Effects of *Detarium senegalense* JF Gmelin aqueous stem bark extract on castor oil induced diarrhoea in albino rats

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Abstract

*Detarium senegalense* JF Gmelin stem bark aqueous extract was investigated for its phytochemical contents as well as its anti-diarrhoea effects. The aqueous extract which is normally used in folkloric medicine was subjected to phytochemical screening. Graded doses of the extract (100, 200 and 400 mg per kg) were administered orally to three groups of rats (n = 5) before induction of diarrhoea with castor oil. Another two groups of animals were treated with normal saline (control) and diphenoxylate, a conventional anti-diarrhoea drug respectively. In two separate experiments, gastrointestinal transits of charcoal meal and gastro-intestinal enteropooling with the same graded doses of the aqueous extracts were used for comparison. The extract produced a significant inhibition of the castor oil induced diarrhoea in the animals. The gastro-intestinal transit of charcoal meal was also reduced by the various doses used in this study. However, the intestinal fluid accumulation was only slightly reduced especially by 400 mg/kg dose of the extract. The aqueous extract alone dose dependently reduced the contractile amplitude of the jejunal tissue. The aqueous extract also decreased the contractile amplitude of isolated jejunal segment exposed to 0.2 ml of 10 µg/ml of acetylcholine. Phytochemical analysis of the stem bark extract revealed the presence of secondary metabolites such as alkaloids, tannins, flavonoids, terpenes and steroids, saponins and glycosides. The findings suggest that, the aqueous stem bark extract of *D. senegalense* possesses antidiarrhoeal effect, which could be related to inhibition of gastro-intestinal motility and secretion.

Keywords: Diarrhoea, *Detarium senegalense*, Diphenoxylate, Enteropooling, intestinal transit

Introduction

Diarrhoea is a major health problem in developing countries including Nigeria. Infants are most vulnerable to the scourge of diarrhoea, with reported death of 9.9 % of the 6.9 million deaths in children under the age of 5 in 2011 (Claudio *et al.*, 2013). Scientific knowledge has revealed a clear understanding of the pathogenesis of diarrhoea and its control measure which has yielded some successes in the reduction of diarrhoea related mortality over the years; however it is still a major cause of morbidity and mortality in the developing world (Walker *et al.*, 2012). The use of traditional medicine in the treatment of diarrhoea by patients and traditional healers is still a major practice all over the world (Adeyemi & Akindele, 2008). The World Health Organisation...
(WHO) has advocated for scientific validation and integration of traditional medicine with the aim of improving and preventing diarrhoeal disease (Shettima et al., 2012). It is therefore important to identify and evaluate the commonly available plants as alternative to currently used antidiarrhoeal drugs which are not completely free from adverse effects. Detarium senegalense (JF Gmelin) is one of the plants used in the north eastern part of Nigeria for the treatment of diarrhoea and other diseases. D. senegalense belongs to the family Leguminoseae and sub-family Ceasalpinocea. It is native to Africa and mostly found in Senegal, Gambia, Sudan, DR Congo and Nigeria along water courses. The plant serves as an ornamental shade tree within its area of distribution (Akah et al., 2012). D. senegalensesis has been reported to have considerable use in food and pharmaceutical industries (Wang et al., 1996). A decoction of the bark is claimed to be effective in cases of heavy blood loss, pneumonia, diarrhoea, stomach-ache, digestive disorders and in expelling the placenta after child birth (Sowemimo et al., 2011). Active phytochemical compounds isolated from D. senegalense include 2-methoxyamine 3,4,5,7 tetrahydroxyanthocyanidines from the stem bark (Okwu & Uchegbu, 2009), while cyclohexanone β-myrcene, cis-cose oxide camphor and citronellol were obtained from the petroleum ether extracts of the seed (Sowemimo et al., 2011). The aim of this study is to evaluate the antidiarrhoeal potentials, and phytochemical components of the aqueous stem bark extract of D. senegalense in order to justify its application in traditional medicinal practice.

