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Phytochemical investigation and diuretic activity of the Palestinian *rataegus aronia* in mice using an aqueous extract

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ABSTRACT:

Man used various natural materials as a remedy for the treatment of various diseases and recently witnessed a vastly growing and renewed interest in herbal medicine globally. In Palestinian folk medicine, *Crataegus aronia* is used as diuretics and for the treatment of hypertension. This study aimed to evaluate the diuretic effect of the aqueous extracts of this plant in mice after intraperitonial administration. It is an experimental trial applied to mice (n=12 male, CD-1, weight range: [25-30] g), which are divided into three groups (4 in each): first group administered with the plant extract (500 mg/kg), the second with normal saline as negative control group and the third with furosemide (10 mg/kg) as positive control. Then, urine output and electrolyte contents were quantified up to 6 hours for the three groups and then compared. Results were analyzed using SPSS program. The aqueous extract of C. *aronia* produced significant diuresis ($p < 0.05$). Moreover, aqueous extracts had an acidic PH (6.5125). The aqueous extracts of *C. aronia* tended an increase in sodium loss compared to the negative control group, but the results were not significant statistically for sodium (P-value 0.068)**.** Our investigations significantly revealed that C. *aronia* aqueous extract had a potential diuretic effect which can be utilized for the treatment of hypertension and other diseases related to diuresis and also for manufacturing natural and safe diuretic drugs.

Keywords: Palestine, C. *aronia*, furosemide, diuresis, mice, herbals and medicinal plants.

INTRODUCTION

In the past, people used to use herbs or plants of many aspects in their life, and the most important one is their use in the treatment of many diseases, whether as a natural sources or as a synthesized one from which most of the new drugs are isolated from plant products or their derivatives [1, 2].

Nowadays, most of the pharmaceutical companies have shifted their interest toward purified synthetic or semi-synthetic chemical compounds. This is because of some basic obstacles of isolation and synthesis of natural product derived drugs [3, 4].

As a result of several problems associated with these synthetic chemical drugs such as harmful side effects, contraindication, and drug-drug interaction, these companies cannot achieve the ideal global health expectations as evident in a decreasing number of novel medications reaching the markets [1, 5].

Due to these reasons, the global interests moved again to natural products based on drug development, even it necessitates broad interdisciplinary research approaches [6].

A common approach is to start pharmacological researches with crude plant extracts and subsequently isolate and characterize the

ingredients responsible for the pharmacological effect of the extract.

Diuretic drugs are used normally to increase the urine output to reform and adjust the normal body composition of fluids. Therefore, it's used to relieve some life-threatening diseases such as hypertension, congestive heart failure, cirrhosis, nephritic syndrome, and pregnancy toxemia.

Rosaceae family, *Crataegus ge*nus classification, a Greek word, means kratos, which means hardness of wood. All species contain 17 basal chromosomes. Usually, these species cannot grow completely or form large trees. Some are shrubby, while other species may grow to heights of 12 m, although most species can attain tree-sized proportions ([7].

As described below, *Crataegus* species have leaves having 2 leafy bracts. They are about 15 mm – 5 cm long, glabrous or smooth in character, broad-ovate or obovate and have toothed margins with widely colored flowers initiating from white to pink, and ending with red, both sexes (Male and female) exist, usually grow in clusters and are fertilized by insects attracted by the odors released by the flowers.

These small sized trees are found mostly in temperate areas including countries like North Africa, Western Asia, India, China and North America. *Crataegus* is labeled an aggressive settler, tenacious, and hard to remove. In many Australian states, it has been stated as a noxious plant [7].

When analyzed chemically, many bioflavonoids -like complexes are found inside the leaves, flowers, and berries of hawthorn. These complexes included oligomeric procyanidins (OPC), vitexin, quercetin, and hyperoside, vitamin C, saponins, tannins, cardiotonic amines (phenylethylamine, tyramine, isobutylamine, O-methoxy phenylethylamine, choline and acetylcholine), purine derivatives (adenosine, adenine, guanine, caffeic acid, amygdalin), triterpene acids, ursolic acid are also found in their extract phytochemical analysis .This mostly explains the potential cardiac actions of this plant [7].

