Global Veterinaria 16 (1): 93-99, 2016
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2016.16.01.102115

The Antidiarrheal Activity and Phytoconstituents of the Methanol Extract of *Tecurium oliverianum*

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Abstract: The purpose of the present study was to evaluate the antidiarrheal activity and the phytochemical constituents of methanol extracts of *Tecurium oliverianum* using castor oil – induced diarrhea and gastrointestinal motility test using charcoal meal method. The methanolic extract was initially assayed for its effect in castor oil – induced diarrhea at different doses (125, 250, 500 and 1000 mg/kg b.wt.) followed by its evaluation on the peristaltic movements in charcoal meal test using atropine sulfate as a reference drug. The results of the present study indicated that, administration of the methanol extract of *Tecurium oliverianum* induced dose-dependent percentages of inhibition of diarrhea. The antidiarrheal activity of this plant might be due to its high contents of flavonoids and tannins. It could be concluded that, the remarkable antidiarrheal activity of *Tecurium oliveranium* attest to their utility in a wide range of stats of diarrhea.

Key words: Antidiarrheal · Phytoconstituents · *Tecurium oliverianum* · Flavonoids · Tannins · Rats · Castor oil

INTRODUCTION

The plant kingdom is rich in chemical constituents of antispasmodics that relieve colicky pain. Intact, most remedies used in conventional medicine include at least one antispasmodic of plant origin. They form a very important part of the treatment of gastrointestinal motility disorders [1]. The antispasmodics are considered useful for relieving or calming colicky pains resulting from spasms of the gut muscles and diarrhea due to hyper motility of the gastrointestinal tract. Among the wide range of plant-derived drugs that have relaxant activities on various smooth muscles, papaverine (*Papaver somniferum*) is the one, which is used in the treatment of colic [2]. Muscarinic antagonists like atropine (*Atropa belladonna*) inhibit the contractions of gastrointestinal tract induced by acetylcholine [3]. Relatively the less polar solvents like methanol and ethanol are very appropriate in extracting the spasmolytic agents of plants. The ethanol extract of *Capparis cartilaginea* inhibited the submaximal contractions of ileum induced by acetylcholine, histamine or serotonin [4]. The crude methanol extracts of *Erythrina sigmoidea* stembark were found to have potent anticholinergic effects by decreasing the tone and spontaneous activity of isolated rat ileum induced by carbachol and acetylcholine [5]. Plant-derived antispasmodics include some tropane alkaloids (atropine, hyoscine or scopolamine, hyoscycamine), opium alkaloids (papaverine, codeine, morphine), flavonoids (luteolin, cirsimartin, quercetin, rutin, apigenin, kaempferol, genkwanin) and essential oils (peppermint, caraway, dill, garlic, chamomile, anise) [6].

Teucrium species (family Lamiaceae) are known for their medicinal utilization and exhibit interesting biological properties such as hypoglycemic, hypolipidemic, hepatoprotective, antipyretic, anti-inflammatory, antiulcer, antitumor, antibacterial and insect antifeedant activities [7-12]. The genus *Teucrium* is one of the richest sources of diterpenes, with a neoclerodane skeleton and more than 220 diterpenes have been described [13]. Also, essential oils have been reported from the aerial parts of several *Teucrium* spp. and the percentage of the major chemical constituents (mainly monoterpene/sesquiterpenes hydrocarbons and oxygenated sesquiterpenes) differs notably from species to species [14]. *Teucrium polium*

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has been reported to possess antispasmodic, antimicrobial and anti-inflammatory [15], hepatoprotective effect [16] and analgesic properties [17]. Also *Teucrium chamaedrys* has been used as antimalarial, antispasmodic and for gastric pain, kidney disorders and heart diseases [18, 19]. Very little studies have been done about *Teucrium oliverianum* in spite of its distribution widely in the dry and stony places of the hills and deserts of almost all Mediterranean countries, South Western Asia, Europe and North Africa. The isolation of several compounds with a biologic significance [20-22] and the alcoholic extracts of *Teucrium oliverianum* induced a potent antinociceptive activity [23]. Ajabnoor *et al.* [24] recorded its antidiabetic and hypoglycemic potentials and more recently, Shahat *et al.* [25] found its activity against hepatocellular carcinoma and such effect could be attributed to hepatoprotective properties, antiproliferative activity and antiangiogenic potential. No available studies were recorded about its antidiarrheal activity. So the present study was designed to explore the antidiarrheal effects and the phytochemical constituents of methanol extract of *Teucrium oliverianum* using castor oil – induced diarrhea and gastrointestinal motility test using charcoal meal method.

