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The Effects of Crude Extracts and Fractions of *Alchemilla abyssinica* on Smooth Muscle of Guinea-pig Ileum: An in Vitro Study

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Abstract **Background:** *Alchemilla abyssinica* is a plant widely used in traditional medicine. Its wide use among the community plus already established scientific evidences for medicinal values of other *Alchemilla* species provided good ground for this investigation. **Methods:** In this research, $CHCl_3$/EtoAc 1:1 extract of dried aerial parts of *Alchemilla abyssinica*, methanolic extract of the $CHCl_3$/ EtoAc residue and fractions of the methanolic extract were tested on isolated guinea-pig ileum (GPI) for possible presence of spasmogenic or spasmolytic effects. Concentrations of each extract and fraction ranging from 20-600 µg/ml final organ bath concentration were tested. The effects of these test samples on the basal rhythmic contractions of the GPI as well as on its contraction elicited using the agonist, histamine, were determined. The antagonist, Papaverine, was also used as a control smooth muscle relaxant. **Results:** While the $CHCl_3$/EtoAc 1:1 extract showed neither spasmogenic nor spasmolytic result, the methanolic extract showed marked spasmolytic effect. This methanolic extract was fractionated using column chromatography and the fraction eluted using Hexane/EtoAc 1:2 gave greatest spasmolytic result. This fraction produced significant ($P<0.05$) dose-dependent spasmolytic effects on the agonist induced contractions of the GPI to 95.7% at 20 µg/ml, 43.6% at 70 µg/ml and 14.2% at 120 µg/ml in the organ bath. **Conclusions:** The results of the present study showed that *Alchemilla abyssinica* possesses spasmolytic property. The oral acute toxicity study showed *Alchemilla abyssinica* exhibited no toxicity up to doses of 1,000 mg/kg body weight in Swiss albino mice. Further chemical work to identify the compound(s) responsible for the activity is recommended.

Keywords: *Alchemilla abyssinica, spasmolytic, guinea-pig ileum*

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1. Introduction

Traditional medicine encompasses knowledge, skills and practices accumulated in different cultures. In one way or another it serves in the maintenance of health plus fight against physical and mental disorders [1]. Natural products have been in use for medicinal purpose as old as some 4,000 years back [2]. According to another description herbalism can be considered to be synonymous with traditional medicine, botanical medicine, folk medicine or phytotherapy and it is an exercise of using plants and plant extracts for health care [3]. *Alchemilla abyssinica* Fresen. (Local name in Oromiffa - Hindrif / Endrif) is a robust herb with decorated basal that grows in moist montane forests as well as on moist places in somewhat overgrazed moorland and on rocky slopes; 2,500-4,400 m above sea level. *Alchemilla abyssinica* grows in Tigray upland, Wollo upland, Gondar, Gojam, Shewa upland, Balle, Harar, Sidama and Kenya [4,5]. Regarding the medicinal value of the plant the leaves of *A. abyssinica* are crushed and tied on to open wounds promoting blood clotting and facilitating wound healing [6]. The medicinal value of genus *Alchemilla* are so diverse which include: promoting blood clotting and facilitating wound healing, treating infections of the mouth and throat, treat menorrhagia and menstrual cramps, treating obesity, gastrointestinal pain, and inflammation among others [6,7,8]. Considering the traditional importance of the specific plant as well as publications on other species of the genus in vitro experiments have been conducted in the present work. Thus, in this paper the results of the fresh leaf extracts and fractions of *A. abyssinica* on smooth muscle preparations are presented.

2. Materials and Methods

2.1. Plant Collection

Areal part of *A. abyssinica* Fresen was collected from the Bale Mountains National Park near Dinsho town (500 km south of Addis Ababa), in March, 2007. The collected

specimen was transported according to the standard protocol and it was compared with the already existing collection of the same species in the National Herbarium of Addis Ababa University and was authenticated by a taxonomist. The representative plant specimen was kept in the Herbarium of ALIP and was labeled.