The use of crataegus dates to ancient times as an important part of alternative medicine. In many cultures, it is used to treat many diseases like such as cardiovascular disease, cancer, diabetes, and sexual weakness. For example, in Mexico, Diabetes mellitus (DM) is treated with hawthorn extract. Such treatment may have worthy benefits particularly in the early stages of the illness. In folk medicine, many hawthorn species were mainly used for treatment of cardiovascular diseases, including *Crataegus pinntiflda* (Chinese hawthorn), *C. pubesens* (Mexican hawthorn), *C. cuneata* (Japanese hawthorn), *C. laevigata* and *C. monogyna* (Europe), *C. oxycantha* and *C. aronica* (Middle East), *C. phaenopyrum* (American hawtrorn) and *C. ambigua* (Russian hawthorn). Hawthorn (*C. pinnatifida*) is also used in traditional Chinese medicine to lower plasma lipids [7].

As been widely used in folk medicine, many researches to approve its clinical and pharmacological efficacy were established. The results of most of these clinical trials supported its role in medicine.

Of the studies conducted on the effects of *Crataegus* on cardiovascular system was a study applied on rats, in which was observed that the hyperoside fraction and aqueous extract of *Crataegus tanacetifolia* can prevent hypertension and have beneficial effects on the cardiovascular system. In another study, *Cratageus* exhibits anti-arrythmic activity when compared with other known cardioactive drugs like ouabain, epinephrine, milrinone, and propranolol for anti-arrhythmic potential. Its extract appeared to be capable of inducing rhythmicity in quiescent cardiomyocytes and showed antiarrhythmic activity. Its extract also showed negative chronotropic effects and did not cause β-adrenergic receptor blockade [7].

Other studies conducted on its efficacy as anti-atheromatous agent. In one of them, the hydroalcoholic extract of *Crataegus meyeri* infused to rats, which resulted in a significant decrease in the total number of ventricular ectopic beats. Hydroalcoholic and ethyl acetate infused extracts significantly reduced the time spent for ventricular fibrillation. Thus, the extracts of *Crataegus meyeri* may possess active principles which have a hypo-

tensive and antiarrhythmic potential on ischemic myocardium.

C .aronia also affects clotting pathway, it inhibits platelet function altering the bleeding time and closure time, which is determined by the PFA-100 and thromboxane B2 levels [8]. It can be involved in the treatment of non -alcoholic fatty liver disease as it reduces the lipid by lipid lowering agent and antitoxin agent [9].

The administration of the *C. aronia* extract induced bradyarrhythmia and hypotension, without significant changes in the ECG components [10].

Crataegus can also be involved as anti-inflammatory, gastro-protective, antimicrobial agent and used as a hepatoprotective agent. *Crataegus* has high antioxidant and immune-stimulating activity. It is also employed in CNS disorders like anxiety and mild depression [7].

In fact, various scientific studies have been proving the diuretic effect of flavonoids [11, 12]. Therefore, it was suspected that this plant may have a diuretic effect due to the presence of this class of biological components. For that, the current study aimed to investigate the diuretic activity of the aqueous extract of *C. aronia* and compare them with furosemide compounds.

MATERIALS AND METHODS

Study design

An experimental trial was applied on mice, which are divided into three groups (4 in each): the first group administered with the plant extract, the second with normal saline as the negative control group, and the third with furosemide as positive control. 12 mice were selected to be males, CD-1 type with a weight range between (25-30) g. The experiment was done in the period between September /2018 –February/ 2019**.**

Instrumentation and chemicals

Freeze dryer (Mill rock technology, model BT85, China), grinder (Moulinex model, Uno, China), balance (Beco, Germany), hot plate (Labtech, South Korea), filter paper (Whatman no.1, USA), furosemide (Jerusalem Pharmaceutical company, Palestine) and NaCl.

Collection and preparing plant materials

The entire *Crataegus aronia* plant was collected in September 2018 from the mountains of salfit region of West Bank / Palestine. The plants were identified by the pharmacognosist Dr. Nidal Jaradat. Voucher specimen was deposited in the Laboratory of Pharmacognosy under the voucher specimen code of Pharm-PCT-711

The collected parts were washed well using distilled water to avoid any contamination and then dried in the shade at room temperature until all plant parts became dry. After drying, the plant materials will be grounded well using a mechanical blender into a fine powder and transferred into airtight containers with proper labeling for use.

