹ of the extract was not significantly different from the inhibition induced by 5 mg kg⁻¹ of the standard drug (p = 0.45) (Figure 1B).

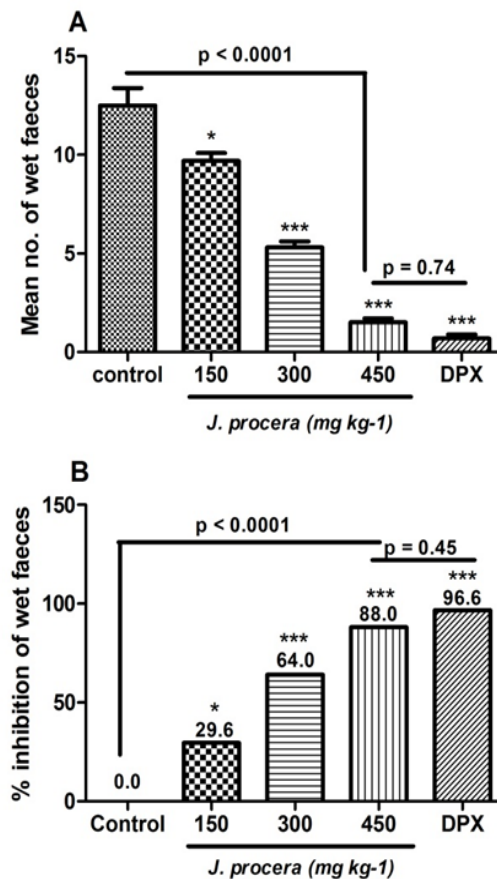


Figure 1: Effects of *J. procera* (150, 300 and 450 mg kg⁻¹) and 5 mg kg⁻¹ diphenoxylate (DPX) treatment 1 hr after castor oil (1 ml) induced diarrheal on the number of wet faeces produced (A) and the percentage inhibition of wet faeces (B). Data are presented as mean ± SEM and proportion. Significantly different from control: *p<0.05 and ***p<0.001 by Tukey post hoc test (n = 6).

Table 1: Effects of *J. procera* (150, 300 and 450 mg kg⁻¹) and 5 mg kg⁻¹ atropine treatment 30 minutes before administration of 1 ml of charcoal meal on the distanced as well as % inhibition of charcoal movement.

Test Group	Total distance of the intestine (cm)	Distance Traveled by charcoal meal (cm)	P value	% Inhibition
Control	69.0 ± 1.0	61.0 ± 1.1		11.6
Extract (150 mg kg ⁻¹)	68.5 ± 1.0	28.5 ± 1.1	0.00	58.4
Extract (300 mg kg ⁻¹)	67.5 ± 1.1	20.0 ± 1.0	0.00	70.4
Extract (450 mg kg ⁻¹)	68.7 ± 1.0	11.8 ± 0.5	0.00	82.8
Atropine (5 mg kg ⁻¹)	69.0 ± 1.2	9.3 ± 0.5	0.00	86.5

Data are presented as mean ± SEM and proportion. P values are significantly different from control using Tukey post hoc test (n = 6).

Effect of the extract on intestinal motility

As presented in Table 2, the length of the intestine of the albino mice in all the groups was similar. Using one way ANOVA, the aqueous extract of *J. procera* was able to decrease the distance travelled by the charcoal meal in a dose dependent manner ($p < 0.0001$) from 28.5 ± 1.1 cm when treated with 150 mg kg⁻¹ to 11.8 ± 0.5 cm when treated with 450 mg kg⁻¹ through 20.0 ± 1.0 cm when treated with 300 mg kg⁻¹ (Table 1). Inversely, the percentage inhibition of the charcoal meal movement also significantly increased ($p < 0.0001$). There were no significant differences when the highest dose of the extract was compared to the 5 mg kg⁻¹ of the standard reference drug (Atropine) in terms of the distance travelled as well as the % inhibition (Table 1).

Anti-enteropooling property

From the one way ANOVA using treatment as a factor, the extract significantly reduced ($p < 0.0001$) the content of the animal intestine from 0.97 ± 0.45 mL when treated with 150 mg kg⁻¹ through 0.48 ± 0.10 mL when treated with 300 mg kg⁻¹ to 0.24 ± 0.02 mL when treated with 450 mg kg⁻¹ in a dose dependent manner (Figure 2).

