Original Research Article

Evaluation of Haemostatic effectsof `(Parsley) in Wistar rats

Comment [A1]: Scientific name of plant

Abstract

Petroselinum crispum (parsley or garden parsley), besides its use as an edible vegetable, is used in the treatment of various ailments with documented evidence validating some of these medicinal uses. The present study evaluates the impact of oral administration of P. *crispum*on hemostatic parameters. Fresh leaves of P.*crispum*were locally sourced, authenticated and extracted using ethanol to obtain an ethanolic extract of P. *crispum*(PCE). Twenty-five (25) male Wistar rats were used for the study and were randomly assigned to five (5) animals of five (5) rats each. Group I served as the control group and was given distilled water, whereas groups II, III, and IV were treated as experimental groups and administered PCE at 200, 400, and 800mg/kg via oral gavage, respectively. Group V also received 800mg/kg of PCE but the administration was terminated on the 28th day and allowed to recover for 14 days. Platelet count (PLT) and other platelet indices were determined using an automated Haematology analyzer. Bleeding time (BT), clotting time (CT), prothrombin time (PT) and activated partial thromboplastin time (aPTT) and plasma fibrinogen (FIB) were assayed following standard laboratory protocols. The result of the study showed that mean values of PLT were significantly reduced compared to the control ($p<0.05$) while BT, CT, PT, aPTT and FIB significantly increased compared to the control ($p<0.05$). Also, BT, CT, PT, and aPTT recovered towards the control values after the 14-day withdrawal period. The present study shows ethanolic leaf extract of *P. crispum*prolonged blood coagulation. The current evidence suggests that P. *crispum* could be a candidate for further explorationas a naturalplant-based antithrombotic agent.

Comment [A2]: italics

Comment [A3]: italics

Keywords: *Petroselinum crispum*, haemostasis, bleeding time, clotting time, prothrombin time, activated partial prothrombin time.

INTRODUCTION

Petroselinum crispum, commonly referred to as parsleyor garden parsley is a fragrant and flavorful herb that belongs to the Apiaceae family[1, 2]. It is native to the Central and Eastern Mediterranean region but naturalized and used in Europe, Middle Eastern and American cuisine. It has been employed as food, perfume and cosmetics and in the treatment of various ailments [3, 4]. It is grown worldwide for its edible leaves, which can be used fresh or dried as a seasoning. It is also used as a garnish for salads, snacks, vegetable soups, stews and casseroles. The ancient Greeks and Romans used it in funeral decoration and as deodorants [1, 5]. *P.crispum*has found use in traditional medicine in different parts of the world in the management of various illnesses, including gastrointestinal disorders, amenorrhea, dysmenorrhea, diabetes mellitus, skin diseases,

fever, ulcers, rheumatism, and hypertension. It has been found useful as an anti-inflammatory, anti-urolithiasis, carminative, gastrotonic, antiseptic and diuretic[1, 4, 6, 7].

Several bioactive compounds and phytochemical constituents have been extracted from various parts of P.*crispum*, including its seeds, roots, leaves, and petioles. These compounds are predominantly composed of carbohydrates, flavonoids such as apigenin, quercetin, isorhamnetin, luteolin,chrysoeriol and eugenol. Also, coumarins and essential oils such as myristicin and apiol, and othercompounds[1, 8, 9].Furthermore, the plant is a good source of iron, phosphorous, calcium, zinc and vitamins A & C [10-12]. Screened pharmacological actions of P.*crispum*include antimicrobial [13, 14], anti-inflammatory [15, 16], antioxidant[9, 17], anti-diabetic[18, 19], immunomodulatory [20, 21], genitourinary[22, 23], neuroprotective[24], analgesic[25, 26], spasmolytic[27, 28], anti-cancer [29, 30], anti-hypertensive [4], and anti-platelet [31] activities. Despite of array of documented pharmaceutical benefits, *P. crispum* and its essential oils have to been demonstrated to be hepatotoxic and nephrotoxic at dosages equal to or higher than 1000mg/kg [32].