Materials and Methods

Collection of plant material and extraction
The stem bark of Detarium senegalense was collected in the month of April, 2009 from Marama, Biu Local Government Area of Borno State, Nigeria. The plant materials was identified and authenticated at the Department of Biological Sciences, University of Maiduguri, Nigeria, where a voucher specimen was deposited. The stem bark was washed with tap water and dried under shade. The dried stem bark was pulverized to a fine powder and 100 g of the powder was exhaustively extracted using a Soxhlet extractor and 500 ml of water as solvent. The extract was evaporated to dryness in a hot air oven at 40 – 50°C., and thereafter stored at 4°C until required (Cho et al., 2003; Motohashi et al., 2004).

Experimental animal
Wistar albino rats of both sexes weighing between 110 – 245 g and an adult rabbit were obtained from the Animal Unit of the Department of Biochemistry, University of Maiduguri, Nigeria. They were kept in the Department of Biochemistry Research Laboratory and allowed to acclimatize to the laboratory condition, one week before the experiment.

The animals were fed standard grower commercial feed (Vital feed, Nigeria Ltd) and tap water provided ad libitum before the experiment. The experiments complied with the regulations of the international guiding principles for biochemical research involving animals (C.I.O.M.S, 1985) as adopted by the Ethical Committee of the Faculty of Science, University of Maiduguri, Nigeria.

Phytochemical screening of the extract
Phytochemical screening of the extract was carried out according to the methods of Trease & Evans (1989).

Acute toxicity studies:
The up and down procedure (Dixon, 1991) was used to evaluate the oral acute toxicity of the aqueous extract of D. senegalense. Five rats weighing between 170-230 g were randomly selected and used for the experiment. They were housed individually in cages for 7 days prior to treatment to allow for acclimatization to the laboratory conditions. The rats were fasted overnight but allowed free access to water. Freshly prepared aqueous extract was orally administered at a limit dose of 5,000 mg/kg. One rat was dosed and observed for 48 hours for signs of toxicity or death. Since the rat survived, the same procedure was adopted until all the other rats were treated at the same limit dose of 5,000 mg/kg and observed for 48 hours (Bruce, 1985).

Effects of the extract on castor oil induced diarrhea
Twenty five rats of both sexes weighing between 122 – 220 g were used for this study. The method of Offia & Chikwendu (1999) was adopted. The rats were denied feed for 12 hours prior to the study but allowed free access to water. They were randomly divided into 5 groups of five rats each. Diarrhoea was induced by administering 1 ml of castor oil to each of the rats. Group 1 received 2 ml of normal saline orally and served as the control and group
II received diphenoxylate HCl, 5 mg/kg body weight intraperitoneally, while groups III, IV and V were orally administered 100, 200 and 400 mg/kg body weight of the aqueous stem bark extract respectively, 1 hour prior to castor oil administration. The rats were housed singly in cages lined with white blotting paper. The number of faeces passed by the rats were thereafter counted after 6 hours.

Effects of the extract on gastrointestinal transit of charcoal
Gastrointestinal transit of charcoal was determined by the method described by Akter et al. (2010). Twenty five rats weighing between 136–273 g were used. The rats were fasted for 18 hours and thereafter randomly divided into 5 groups of 5 rats each. They were allowed free access to water. Rats in group I received 2 ml normal saline orally while those in group II received atropine sulphate (3 mg/kg body weight) intraperitoneally. Groups III, IV and V were respectively given 100, 200 and 400 mg/kg body weight of the aqueous extract and of the aqueous extract respectively. One hour after drug and extract administration, 1 ml of 5% activated charcoal suspension in 10% aqueous solution of Acacia powder was given orally to each rat. The rats were sacrificed 30 minutes later and the abdomen opened. The distance travelled by the activated charcoal from the pylorus was measured and expressed as a percentage of the total length of the intestine.

Effects of the extract on castor oil induced enteropooling
Twenty five rats weighing between 150 and 185 g, were used to determine the intraluminal fluid accumulation as described by Nwafor & Hamza (2007). The rats were fasted overnight and divided into 5 groups of 5 rats each. Group I rats were treated with 2 ml/kg body weight of normal saline orally. Group V received 3 mg/kg atropine sulphate (intraperitoneally). Groups II, III and IV were respectively given 100, 200 and 400 mg/kg body weight of D. senegalense extract orally. After one hour, each rat was administered 1 ml of castor oil. One hour after the castor oil treatment, the rats were sacrificed and the small intestine removed, tied on both ends with a thread and weighed. The intestinal content was collected by milking and the volume measured. The intestine was thereafter weighed and the difference between the full and the empty intestine was calculated.