DISCUSSION

The results from this study clearly reveal that the aqueous extract of *J. procera* possesses anti-diarrhoeal property. The aqueous extract of the leaves of this plant may contain different agents that effectively reduced diarrhoea that was induced by a potent diar-

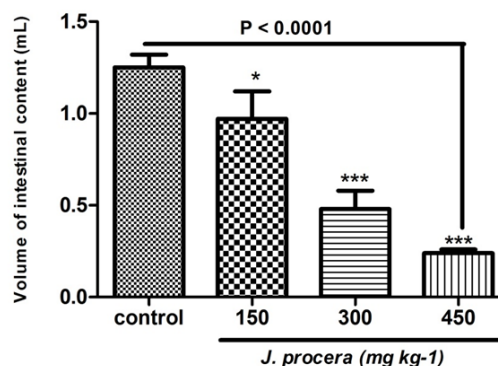


Figure 2: Effects of *J. procera* (150, 300 and 450 mg kg⁻¹) on the volume of intestinal content. Data are presented as mean ± SEM. Significantly different from control: * $p < 0.05$ and *** $p < 0.001$ by Tukey post hoc test (n = 6).

rhoeal agent, castor oil. Diarrhoea can be characterized by different phenomena including frequent out flow of wet (waterish) faeces, high intestinal motility, high accumulation of important nutrients in the lumen of the intestine, and others (Capasso *et al.*, 1994; Jarbur and Sjovall, 2000). The findings from the present study are in agreement with previous works by Qnais *et al.*, (2005). As reported by Venkateran *et al.*, (2005), Oben *et al.*, (2006) and Das *et al.*, (2009) the anti-diarrhoeal properties of plant extracts are expressed by their action of reducing intestinal motility and enhancing intestinal re-absorption, which can be done through inhibition

of prostaglandin release.

A high rate of intestinal absorption might lead to a decrease in intestinal accumulation and together with reduced intestinal motility may result in increased transit time (Jarbur and Sjoval, 2000). This in turn might give chance for further absorption as evidenced by small volume of intestinal contents recorded in this study. Hence, the obtained anti-diarrhoeal activities of *J. procera* in this study might be due to possession of chemicals that facilitate the aforementioned actions. Phytochemical groups like flavonoids, tannins, alkaloids and saponins have been reported to show anti-diarrhoeal activities (Langana *et al.*, 2000; Venkateran *et al.*, 2005; Salgado *et al.*, 2006). Moreover, these substances have also been reported in other *Juniperus spp.* (Qnais *et al.*, 2005). Though further analysis is needed to assert the presence or otherwise of these aforementioned phytochemicals, the positive result of the present study indicates that these secondary metabolites might exist in the leaves of *J. procera*. Reductions in the volume of intestinal contents were also recorded in this study that might be correlated to the ability of the extract to increase intestinal absorption (Oben *et al.*, 2006). In addition to this, the extract might have tannate that can make the intestinal mucosa more resistant and reduce secretion, which is similar with reports made for *J. phoenicia* by Qnais *et al.*, (2005).

CONCLUSION

The present study clearly shows that like other members of the genus this ever green plant may contains phytochemicals with anti-diarrhoeal properties. Hence, further studies are needed not only to isolate the active principles but also to find such property in parts other than its leaves.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