Haemostasis refers to the process of stopping or preventing bleeding in response to damage to blood vessels. This involves a series of physiological events that result in the production of a localized clot at the site of injury in a very short amount of time, usually seconds to minutes[33, 34]. When a blood vessel is damaged, the body naturally produces a reflex vasoconstriction to slow the loss of blood and allow more time for the formation of a platelet plug and the initiation of blood coagulation.The process of haemostasis involves the formation of a platelet plug, followed by the activation of coagulation to form a fibrin mesh, fibrinolysis, and eventually the repair of the blood vessel[33, 35]. The haemostatic cascade involves the intrinsic and extrinsic pathways, which originate differently but converge at the point of fibrin activation to stabilize the platelet plug. The intrinsic pathway is activated by trauma within the vascular system, while the extrinsic pathway is activated by trauma outside the vascular system[36, 37]. This process largely relies on the platelet count and function, as well as other coagulation parameters to achieve a steady balance between the fluid and solid states of blood and prevent pathological thrombosis.Thrombosis is the formation of a blood clot within a blood vessel, which can partially or completely obstruct blood flow leading to cardiovascular complications and death [38, 39]. Due to the negative side effects, expensive prices, and limited availability of synthetic medications, the utilization of plant-derived natural substances derived from plants has increasingly gained popularity and has become the primary approach in human pharmacotherapy[40-42]. This has led to the screening of possible natural anti-thrombotic agents derived from natural products .Although there has been increasing interest in exploring the pharmacological properties of P. *crispum* , the impact it has on haemostasis and its potential as an anti-thrombotic agent remain unclear and have yielded conflicting findings. This present study therefore, aims to evaluated the haemostatic effect of P. *Crispum*using Wistar rat models.

Comment [A4]: evaluate

MATERIALS AND METHODS

Source and Preparation of Plant Extract

Fresh leaves of *P. crispum* were procured from Fruit Garden Market, Port Harcourt, Nigeria and authenticated at the Plant Science and Biotechnology Department of the University of Port Harcourt. The leaves were carefully washed to eliminate any sand or dirt and allowed to air dry at room temperature for four (4) weeks. Following air-drying, the leaves were subjected to Soxhlet extraction using 99.5% ethanol[43]. The resulting extract of *P. crispum* (PCE) was stored at a temperature of 5ºC until administration**.**

Experimental Animals

For the study, a total of 25 male Wistar rats weighing between 150 to 200g were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt. These rats were given two weeks to acclimate during which they had access to standard rat pellets and water. The rats were kept in standard cages with appropriate ventilation, temperature, and humidity, and were subjected to a regular 12-hour light/dark cycle. Throughout the study, the animals were treated in accordance with the Animal Use and Care Guidelines of the National Veterinary Research Council of Nigeria.

Experimental Design

A total of 25 male Wistar rats were divided into five groups of five animals each, through a random allocation process. Group I served as the control group and was given distilled water, whereas groups II, III, and IV were treated as experimental groups and administered with P. *crispum*ethanolic extract at 200, 400, and 800mg/kg via oral gavage, respectively. Group V also received 800mg/kg of PCE. However, the administration of PCE was stopped for Group V after 28 daysand they were given access to standard rat pellets and water for 14 days to recover.

Comment [A5]: what solvent is used.. is it directly fed..or mixed with pellet

Laboratory Analysis

Determination of Bleeding Time

Bleeding time (BT) was determined using a modified Duke's method [44, 45]. A surgical blade was utilized to create a small incision at the end of each rat's tail. This incision was sufficient to cause bleeding, and a stopwatch was started as soon as the first drop of blood was visible. Every 15 seconds, the bleeding area was gently cleaned using a dry cotton wool swab, and the stopwatch was stopped once the bleeding stopped completely. The time taken for the bleeding to stop was recorded for each rat as thebleeding time.

Determination of Clotting Time

Clotting time (CT) was assayed using a modifiedIvy's method[45, 46]. The tail tip of each rat was cut using a dissection blade to initiate bleeding. A single drop of blood was collected from the incision on a clean and dry microscope slide, with the stopwatch started simultaneously as the blood came in contact with the slide. At 15-second intervals, the tip of a clean and dry pin was cautiously passed through the blood droplet on the slide, and the stopwatch was stopped immediately upon observation of a fibrin thread, this was recorded as the clotting time.

Determination of Platelet Count and Clotting Parameters

First, the animals were humanely anaesthetized by cervical dislocation and blood samples were taken directly from animals by cardiac puncture. These samples were collected in two types of bottles: EDTA bottles for platelet analysis and sodium citrate bottles for coagulation tests. The platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV), platelet distribution width (PDW) and platelet large ratio(PL-R)were measured using an automatic haematology analyzer (Mindray, China) while the prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen (FIB) were analyzed using the protocol provided by standard assay kit (Helena Laboratories, USA).