MATERIALS AND METHODS

Animals: Westar albino mature male rats (180±20 g) were obtained from the Animal House of the Faculty of Medicine, Alexandria University, Egypt and housed at a temperature of 22 - 28°C and relative humidity of 50–60%, with artificial light from 5.00 a.m. to 4.00 p.m. Animals had free access to tap water and standard rat chow, used for the study. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication no. 85-23, revised 1996). The local ethics committee approved the study.

Preparation of Plant Material: The plants were collected at the flowering stage, shade dried and powdered plant materials were successively extracted as the following: About 300 gm of the powdered plant were soaked in 3000 ml methanol. It was left for 72 hours, the methanol extract was filtered using Whatman No.1 filter paper and the residue was removed with intermittent shaking till obtain methanolic extract. The extracts were filtered through Whatman No. 1 filter paper and concentrated until obtaining paste under vacuum using the rotary evaporator (Rotavapor R-215, Butchi, Switzerland). The extract was used for evaluation of the anti-diarrheal activity and phytochemical constituents-.

Phytochemical Analysis:

Total Phenolics and Total Tannins Content: Total phenolic content was determined using Folin-Ciocalteu's reagent [26] with some modifications. Few amount of residue (50 mg) was mixed with 2.5 ml of deionized water followed by 0.25 ml of Folin-Ciocalteu's reagent and allowed to react 6 min. Then 2.5 ml of sodium carbonate 7% was added and allowed to stand for 1 hr, then absorption at 765 nm was measured. Measurements were calibrated to a standard curve of prepared gallic acid solution and the total phenolic content was expressed as mg gallic acid equivalent per g of residue. Total tannin in the extracts was determined by a modification of the Folin-Ciocalteu method using poly vinyl poly pyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols [27]. About 100 mg of PVPP was added to 1ml sample extract diluted with 1ml water and left 15 min at 4°C. After centrifugation, PVPP forms a precipitate with tannins and the supernatant has only simple phenols. Simple phenols were determined using the Folin-Ciocalteu reagent as previously mentioned. The difference between total and simple phenol values represents the total tannin content, expressed as mg gallic acid equivalents/ g residue.

Total Flavonoids: The flavonoid content was measured using a colorimetric assay [28]. A known weight of extract residue dissolved in 1 ml methanol was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% w/v sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% (w/v) $AlCl_3$ was added and, after 6 min, 2 ml of 1M NaOH were added to the mixture, followed by the addition of 2.1 ml distilled water. Absorbance was read at 510 nm against the blank (water) and flavonoid content was expressed as mg quercetin equivalents/ g residue.

Anti-Diarrheal Activity of Plant Extract

Castor Oil-Induced Diarrhea: The method of Mukherjee *et al.* [29] and Rani *et al.* [30] was applied. Westar albino mature male rats (180±20 g body weight) were used. Six groups of five animals were used. Groups 1-4 were given respectively, 125, 250, 500 and 1000 mg/kg b.wt. plant

extract orally. Group 5 was given atropine sulfate orally (5 mg /kg) as a reference anti-diarrheal drug. While group 6, was given 2% tween 80(v/v) and kept as control. One hour after administration of the extracts, all animals received 2 ml of castor oil orally and placed in the standard cages supplied with changeable filter paper. The number of total and soft fecal pellets was calculated at 1, 2, 3 and 4 h after castor oil administration.

Effect of Plant Extract on Gastrointestinal Motility in Rats (Charcoal Meal Test): Five minutes after drug administration, 0.5 ml of a 5% charcoal suspension in 10% aqueous solution of gum acacia powder was administered orally to each animal. The animals were killed 30 min later and the abdomen was opened. The percentage distance of the small intestine (from the pylorus to the caecum) moved by the charcoal meal was calculated [31]. Percentage-inhibition = Mc – Md / Mc x 100. Mc: mean distance travelled by charcoal meal; Md: mean distance travelled by drug or extract.