Effect of the Aqueous Extract of D. senegalense on Isolated Rabbit Jejunum
Adult male rabbits, which had free access to water and starved overnight prior to the experiment were used. Each rabbit was stunned by a blow on the base of the head and exsanguinated. Segments of the jejunum, 2–3 cm were obtained and each mounted in a 50 ml organ bath containing Tyrode’s solution of the following composition (mM) : NaCl (136.8), KCl(2.7), CaCl2 (1.3), NaHCO3 (11.9), MgCl2(0.5), Na2PO4 (0.45) and glucose (5.5) at a temperature of 37°C (+ 1°C) and aerated with 95% O2 and 5% CO2. The preparation was set up under a tension of 0.5g and responses recorded using an Ugo Basile microdynometer with isotonic transducer (Amos et al., 1998). The tissue was equilibrated for 30 minutes at 37°C before use.

Responses to acetylcholine (2 µg/ml) at 0.1, 0.2 and 0.4 ml were recorded after which the tissue was washed three times with the physiological fluid and allowed to rest. Similarly, dose response to the aqueous extract (100 mg/ml) at 0.1, 0.2, 0.4 ml was recorded. Response of the tissue to the aqueous extract (100 mg/ml) at 0.1, 0.2, 0.4 ml was also recorded in the presence of 0.1, 0.2, 0.4 ml of Ach (2 µg/ml). Each of the experiments was performed in triplicate.

Statistical analysis
Results were presented as mean ± standard deviation. Analysis of the result was done using one-way analysis of variance (ANOVA) and Tukey Kramer Multiple Comparisons was used to compare the difference between the means, and p<0.05 were considered to be statistically significant (Graphpad Instat, 2003).

Results
Phytochemical analysis
The results of the phytochemical analysis of the aqueous stem bark extract of D. senegalense is shown in Table 1. The results revealed the presence of carbohydrates, tannins, saponins, glycosides, terpenes and steroids, flavonoids and alkaloids.

Acute toxicity study
The administration of the aqueous extract at a dose of 5,000 mg/kg orally to a group of rats did not cause any death. However toxicity signs like depression,
Table 1: The phytochemical content of *Detarium senegalense* aqueous stem bark extract

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Terpenes/steroids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
</tbody>
</table>

Keys: - Not detected; + Present

Table 2: Effect of *Detarium senegalense* aqueous stem bark extract on castor oil-induced diarrhea in rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Mean number of defaecation in 6 hours</th>
<th>Percent protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline) + Castor oil</td>
<td>10.00 ± 1.23</td>
<td>0.00</td>
</tr>
<tr>
<td>Diphenoxylate (5mg/kg) + castor oil</td>
<td>0.00 ± 0.00*</td>
<td>100</td>
</tr>
<tr>
<td>Extract (100mg/kg) + Castor oil</td>
<td>5.00 ± 1.41*</td>
<td>49.90</td>
</tr>
<tr>
<td>Extract (200mg/kg) + Castor oil</td>
<td>1.20 ± 2.17*</td>
<td>87.62</td>
</tr>
<tr>
<td>Extract (400mg/kg) + Castor oil</td>
<td>0.92 ± 1.27*</td>
<td>90.50</td>
</tr>
</tbody>
</table>

Values are mean ± SD based on five observations

* - P<0.05 significant decrease compared to control

anorexia and difficulty in breathing were noticed in the treated rats.

**Effect of the aqueous extract on castor oil induced diarrhoea in rats**

The effect of the aqueous extract on castor oil induced diarrhoea in rats is shown in Table 2. The administration of the extract at doses of 100, 200 and 400 mg/kg body weight significantly (p<0.05) decreased the number of faeces by 49.90, 87.62 and 90.50 % respectively when compared to the control value. The standard drug (diphenoxylate) significantly (p<0.05) inhibited diarrhoea (100 % protection) in the rats when compared to the control group.