Ethical Considerations

The animals received care and handling in compliancewith the Animal Use and Care Guidelines set forth by the National Veterinary Research Council of Nigeria and the guide for the care and use of laboratory animals[47]. The research protocol and design were approved by the University of Port Harcourt Research Ethics Committee (UPH/CEREMAD/REC/MM87/030).

Statistical Analysis

Data were analyzed using IBM Statistical Product and Service Solutions (SPSS version 25). For each research group, the mean and standard error of the mean for each parameter was calculated. The mean values of the experimental groups (II, III, IV, and V) were compared to the control group (Group I) using analysis of variance (ANOVA), followed by a least significant difference (LSD) posthoc analysis. A p-value less than 0.05 was considered statistically significant.

RESULTS

Table 1: The effect of *P. crispum* on platelet count and platelet indices of male Wistar rats

Parameters	Control (n=5)	200mg/kg PCE (n=5)	400mg/kg PCE (n=5)	800mg/kg PCE (n=5)	800mg/kg PCE + Recovery (n=5)
PLT ($x10^9$/L)	456.00±46.05	492.80±79.81	230.40±52.29*	361.40±70.69	487.60±93.07
PCT (%)	0.33±0.4	0.37±0.07	0.14±0.06	0.26±0.05	0.38±0.07
MPV(fL)	7.24±0.11	7.46±0.11	7.24±0.16	7.42±0.22	7.68±0.13
PDW (%)	10.00±0.13	10.32±0.16	9.98±0.22	10.3±0.36	10.60±012
PL-R	12.30±0. ±97	12.32±0.84	10.30±1.32	12.16±1.73	13.84±0.61

Result is given as mean±standard error of mean;*significantly different compared to control ($p<0.05$)

Table 1 displays the impact of oral administration of ethanolic leaf extract from *Petroselinum crispum*on male Wistar rats' platelet count and other platelet indices for 28 days, followed by a 14-day stoppage of administration for animals in Group V. The findings indicate a significant decrease in platelet count among animals in Group III (400mg/kg PCE) compared to the control ($P<0.05$). Additionally, during the 14-day recovery period, the platelet counts increased, with no significant difference observed between Group V (800mg/kg PCE + recovery) animals and the control ($p>0.05$).

Table 2: The effect of *P. crispum* on some coagulation parameters of male Wistar rats

Parameters	Control (n=5)	200mg/kg PCE (n=5)	400mg/kg PCE (n=5)	800mg/kg PCE (n=5)	800mg/kg PCE + Recovery (n=5)
BT (secs)	73.20±6.47	93.80±9.52	95.80±5.52	109.80±6.36*	72.160±10.83
CT (secs)	45.00±3.2	62.007.89	68.00±8.74	79.40±14.97*	40.40±5.33
PT (secs)	18.80±2.08	24.40±1.80	26.60±2.37	31.80±7.33*	17.00±1.14
aPTT (secs)	16.20±0.60	18.40±3.12	21.20±1.20	22.60±1.96*	19.40±1.81
FIB (mg/dL)	174.40±8.63	246.40±7.13*	240.80±9.28*	252.00±7.67*	257.2±8.16

Result is given as mean±standard error of mean;*significantly different compared to control ($p<0.05$)

Table 2 shows the effect of oral administration of ethanolic leaf extract from *Petroselinum crispum*on the bleeding time, clotting time, prothrombin time, activated partial thromboplastin time and fibrinogen on male Wistar rats after 28-day oral administration followed by 14 days recovery period for animals in Group V. The results show that the BT, CT, PT and aPTT was significantly raised for rats in Group IV (800mg/kg PCE) compared to the control ($P<0.05$). Similarly, the mean FIB level increased for all the experimental groups (II, III, IV & V). While the 14-day recovery period lead to a reduction in BT, CT, PT and aPPT, the FIB continued to increase when compared with the control ($P<0.05$).

DISCUSSION

Due to the high cost of antithrombotic treatment, there is now a focus on exploring various natural and plant-based alternatives for potential antithrombotic agents. With over 400 promising natural substances in different phases of clinical evaluation as possible antithrombotic agents [2, 48], the present study evaluated the effect of ethanolic extract of *P.crispum*on haemostasis and its potential as an anti-thrombotic agent using Wistar rat models.