Statistical Analysis: Results were expressed as the mean ± standard error (S.E.M.). All statistical analyses were performed by ANOVA and Duncan's multiple-range test with a value of $p \leq 0.05$ selected as the cut-off for statistical significance.

RESULTS

Phytochemical Investigation: The quantitative phytochemical investigation of the tested plant showed the presence of total phenolics, tannins and flavonoids in a different quantities respectively, 27.25 ± 2.12 mg/g, 14.24 ± 0.93 mg/g and 12.55± 0.78 mg/g of methanolic residues (n= 5).

Anti-Diarrheal Activity of Plant Extract on Castor-Oil Induced Diarrhea in Rats: As shown from Table 1, it was observed that, administration of the methanol extract of *Tecurium oliverianum* (125 and 250 mg/kg b.wt.), induced insignificant decrease in the total number of feces and number of diarrheal feces and percentages of inhibition of diarrhea. While, the administration of (500 and 1000 mg/kg b.wt.) significantly affect the total number of feces and number of diarrheal feces.

Gastrointestinal Transit Using Charcoal Meal (Marker) in Rats: As shown from Table 2, it was observed that administration of the methanol extract of *Tecurium oliverianum* (125 and 250 mg/kg b.wt.), induced insignificant effect in gastrointestinal transit using charcoal meal (marker) in rats. While at 500 and 1000 mg/kg b.wt. the extract significantly decreased the gastrointestinal transit using charcoal meal (marker) in rats.

DISCUSSION

Diarrhea is a very common problem in many tropical countries and causes deaths through the world annually [32]. The use of herbal drugs in the treatment of diarrheal diseases is a common practice in many countries. Several medicinal plants have been reported to be effective against diarrhea. It was found that the aqueous extract of *Evodia rutaecarpa* fruit had both anti-transit effect and anti diarrheal effects in mice [4]. Relatively the less polar solvents like methanol and ethanol are very appropriate in extracting the spasmolytic agents of plants. Sunilson *et al*. [33] reported that the ethanol extract of *Capparis cartilaginea* inhibited the submaximal contractions of ileum induced by acetylcholine, histamine or serotonin. Many plants like *Andrographis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata* and others were used for the same purposes [34]. Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the alimentary tract, accompanied by an excess loss of fluid in the feces. In some diarrheas, the secretory components predominate, while other diarrheas are characterized by hyper motility [34]. The use of castor oil induced – diarrhea as a model is logical in our study because the autacoids and prostaglandin are involved in the causation of diarrhea [35, 36]. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandin which stimulates motility and secretion [37].

The results of the present study showed that, the methanol extract of the *Tecurium oliverianum* in a dose – dependent manner produced a significant reduction in the severity and frequency of diarrhea produced by castor oil. Additionally, this extract significantly reduced the castor oil induced intestinal transit. An increase in intestinal transit time by atropine sulfate could also result from reduced in gastric emptying [38]. Castor oil is also reported to induce diarrhea by increasing the volume of intestinal content by preventing the re-absorption of water. Thereby, it prevents the reabsorption of sodium chloride (NaCl) and water. Probably, the extract increased re-absorption of NaCl and water by decreasing the intestinal motility as observed by decreasing the intestinal

Table 1: Effect of the methanol extract of *Teucrium oliverianum* on castor-oil induced diarrhea in rats.

Plant name	Total number of feces	Number of diarrheal feces	% inhibition of diarrhea
Teucrium oliverianum (125 mg/ kg)	18.9 ± 0.90	16.6 ± 1.28	7.77
Teucrium oliverianum (250 mg/ kg)	18.7 ± 1.03	15.8 ± 1.12	12.22
Teucrium oliverianum (500 mg/ kg)	13.2 ± 0.45*	9.1 ± 0.65*	49.44
Teucrium oliverianum (1000 mg/ kg)	12.6 ± 0.70*	7.0 ± 0.73*	61.11
Atropine sulfate (5 mg/kg)	10.2 ± 0.73*	5.4 ± 0.59*	70.00
Control	20.6 ± 1.50	18.0 ± 1.51	---------

* Significantly different compared to corresponding to control $p \leq 0.05$. Values are expressed as means ± s.e. (n=5)

Table 2: Effect of the methanol extract of *Teucrium oliverianum* on gastrointestinal transit using charcoal meal (marker) in rats