**Effect of the aqueous extract on intestinal transit of charcoal meal in rats**

The effect of the aqueous extract on intestinal transit of charcoal meal in rats is shown in Table 3. In the control group given saline, the charcoal meal travelled a distance of 62.50±12.61 cm which represents 57.54 % of the total intestinal length. The administration of the extract at 200 and 400 mg/kg body weight significantly (p<0.05) reduced the distance travelled by the charcoal meal to 36.83±1.75 cm and 29.10±3.82 cm respectively when compared to the control. These respectively represent 41.58 and 28.90 % of the total intestinal transit. The group treated with atropine exhibited the shortest transit of 9.03±0.50 cm when compared to the control. The group administered with 100 mg/kg of the extract had the highest distance travelled by the charcoal meal (66.70±1.66 cm) when compared with all other groups.

**Effect of the aqueous extract on castor oil induced enteropooling in rats**

Table 4 shows the effect of the aqueous extract on castor oil induced enteropooling in rats. Treatment of rats with 100, 200 and 400 mg/kg body weight of the extract resulted in 31.64, 41.24 and 23.01 percent fluid accumulation respectively. These values were not significant (p>0.05) when compared
Table 3: Effect of *Detarium senegalense* aqueous stem bark extract on gastrointestinal transit of charcoal in rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Total length of intestine (cm)</th>
<th>Distance travelled by charcoal (cm)</th>
<th>Percent Intestinal transit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>108.43 ± 7.78</td>
<td>62.50 ± 12.64</td>
<td>57.54</td>
</tr>
<tr>
<td>Extract (100mg/kg)</td>
<td>107.47 ± 8.68</td>
<td>66.70 ± 1.66</td>
<td>62.12</td>
</tr>
<tr>
<td>Extract (200mg/kg)</td>
<td>87.63 ± 5.91</td>
<td>36.83 ± 1.75*</td>
<td>41.58</td>
</tr>
<tr>
<td>Extract (400mg/kg)</td>
<td>97.80 ± 5.72</td>
<td>29.10 ± 3.82*</td>
<td>28.90</td>
</tr>
<tr>
<td>Atropine (3mg/kg)</td>
<td>71.10 ± 6.81</td>
<td>9.03 ± 0.50*</td>
<td>12.21</td>
</tr>
</tbody>
</table>

Values are Mean ± SD based on five observations
* - (P<0.05) significant decrease compared to control

Table 4: Effect of *Detarium senegalense* aqueous stem bark extract on castor oil-induced enteropooling in rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Weight of Intestine and content (g)</th>
<th>Weight of empty intestine (g)</th>
<th>Difference in weight (g)</th>
<th>Percent fluid accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>6.02 ± 2.27</td>
<td>3.78 ± 0.52</td>
<td>2.25 ± 1.93</td>
<td>37.03</td>
</tr>
<tr>
<td>Extract (100mg/kg)</td>
<td>5.24 ± 0.97</td>
<td>3.68 ± 0.31</td>
<td>1.69 ± 0.93</td>
<td>31.64</td>
</tr>
<tr>
<td>Extract (200mg/kg)</td>
<td>3.88 ± 0.33</td>
<td>3.38 ± 0.17</td>
<td>1.60 ± 0.30</td>
<td>41.24</td>
</tr>
<tr>
<td>Extract (400mg/kg)</td>
<td>4.34 ± 0.21</td>
<td>3.32 ± 0.42</td>
<td>1.00 ± 0.39</td>
<td>23.01</td>
</tr>
<tr>
<td>Atropine (3mg/kg)</td>
<td>5.24 ± 0.36</td>
<td>4.65 ± 0.38*</td>
<td>0.66 ± 0.24*</td>
<td>12.20</td>
</tr>
</tbody>
</table>

Values are Mean ± SD based on five observations
* - Significant (P<0.05) compared to control

- Acetylcholine at 0.1 ml produced amplitude of 4.13 ± 0.14 cm and at 0.2 ml it was 4.77 ± 0.26 cm, while at 0.4 ml, it was 5.07 ± 0.29 cm. The extract at 0.1, 0.2, 0.4 ml significantly (P<0.05) reduced the response of the jejunal tissue to acetylcholine to 2.27 ± 0.17 cm, 2.03 ± 0.27 cm and 2.33 ± 0.08 cm respectively when compared to both acetylcholine and the extract.

**Discussion**

The administration of the aqueous extract of *D. senegalense* stem bark orally at a dose of 5,000 mg/kg body weight did not cause any death in the treated animals. The absence of mortality or serious toxicity at this dose may be an indication of relative safety of the extract. According to OECD (2008), the LD$_{50}$ of any substance administered at a limit dose...
of 2000 or 5000 mg/kg using the up and down method of Dixon (1991) is higher than the limit dose if 3 or more of the rats out of 5 survived (within 14 days); such substance is therefore considered safe.