2.2. Extraction and Fractionation

Alchemilla abyssinica aerial parts were openly dried at room temperature; the dried parts were ground into fine powder using mortar and pestle. Pilot study was conducted on both H_2O and hydro alcoholic (80% MeOH:20%H_2O) extracts of the dried samples and the hydro alcoholic extract shown greater spasmolytic activity on smooth muscles. The investigation involved bioassay guided fractionations, the fractionation which proceeded forward as a minimum of two tissue experiments strongly suggested that an extract or a fraction can be regarded to have spasmolytic effect. Moreover, whenever two or three of the many fractions shown to have spasmolytic effects, only the one that highly surpasses all have been taken in to consideration and the others that have shown slight spasmolytic effect were omitted for this investigation. Powdered aerial part of *Alchemilla abyssinica*, 74 g, was soaked using $CHCl_3$/ EtoAc 1:1 (300 ml) for 8 h on shaker. This content was filtered using Whatmann No. 1 filter paper and gave $CHCl_3$/EtoAc extract, this was checked for its effect on smooth muscles on the Polygraph and the residue in the filtration above was soaked using MeOH (300 ml) for 8 hrs on a shaker two times and gave MeOH extract. This MeOH extract was checked for activity on smooth muscles on the Polygraph. The MeOH extract, 8 g, was adsorbed on 15 g of silica gel and was eluted using six solvent systems: Hexane/EtoAc 1:1 and 1:2, EtoAc 100%, EtoAc/MeOH 2:1 and 1:1, and MeOH 100%. Out of these six fractions, fraction 2 (Hexane/EtoAc 1:2 fraction) gave the highest spasmolytic effect and it was taken as the final test fraction.

2.3. Test animals

Swiss albino mice (25–30 g) and guinea-pigs (300–400 g) were obtained from the animal house of Faculty of Life Science of Addis Ababa University and Ethiopian Health and Nutrition Institute respectively. These animals were housed at a temperature of 24 ± 2°C and maintained under uniform conditions of 12 h daylight and 12 h dark cycles. The mice were used for toxicity studies; while the guinea-pigs for the organ bath experiment involving fractions and final test sample. They were given a standard diet and tap water *ad libitum* based on previous works [9].

2.4. Ethical Considerations

This investigation was conducted after ethical clearance on the use of experimental animals was obtained from the Institutional Review Board of College of Health Sciences, Addis Ababa University.

2.5 Acute Toxicity Test

Swiss albino mice of both sexes (25-30g) were divided into five groups of five mice each and fasted for 4 h. The test was performed using increasing doses of *Alchemilla abyssinica* Hexane/EtoAc 1:2 fraction, the final test sample used for test on the Polygraph was dissolved in DMSO /distd H_2O (1:9) solvent system. This solution was administered orally at concentrations of: 100, 400, 700 and 1,000 mg/kg body weight. To the fifth, control, group the vehicle, DMSO/distd H_2O (1:9), was administered [10,11,12]. The mice were allowed food and water *ad libitum* during a 24 h test period and kept under regular observation for mortality and any behavioral change during the test period [13].

2.6. Organ Bath Procedures

Every time a tissue was required a guinea-pig of either sex was fasted overnight and was sacrificed by a gentle blow on the head and then bled from the neck. The abdomen of each animal was opened, and the ileum was removed and cleaned of attached tissues. A segment of the removed ileum (2–3 cm) from each guinea-pig, was used. Tyrode's solution of the following composition (mM), NaCl, 137; KCl, 2.6; $MgCl_2$, 1.05; $CaCl_2$, 0.3; NaH_2PO_4, 0.04; $NaHCO_3$, 11.9; $C_6H_{12}O_6$, 5.5 was used as previously described by [9,14]. The above chemicals were procured from Sigma Chemical Company, St. Lous, MO, USA. The segments of ileum were tied with threads at both ends in opposite directions and suspended in a thermo regulated 25 ml organ bath containing Tyrode's solution which was maintained at 37°C and gassed with air. A tension of 1 g was applied to each tissue and then allowed to equilibrate for at least 30 min before adding histamine, the agonist. The responses were recorded isometrically using a Grass FT.03 strain gauge transducer connected to a Grass Model 7 Polygraph (Grass Instruments Quincy, MA, USA). Dose response curves of the histamine induced contractions were done for all the tissue preparations and the histamine concentration that effected submaximal stimulation was taken as the control histamine in each experiment. Histamine and extract amounts are expressed as final organ bath concentrations.

2.7. Statistical Analysis

The results were analyzed statistically using one-way ANOVA Scheffe post-hoc comparison between the control histamine contraction and contractions in response for the presence of the fraction using SPSS 14 statistical software package. The values $P<0.05$ were regarded as statistically significant.

3. Results

As observed from the present acute toxicity study the final fraction of *Alchemilla abyssinica* can be considered tolerable in mice when tested up to the oral dose of 1,000 mg/kg body weight with no mortality and behavioral changes within 24 h. The results presented here are only those of the final test fraction of the bioassay guided fractionation and they are expressed as the percentage contractions, taking the control histamine response as 100 % in each experiment. The final test fraction caused significant concentration dependent spasmolytic effects on the GPI ($P<0.05$, $F= 61.5$). It was capable of inhibiting histamine induced contractions down to 95.7 ± 3.4% at 20 µg/ml, to 43.6 ± 7.1% at 70 µg/ml and 14.2 ± 4.7% at 120 µg/ml organ bath concentrations (see Table 1 and

Figure 1). In this study the NMR spectra of the test fraction showed the fraction is a mixture of secondary metabolites possibly of the flavonoid class.