Plant name	Total length of intestine (cm)	Distance travelled by marker (cm)	% intestinal transit	% of inhibition
Teucrium oliverianum (125 mg/ kg)	89.7 ± 5.23	65.6 0± 4.23	72.52 ± 5.19	7.42
Teucrium oliverianum(250 mg/ kg)	88.2 ± 7.21	63.40 ± 4.12	70.34 ± 5.70	10.21
Teucrium oliverianum (500 mg/ kg)	92.2 ± 8.32	57.45± 6.43	61.25 ± 3.00*	21.81
Teucrium oliverianum (1000 mg/ kg)	84.6 ± 5.22	46.33 ± 4.29	52.15 ± 6.62*	33.43
Atropine sulfate (5 mg/kg)	87.4 ± 4.79	33.80 ± 7.32	39.95 ± 9.25*	49.00
Control	91.8 ± 5.85	72.20 ± 7.07	78.34 ± 5.30	---

* Significantly different compared to corresponding to control $p \leq 0.05$. Values are expressed as means ± s.e. (n=5)

transit by charcoal meal. The antidiarrheal activity of the extract may be also due to the presence of high concentrations of tannins and flavonoids reported at this study. The antidiarrheal activity of medicinal plants was found to be due to tannins, alkaloids, saponins and flavonoids [39]. These ingredients are known for inhibiting autacoids and prostaglandin, thereby inhibiting the motility and secretion. Flavonoids are natural products, which exhibit various pharmacological effects [40]. Quercetin, one of the flavonoids isolated from aerial parts of *Conzya flaginoides*, caused a concentration dependent inhibition of spontaneous contractions of rat ileum [41] and showed anti diarrheal activity against castor oil-induced diarrhea in mice. It also exerted inhibitory effects on guinea pig ileum contractile response [42]. Rutin, another flavonoid in *Artemisia scoparia*, was found to cause a concentration dependent inhibition of spontaneous movements of rabbit jejunum [43]. Besides, tannins extracted from many plants also have exhibited an anti diarrheal activity. Tannins, a unique group of phenolic metabolites with the property of precipitating proteins, are commonly found in plants such as apple fruit, pine bark, grape seed, tea, oak and medicinal plants and possess a variety of biological effects, including anticarcinogenic, antimutagenic, antimicrobial and antioxidative activities [44]. The mechanisms of their antidiarrheal activity are those by inhibiting cystic fibrosis transmembrane conductance regulator protein chloride channels and by generating protein-precipitating reaction to the gastrointestinal mucosa due to the protein precipitating action [45]. For evaluation of relationship between antidiarrheal activity and the total content of tannins and flavonoids in the studied extract, the results showed a clear positive relationship between the highest total content of tannins and flavonoids and the strongest antidiarrheal in a dose-dependent manner.

Studies have been done previously on some plants having higher contents of flavonoids and essential oils and agree with our results. Flavone cirsimartin, which is isolated from *Artemisia judaica*, *Artemisia capillaris*, *Artemisia xerophytica* and *Artemisia scoparia* is responsible for the spasmolytic activity of isolated guinea pig ileum [46]. Four flavones with spasmolytic activity were isolated from the aerial parts of *Artemisia abrotanum*, which are the active principles for smooth muscle relaxing activity of the plant [47]. The essential oil of *Achillea ageratum* L. was found to be an effective spasmolytic agent capable of inhibiting acetylcholine and $BaCl_2$ induced contraction of isolated rat duodenum and the leaves extract has also showed anti diarrheal effect against castor oil induced diarrhea in mice [48]. The essential oils of *Artemisia thuscula Cav.* flowers and *Artemisia alba* were investigated and shown to have dose-dependent and essentially non-competitive spasmolytic effects in guinea pig ileum [49].

CONCLUSION

It could be concluded that, the remarkable anti diarrheal effects of *Tecurium oliverianum* attest to their utility in a wide range of stats of diarrhea and a further studies are required to isolate a more active constituents in this plant.

Conflict of Interest: The author has declared that there is no conflict of interest related to this paper.

ACKNOWLEDGEMENT

The author would like to express his profound gratitude and sincere appreciation to Dr. Ibrahim M. El-Ashmawy, Prof. of Pharmacology, Faculty of Veterinary Medicine, Alexandria University for the preparation of the plant extract.

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