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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 alternatives 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 *Cuperssaceae* are claimed to cure diarrhea. 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 diarrhea since the 1980s (Syder and Merson, 1982; Park, 2000).

Castor oil is known to induce GIT enteropooling similar to that observed in diarrhea (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 diarrhea (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 Ayehu, 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 $NaHCO_3$, 1 $NaH_2PO_4.H_2O$, 0.5 Na_2HPO_4, 11.1 glucose, 2.5 $MgCl.6H_2O$, and 2.5 $CaCl_2.2H_2O$. 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).

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

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):

$$\% \, Inhibition = \frac{MTLI - MDCC}{MTLI} \, x \, 100$$

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).

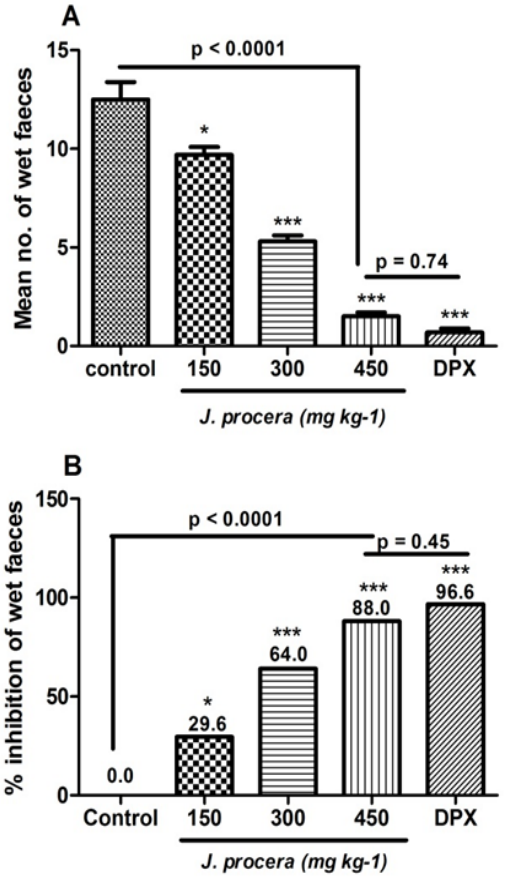

Figure 1: Effects of *J. procera* (150, 300 and 450 mg kg^{-1}) and 5 mg kg^{-1} diphenoxylate (DPX) treatment 1 hr after castor oil (1 ml) induced diarrheal on the number of wet faeces produced (A) and the percentage inhibition of wet faeces (B). Data are presented as mean ± SEM and proportion. Significantly 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).

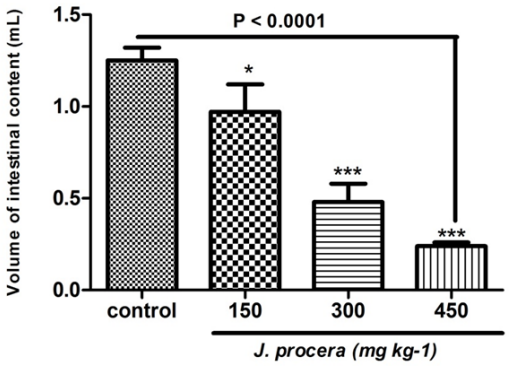

Figure 2: Effects of *J. procera* (150, 300 and 450 mg kg^{-1}) on the volume of intestinal content. Data are presented as mean ± SEM. Significantly different from control: *p<0.05 and ***p<0.001 by Tukey post hoc test (n = 6).

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 diarrhoeal 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 reabsorption, 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 Sjovall, 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.

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