Table 1. The effect of the final test fraction of *Alchemilla abyssinica* on histamine-induced contractions of GPI. Control histamine = 8ηg/ml of final organ bath concentration. Responses were expressed as % of initial contractions induced by agonist histamine prior to the addition of the final test fraction. Data of contractile responses are expressed as mean ± SEM of 3 guinea pig ileum preparations. The mean difference is significant at *P < 0.05

Group	Fraction conc. (µg/ml)	Contractile response (%)	F-value
1	20	$95.7 \pm 3.4^{*2,3}$	61.5
2	70	$43.6 \pm 7.1^{*1,3}$	
3	120	$14.2 \pm 4.7^{*1,2}$	

4. Discussion

The toxicity result obtained in this investigation using Hexane/EtoAc 1:2 fraction of *Alchemilla abyssinica* can be considered tolerable in mice when tested up to the oral dose of 1,000 mg/kg body weight with no mortality and behavioral changes within 24 hours. This is in agreement with the toxicity study on *Alchemilla vulgaris* by Saad and coworkers [15] that states the LD_{50} of *Alchemilla vulgaris* dried leaf extract tested on rats is found to be 17.3 g/kg body weight. According to Said (2010) also *Alchemilla vulgaris L.* (lady's mantle) is regarded as safe by the German Commission even at large doses without known adverse effects. The LD_{50} reported by Saad and coworkers [15] is within the range of relatively harmless according to Hodge and Sterner Scale [16]. It has also been stated Lady's mantle (*Alchemilla vulgaris* L. or *A. xanthochlora* Rothm.) has no known hazards and/or side effects for proper therapeutic dosages [17].

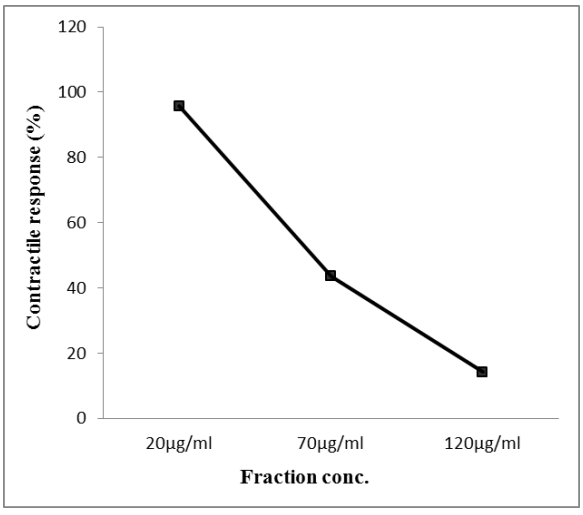

Figure 1. Dose-response curve (line graph) showing the mean percentage contraction recorded by histamine in presence of the final test fraction of *Alchemilla abyssinica* at different organ bath concentrations as compared to the control histamine on isolated GPI

The test fraction has shown significant spasmolytic activity at the three doses 20, 70 and 120 (µg/ml). Similar results were reported for closely related species of *Alchemilla abyssinica* by different investigators. *Alchemilla vulgaris* is claimed to have spasmolytic effect [18]. Ivancheva and co-workers [19] also reported, the infusion of *Alchimella vulgaris* is used as antidiarrheal agent.

The use of *Alchemilla vulgaris* as traditional medicinal plant to expel retained placenta [20] indicates its spasmogenic role in uterine smooth muscles. Its use as antidiahreal agent [19] also predicts spasmolytic effect of the plant extract on gastrointestinal smooth muscles. Hence the plant has both spasmolytic and spasmogenic on different types of smooth muscles. The same property, spasmolytic in GPI and oxytocic in rat uterus, are exhibited by ethanolic extract of *Moringa stenoptella* [9]. This strongly suggests that the spasmolytic effect of the present plant, *Alchemilla abyssinica* may not be universal to all types of smooth muscles and hence it would be worth investigating the effect of the plant extract on other muscle types. Generally however this study shows *A. abyssinica* possesses spasmolytic constituents and these could be of the flavonoid class as indicated in the NMR studies in this work as well as previous works stating *A. vulgaris* and *A. xanthochlora* contain tannins and flavonoids, mainly quercetin [21]. However, further work is required to isolate active compound(s) that gave the plant its spasmolytic property.

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Conflict of Interest

The authors declare that there is no conflict of interest for this paper.

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