In this study, castor oil successfully induced diarrhoea in the experimental rats. This could be attributed to the fact that castor oil contains ricinoleic acid, which when released causes secretion of water and electrolyte in the small intestine as a result of the release of prostaglandins (Jebunnesse et al. 2009). Other mechanisms involved in castor oil induced diarrhoea include inhibition of intestinal Na⁺K⁺ATPase activity thus reducing normal fluid absorption, activation of adenylate cyclase and mucosal cAMP mediated active secretion (Meit et al. 2009). The ability of the aqueous extract of D. senegalense to cause a significant inhibition of castor oil-induced diarrhoea at all the doses used suggests that its mechanism of anti-diarrhoeal action may include decreased gastrointestinal secretion and/or inhibition of gastrointestinal motility. These are similar to the action of diphenoxylate, the standard drug used in this study which is known to inhibit gastrointestinal secretion and motility (Jafri & Pascicha, 2002).

The aqueous extract of D. senegalense stem bark also caused significant (p<0.05) decrease in peristalsis as evidenced by a significant decrease in intestinal transit of charcoal meal. This action prevents speedy evacuation of the stomach content and allows the intestinal contents enough time to be exposed to the absorptive surface of the intestinal tract which will enhance absorption of essential nutrient. The effect of the extract in this respect may be similar to that of atropine which was used as a standard drug in this work. Atropine is a known antimuscarinic agent (Thomas, 2016), which inhibits gastrointestinal motility. The antiperistalsis effect of the extract is further demonstrated by its ability in decreasing the contractile amplitude of isolated rabbit jejunum in the presence of acetylcholine. Acetylcholine acts through muscarinic subtype 2 (M₂) cholinergic receptors. It is therefore suggested that the extract acts on the muscarinic cholinergic receptor of the rabbit jejunum.

The aqueous extract of D. senegalense did not significantly reduced the percentage fluid accumulation caused by castor oil except in the groups of animals treated with the highest dose (400 mg/kg) which recorded the lowest fluid accumulation compared to the control. This may suggest that higher doses may be required to effectively reduce the fluid accumulation in the castor oil induced enteropooling. The phytochemical compounds present in D. senegalense extract may be responsible for the anti-diarrhoea effects seen in this study. Phytochemical investigation revealed that the extract contains alkaloids, tannins, flavonoids, terpenes and steroids, glycosides, carbohydrates and saponins. Flavonoids have been demonstrated to inhibit contraction induced by spasmogens (Abdullahi et al. 2001). Alkaloids have been known to have analgesic, anti-inflammatory and anti-diarrhoea effects (Neha, 2015). Tannins possess astringent properties (Pius et al. 2011). The proteins precipitated by tannins cover the surface of the cell or tissue and act as a barrier between tissue and irritants, with the underlying tissue soothed and protected from damage. This process could reduce intestinal mucous membrane secretions (Kerry & Simon, 2013). Sanni (2013) reported the presence of Zinc in the aqueous extract of D. senegalense. The decrease in the amount of accumulated intestinal fluid may also be due to the presence of zinc in the extract. Zinc has the ability to inhibit cAMP induced chloride dependent fluid secretion by blocking basolateral potassium ion channels (Chaitali & Vijay, 2011). Plant extracts are also reported to inhibit release of autacoids and prostaglandins, thereby

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*Figure 1: Mean ± SEM contractile response of rabbit jejunum to (1) Acetylcholine (2µg/ml), Detarium senegalense (100mg/ml) and (3) Acetylcholine (2µg/ml), plus Detarium senegalense (100mg/ml) a,b,c --- points with different alphabets are significant*
inhibiting motility and secretion by castor oil (Akter et al., 2010). The extract of D. senegalenense may have similar effects since it reduces fluid accumulation in castor oil-induced diarrhoea. In conclusion, the result of this study showed that the aqueous extract of the stem bark of D. senegalenense possesses antidiarrhoeal properties since it inhibited castor oil induced diarrhoea, decreased the gastrointestinal transit of charcoal and slightly reduced fluid enteropooling in the gut. This supports the traditional use of the plant for the treatment of diarrhoea.

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