- Abebe D and Ayehu A (1993). *Medicinal plants and enigmatic health practices in northern Ethiopia*, 511pp
- Akomolafe RO, Adeosun IO, Elujoba AA, Iwalewa EO and Ayoka AO (2003). Effects of *Cassia sieberiana* leaf extracts on the intestinal motility of rat. *African Journal of Biomedical Research*, 6: 141-145
- Capasso F., Mascolo N, Izzo AA and Gaginella TS (1994). Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: Effect of NG-nitro-L-arginine methyl ester. *Britain Journal of Pharmacology*, 113: 1127- 1130
- Chitme HR, Chandra R and Kaushik S (2000). Studies on anti-diarrhoeal activity of *Calotropis gigantea* R.BR in experimental animals. *Journal of Pharmacology and Pharmaceutical Science*, 47(1): 70-75
- Cosentino, S, Barra, A, Pisano, B, Cabizza, M, Pirisi, FM and Palmas, F (2003). Composition and antimicrobial properties of Sardinian *Juniperus* essential oils against food borne pathogens and spoilage microorganisms. *Journal of Food protection*, 66: 1288-1291
- Das AK, Rohini R and Hema A (2009). Evaluation of anti-diarrhoeal activity of *Rhizophora mucronata* bark extracts. *The Internet Journal of Alternative Medicine*, 7(1): 1-9
- Galvez J, Zavezuelo A, Crespo E, Lorente MD, Ocete, MA and Jimenez, J (1993). Anti-diarrholic activity of *Euphorbia hirta* extract and isolation of an active flavonoids constituent. *Planta Medica*, 59: 333-336
- Gorard DA, Libby GW and Farthing, MJG (1994). Ambulatory small intestinal motility in diarrhoea predominant irritable bowel syndrome. *Gut*, 35: 203-210

- Guerrant RL, Van-Gilder T, Steiner TS, Theilman MN, Slutsker L and Tauxe RV (2001). Practice guidelines for the management of infectious diarrhoea. *Clinical Infectious Disease*, 32: 331-35
- Hejazian SH, Morowatisharifabad M and Mahdavi SM (2007). Relaxant effect of *Carum copticum* on intestinal motility in ileum of rat. *World Journal of Zoology*, 2(2): 15-18
- Jarbur, K and Sjoval, H (2000). Pressure and frequency dependent linkage between motility and epithelial secretion in human proximal small intestine. *Gut*, 46(3): 376084
- Jimba Y., Nagao J. and Sumiyama Y (2002). Change in gastrointestinal motility after subtotal colectomy in dogs. *Surgery Today*, 32(12): 1048-1057
- Karaman I, Sahin F, Gulluce, M, Ögütçü, H, Şengül, M and Adıgüzel, A (2003). Antimicrobial activity of aqueous and methanol extract of *Juniperus oxycedrus* L. *Journal of Ethnopharmacology*, 85: 231-235
- Langana OA, Verduyze A and Foriers A (2000). Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in treatment of dysentery and diarrhoea in Lometa area, Democratic Republic of Congo. *Journal of Ethnopharmacology*, 71(3): 411- 423
- Maresca M, Mahfoud R, Garmy N, Fantini J and Clayton F (2003). The virotoxin model of HIV. *Journal of Biomedical Science*, 10(1): 156-166
- Meite S, N'guessan JD, Bahi C, Yapi HF, Djaman AJ and Guede-Giuna F (2009). Antidiarrhoeal activity of the ethyl acetate extract of *Morinda morindoides* in rats. *Tropical Journal of Pharmaceutical Research*, 8(3): 201-207
- Oben JE, Assi SE, Agbor GA and Musoro DF (2006). Effect of *Eremomastax speciosa* on experimental diarrhoea. *African Journal of Traditional, Complementary and Alternative Medicine*, 3(1): 95-100
- Park K. (2000). *Text book of Preventive and Social Medicine*. Jabalpur, India: Banarsidas Bharat Publishers, pp.172-175.
- Qnais EY, Abdulla FA and Ab-Ghalyn YY (2005). Anti-diarrhoeal effects of *Juniperus phoenicia* L. leaves extracts in rats. *Pakistan Journal of Biological Sciences*, 8(6): 867-871
- Salgado HRN, Roncari AFF, Midelin DC and Moreira RRD (2006). Evaluation of anti-diarrhoeal effects of *Psidium guajava* L (Myrtaceae) aqueous extract in mice. *Journal of Basic and Applied Pharmaceutical Science*, 27(1): 89-92
- Syder JD and Merson MH (1982). The magnitude of global problems of acute diarrhoeal disease; A review of active surveillance data. *Bulletin of WHO*, 60: 605-613
- Venkatesan N, Thiyagarajan V, Narayanan S, Arul A, Raja S, Vijaya Kumar SG, Rajarajan T, and Perianayagam JB (2005). Anti-diarrhoeal effect potential of *Asparagus recemous* wild root extract in laboratory animals. *Journal of Pharmacology and Pharmaceutical Science*, 8(1): 39-45