Preparation of plant dry extracts

The aqueous plant extracts were infused by taking 100 ml of boiling water in a beaker and added to 10 g of dry plant material and then covered and incubated for 30 min. The infusion was then filtered using Whatman filter paper No.1 and concentrated to 10 ml in a water bath at 45°C (1 ml of this extraction equivalent to 1 g of dry starting material; all doses are expressed in terms of starting material). Extracts were stored at 4°C in the refrigerator for not more than 1 week. After that, the plant extract was dried by using a freezing dryer to produce a dry extract. Then 15 mg plant powdered extract was dissolved in 15 ml 0.9% normal saline solution (0.9 mg of solid NaCl was dissolved in 100 ml distilled water to produce 0.9% concentration) to produce a 1mg/ml concentration [13].

Animals

In this study, about 12 Male CD-1 mice (weight range: 25-30 g) will be used in the experiment. They will be kept four per cage in the animal house. Mice will be allowed to acclimatize to the animal facility for 7 days prior to testing under controlled conditions of temperature (22±2°C). Mice will be reused with a minimum 7 days interval between drug testing. All experiments will be performed during the light portion of the day cycle. All animals will be fasted over the

night of the experiment. The animals will be pretreated with physiological saline (0.9% NaCl) at an oral dose of 0.15 ml/10 g body weight, to impose a uniform water and salt load (18).

Twelve mice were divided into three groups, 4 mice in each. The first group (serving as negative control group) was given 200µl normal saline, the second group is the positive control group received 200µl furosemide (5ml/Kg), and the third group received C. *aronia* extract at the doses of (5ml/Kg).

Diuretic test

For diuretic effect determination, male mice weighing 25-30 g each were fasted and deprived of water for eighteen hours prior to the experiment. The following day, the mice were given orally 5 ml/kg of Furosemide (40 mg furosemide in 40 ml NaCl to make 1mg/ml concentration solution), normal saline solution, *and C. aronia* (200µl of each). (19).

Therefore, three different types of administration groups were occurred. Mice were reused with a minimum 7 day interval between drug testing. Mice were placed in cages which had a wire mesh fitted with a small container for urine collection .

The urine was collected in measuring cylinders up to 6 hours after dosing. During this period, no food or water was made available to animals. The volume of each group was collected and observed each hour. Urine was then collected and measured 1, 2, 3, 4, 5, and 6 hrs after dosing. The urine was then filtered and finally stored at -20°C for electrolyte analyses. The following parameters were calculated to compare the effects of the extracts with vehicle and standards on urine excretion.

Measurement of urine pH, Na+, and K$^+$

The total urine voided by individual mice over 6 hours was collected, weighed, transferred to Eppendorf tubes, and stored at -20°C until analysis. The samples were diluted (1:5 in deionized water) and urine pH and Na^+ and K^+ concentrations were measured using ion selective microelectrodes according to the manufacturer's protocol (Lazar Research Laboratory, Inc, Los Angeles, CA, USA). Total amounts of each electrolyte were quantified for each 6 h sample using the formula: 5×diluted sample concentration (µEq/ml) × total volume (ml) of sample [14].

STATISTICAL ANALYSIS

All data was entered into the computer and analyzed via Statistical Package for Social Science (SPSS) version 20 frequencies and percentages were calculated for categorical variables, while mean ± standard deviation (SD) were computed for continuous data. A p value of less than 0.05 was considered statistically significant.

RESULTS

Phytochemical analysis

The plant extract was analyzed by phytochemical analysis, and the results are shown in table 1.

Table (1): Phytochemical analysis of *C. aronia*

Test on C.Aronia Extract	Result
Nihydrin	_
Fehling	+
Molisch	+
Iodine	_
Test of alkaloids	+
Flavonoids	+
Alkaline reagent	_
Test of saponin	+
Keller kilani	_
Penol & Tanin	+

Negative (-) sign indicates the absence of a phytochemical constituents, Positive (+) sign indicates the presence of a phytochemical constituents.

Diuretic activity

Effect on urine volume

The aqueous extract of the whole plant of *C.aronia* showed a significant increase in urine volume ($P < 0.043$) compared to the negative control group as shown in table 2 as well as in figures 1 and 2.

Table (2): Effect of aqueous extract of *Crataegus aronia* on urine volume on 6 hours.

Test	Mean ± SEM for time urine collection (in hours in micrliter)						
	1	**2**	**3**	**4**	**5**	**6**	**Total urine collection**
+Ve control	2140±767	3820±621	4190±580	4274±577	4314±573	4330±582	4330±582
-Ve control (Furosomide)	0.0±0.0	140±67.8	170±70	362±179	562±325	562±325	562±325
Crataegus aronia	140±53.4	370±76.8	492±111	754±255	1092±415	1162±448	1162±448

SEM indicates Standard Error of Mean.