Effect on Platelet Count and Other Platelet Indices

There was a significantly reduced platelet count for animals supplemented with 400mg/kg (Group III) of PCE compared to the control ($p<0.05$) (Table 1). Discontinuation of the administration of PCE appeared to improve the platelet raising the mean values to 487.60±93.07($x10^9$/L) for group IV compared to the control value of 456.00±46.05 ($x10^9$/L)after 14 days of recovery. No significant difference was observed in mean PCT, MPV, PDW and PL-R between the experimental groups and control. Platelets are the smallest blood cells with primary function in the process of haemostasis, the body's natural response to stop bleeding. When there is an injury to a blood vessel, platelets become activated and clump together to form a plug, which stops the bleeding. They also release chemicals that attract other cells to the site of injury and promote healing[49, 50]. Platelets can contribute to the development of diseases either directly, as a primary disorder of their function or quantity, or indirectly, through their critical role in blood clotting, which can lead to conditions like coronary artery disease, stroke, peripheral vascular disease, and diabetes[51, 52]. The significantly reduced platelet count

observed among the animals that received 400mg/kg PCE could be attributed to the reported anti-platelet aggregation activities of *P. crispum*[2, 31], hence PCE may have interfered with clot formation by reducing platelet counts. Furthermore, platelet counts have been reported to increase with inflammation [52, 53] while *P. crispum* has been shown to possess anti-inflammatory activity [15, 16], hence it is possible that *P. crispum*attenuates inflammation by reducing platelet count. The improved platelet count among the Group V animal appears to be due to the cessation of the effect of PCE to interfere with platelet production or destruction. Shcherazade*et al*., observed no significant difference in platelet count among phenylhydrazine-induced anaemic mice treated with *P. crispum*[54]while Al-Daraji*et al*., reported that dietary supplementation of local geese with *P. crispum* as a food additive resulted in increased platelet count [55].

Effects of Coagulation Parameters

The result of the present study shows significantly raised BT, CT, PT and aPTT was significantly raised for the highest dose of PCE (800mg/kg) compared to the control ($p<0.05$)(Table 2). coagulation parameters evaluate the clotting ability of the blood while measuring various compoentsof blood clothing These tests measure various components of the clotting process. These prolonged clotting parameters may be due to the potent anti-platelet aggregation activities of *P. crispum*[2, 31]. The activation and aggregation of blood platelets serve a significant physiological purpose in the process of haemostasis. There is substantial evidence suggesting that numerous cardiovascular disorders are connected to abnormal and excessive activation of platelets[31, 53]. Platelet activation and aggregation may have been disrupted by PCE, which could have led to the prolongation of BT, CT, PT, and aPTT. The study also observed that following a 14-day recovery period, BT, CT, PT and aPTT values returned towards normal compared to the control suggesting that reduced coagulation could be due to the termination of the effect of PCE to inhibit platelet aggregation. Plasma fibrinogen was significantly increased among the experimental groups compared to the control with the increase continuing after the caseation of the PCE supplementation (Table 2). Plasma fibrinogen is a vital component of the coagulation cascade and serves as a major factor in thedetermination of blood flow and viscosity [56]. It basically serves to create fibrin, which aids in the formation of a hemostatic plug by binding platelets and plasma proteins which ensures the caseation of bleeding [57]. Although increased plasma fibrinogen concentration is associated with enhanced blood clotting (procoagulant), plasma fibrinogen at certain concentrations has been associated with anti-coagulant activities [58]. While the elevated plasma fibrinogen following PCE administration may be clearly understood, it is possible that the elevated FIB, in this case, acted as an anticoagulant leading to the observed prolonged BT, CT, PT and aPTT. Chao*et al.*, observed that lower plasma concentrations enhanced platelet aggregation while higher concentrations inhibited platelet aggregation [59]. Similarly, it has been observed that following low tissue factor stimulus, elevated fibrinogen served as an anticoagulant by prolonging clotting time and decreasing clot stability suggesting that increased fibrinogen may not be thrombogenic as previously conceived [58]. It also possible that PCE may have in some way affected the production of fibrinogen in the liver.

Comment [A6]: components

CONCLUSION

The result of the present study shows that ethanolic extract of *Petroselinum crispum* reduced platelet count, increased, clotting, clotting, prothrombin and activated partial thromboplastin time as well as plasma fibrinogen concentration. The current evidence suggests that P. *crispum* could be a candidate for further explorationas a natural plant-based antithrombotic agent.

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