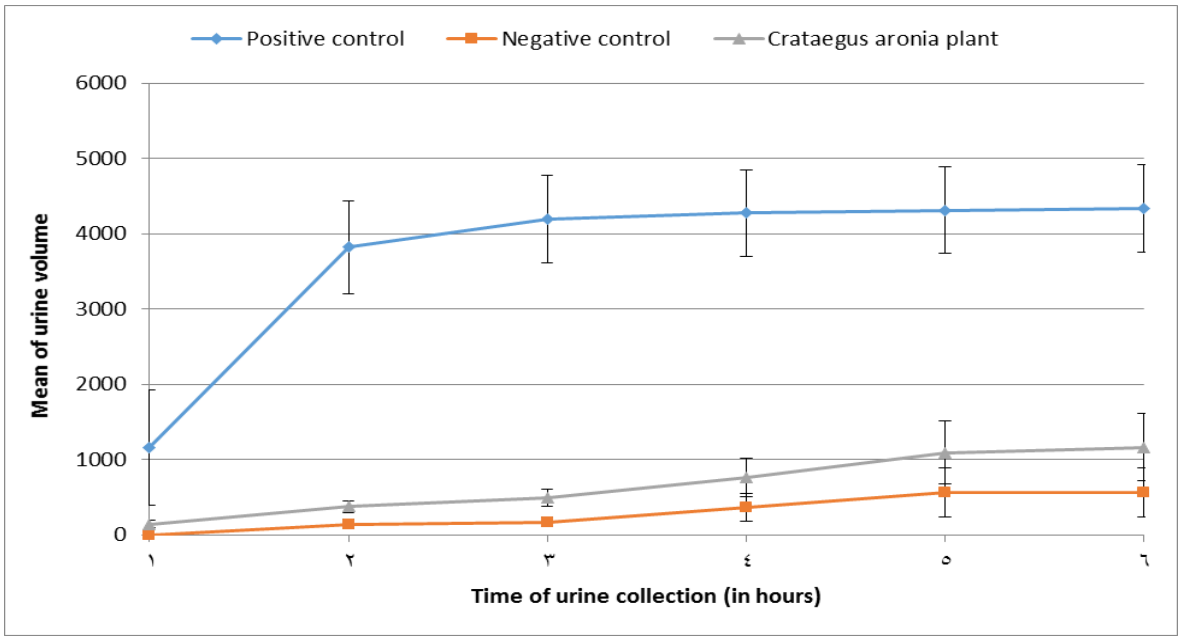

Figure (1): The 6 hours urine output volumes (in microliters) for three mice groups.

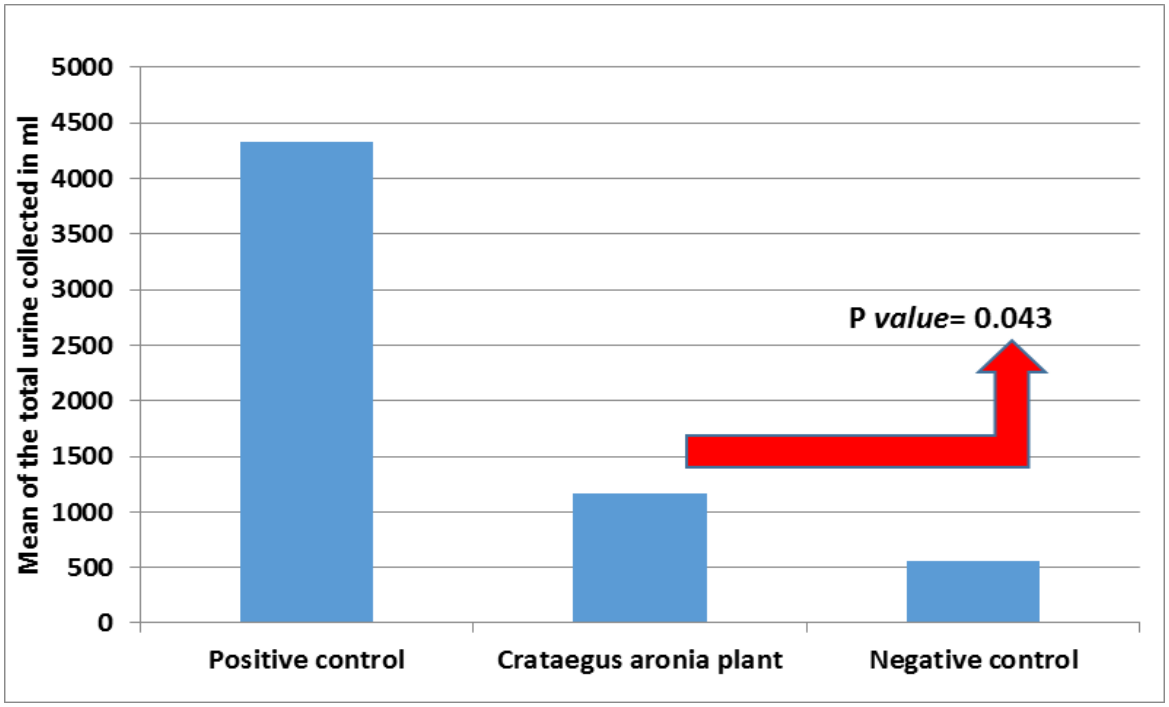

Figure (2): Statistical analysis of the 6 hours' total urine output volumes (in microliter) for the three mice groups.

Saluretic activity: effect on electrolyte content of urine

The urine samples collected over six (6) hours are analyzed for Na+ and K+, shown in tables 3 and 4, as well as in figures 3 and 4. The aqueous extracts of *C. aronia* tended to mildly increase sodium and potassium loss compared to the negative control group, but the results were not significant statistically for both potassium and sodium.

Table (3): Mean concentrations of Na+ and K+ in urine of the three groups (results in microliters).

Group	Na+	K+	SE of mean for N+	SE of mean for K+
C.aronia	1380.0000	1544.7500	138.26303	218.82542
Negative control	885.0000	1082.5000	116.08187	269.52721
Positive control (Furosomide)	2492.5000	1656.2500	176.60573	305.58261

SE indicates Standard Error.

Urinary PH

The urine PH measurement of the studied groups showed that the aqueous extracts of C. *aronia* had produced relatively acidic urine as shown in figure 5 and table 4.

Table (4): Effect of aqueous extract of C. *aronia* on urine PH (results in microliters)

Group	Mean	SE of mean
C. aronia PH	6.5125	0.15353
Negative control PH	6.7025	0.35652
Positive control (Furosomide) PH	6.9700	0.29852

DISCUSSION

In developing countries, about 80% of the population use herbal medicines, primarily for primary health care because of their publicity with better acceptability among people and lesser side effects. In recent times, many collective efforts have been

channeled into researching for traditional plants with therapeutic values [15, 16].

Diuretics are substances utilized to increase the rate of urine output or/and sodium excretion, to adjust the composition and volume of body fluids, and also to eliminate the excess of fluid from tissues. This makes them to be used for adjustment and treatment of volume overload in some diseases, including heart failure, hypertension, renal failure, liver cirrhosis, lung, and kidney diseases [17][18].

In these days, there is an increasing interest in the health and wellness benefits of herbs and botanicals. They are looked at as a natural safeguard against the development of certain conditions and be a putative treatment for some diseases. This is may be the lowering of blood pressure when elevated (i.e., hypertension). Many studies stand for purporting the diuretic effects of many traditional medicines and such researches are expected to increase [16, 19] .

The diuretic effect of C. *aronia* aqueous extract has shown strong diuretic action compared with furosemide. The present study supports the ethno medical use of the studied plants for its diuretic effect and based on the pattern of water, sodium, and potassium excretions, it appears that the plant could possibly have more than one physiological mechanism of action which contributes to the potassium- saving and natriuretic effect especially at the maximal doses.

Further pharmacological and phytochemical studies are required to identify and to isolate the active molecules in the studied plants which were responsible for this effect and also additional clinical trials are required to evaluate the diuretic action on human as well as *C. aronia* aqueous extract used intensively in the Palestinian folk medicine as a diuretic agent.

CONCLUSION

Herbal products identified from traditional medicinal plants have always paved the way for the development of new types of therapeutic agents. The results obtained in this study provide a quantitative basis to explain the traditional folkloric use of *C. aronia* as a diuretic agent which can be used for the treatment of hypertension and renal disease and also many of the pharmaceutical companies will be attracted by these results for manufacturing novel diuretic drugs from natural sources.

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