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Research Article

Diuretic Activity of *Rubus idaeus L* (Rosaceae) in Rats

Ying Zhang, Zhenya Zhang*, Yinnan Yang, Xiaoyan Zu, Di Guan and Yuepeng Wang

Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1, Tenoudai, Tsukuba, Ibaraki, 305-8572, Japan

Abstract

Purpose: *To evaluate the diuretic activity of Rubus idaeus L in experimental rats.*
Methods: *Hot-water and methanol extract of three kinds of Rubus idaeus L. fruits were administered to experimental rats orally at a dose of 2 and 5 mg/kg. Hydrochlorothiazide (10 mg/kg) was used as positive control in study. The diuretic effect of the extracts was evaluated by measuring urine volume, sodium and potassium excretion in the urine.*
Results: *Compared with the control group, significant increase in urine volume was observed from the experimental animal treated with wild raspberry methanol extract. In addition, we find that the methanol extract of wild raspberry fruits shows a potassium-conservation diuretic effect, which is a very interesting property in a phytodiuretic.*
Conclusion: *Methanol extract of wild raspberry fruits have diuretic effect on experimental rats. This might be the first formal reports on diuretic effect of raspberry fruits, which can also, to some extent, explain the use of raspberry as a cure for renal diseases in Chinese traditional medical practice.*

Keywords: *Diuretic activity, Rubus idaeus L., Rasberry, Herbal medicine.*

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Corresponding author:* ***E-mail:* *tyou6688@sakura.cc.tsukuba.ac.jp;* ***Tel:*** *+81-29-853-4712*

INTRODUCTION

Raspberry, *Rubus idaeus L.*, is a plant belonging to the *Rosaceae* family, genus *Rubus*. The geographical distribution of raspberry covers a wide range from Europe to northern Asia and most temperate areas. As a well known fruit crop, raspberry fruit is used not only for nutritional purposes, it is also used as a folk medicine in many countries to treat wounds, colic pain and some other diseases such as diarrhea and renal disease [1].

During the past two decades, many researchers have studied the biological effects of raspberry components. Parsons [2] and Simpson [3] examined the safety and efficacy of raspberry leaf products. Their findings suggest that raspberry leaf may have an effect on shortening the labor process and hence reduce the need for medical intervention for pregnant mothers during delivery. Further details on the activity of raspberry leaf products on the uterus have been discussed elsewhere [4].

According to Zorica [5], the water extract of raspberry seeds possesses the potential for anti-proliferative action against human colon carcinoma cells *in vitro.* Raspberry seeds have also been found to exhibit other properties [6]. With regard to raspberry fruit, its high levels of phenolic and anthocyanin compounds have been have been reported thus indicating its potential health benefits [7-11]. Heinonen [7] also demonstrated that raspberry fruit inhibits low-density lipoprotein and liposome oxidation. Recent studies have confirmed that raspberry fruit has anti-oxidant, anti-proliferative, anti-obese, antibacterial and anti-inflammatory activities [10-12].

In Chinese traditional medical practice, raspberry fruit is claimed to be a cure for renal diseases while in some countries, it is also believed that raspberry leaf may have mild diuretic effect and increase urination [1]. It has been established that a high level of phenolics is present in raspberry fruits [7], and that some of the phenolic compounds have diuretic activity [13]. The folk-medicinal use of raspberry fruits as well as the foregoing reports indicate that this fruit probably has diuretic activity.

To the best of our knowledge, no formal research has been undertaken on the diuretic activity of raspberry fruit. Therefore, the main objective of this study was to evaluate the diuretic activity of raspberry fruit in order to enhance the understanding of this widely used fruit crop.

EXPERIMENTAL

Plant materials

Three types of raspberry fruits were used in our experiments: frozen cultivated type from Chile (T), immature cultivated fruit, named FuPenZi (F), which is also used as a traditional Chinese medicine; and mature wild raspberry fruits (R). The first two types were obtained from a local market, while the third (wild raspberry fruits) was collected from LiaoNing Province, China by local traditional medicine practitioners in 2008. They were identified and authenticated as *Rubus ideaus L.* by Professor Jincai Lu (College of Traditional Chinese Meteria Medica, ShenYang Pharmaceutical University, China). A voucher speciman (no. 20080920RI) was preserved at the Herbarium of Medicinal Plant, ShenYang Pharmaceutical University, China.

Drug and Chemicals

Hydrochlorothiazide (HCTZ), obtained from Wako Pure Chemical Industries, (Osaka, Japan) was used as a reference diuretic drug. Sodium chloride, potassium chloride, sodium hydrogen carbonate and methanol used in our experiments were also obtained from Wako Pure Chemical Industries.

Preparation of fruit extracts

All the experimental fruit materials were air-dried in an oven at a temperature of 40 ± 2 ℃ until constant weights were obtained. The three dried fruit types were ground to powder in an electric grinder, respectively. Each powder was further divided it into two equal parts; one part was boiled in distilled water and the other part in 99.5 % methanol, for 2 h, respectively. The decoction obtained was centrifuged and filtered through Whatman filter paper no. 4. This procedure was repeated three times and the combined filtrate for each fruit type was evaporated in a rotary evaporator to dryness under reduced pressure. Thus, six extracts were obtained: three *Rubus idaeus L.* hot water extracts (TW, FW and FW), and three *Rubus idaeus L.* methanol extracts (TM, FM and RM). The extracts were stored at -20 ℃, and thawed just before use for animal studies.

Experimental animals

Male rats, weighing approx 250 - 300g, and obtained from the Animal Experimental Center, Tsukuba University, Tsukuba, Ibaraki, Japan, were used for the animal experiments. The animals were housed in a temperature- and light-controlled room (25 ℃; 14h/10h light/dark cycle) with free access to food (MF, Oriental Yeast Co. Ltd, Tokyo, Japan,) and drinking water. Prior to the animal studies, the animals were acclimatized in the laboratory for a period of at least one week.

All experiments were carried out in accordance with the regulations for animal experiments and fundamental guidelines [14] under the jurisdiction of the Japanese Ministry of Education, Culture, Sports, Science and Technology and approved by the Institutional Animal Experiment Committee of Tsukuba University, Japan (approval no.10-291).

Diuretic test

Diuretic activity was determined, following the methods of Kawashima [15], but with a minor modification. The rats (48) were fasted for 18 h with free access to drinking water, and then orally administered 30 ml/kg of bicarbonate saline solution (containing 110mM NaCl and 30mM $NaHCO_3$). Thirty minutes later, they were divided into 8 groups (n = 6) and the control group was given 10ml/kg of distilled water. The positive control group received hydrochlorothiazide (10mg/kg) in distilled water. The 6 test (extract) groups were given the dry plant extracts (TW, FW, RW, TM, FM and RM) at a dose of 2 g/kg. The animals were then placed in individual metabolic cages. Urine samples were collected with collecting tubes at the bottom of metabolic cages hourly for 4 h, the urine volume measured and the specimen assayed for Na^+ and K^+ concentrations using an atomic emission spectrometer (model ICPS-8100, Shimadzu, Japan).

Statistical analysis

Statistical analysis was applied to the data using PASW (version 18.0, IBM). The results are expressed as mean ± SEM (standard error of mean). Statistical evaluation was carried out by analysis of variance (ANOVA) followed by Student's t-test for multiple comparisons. When compared with control group, *p*-values less than 0.05 were considered statistically significant.

RESULTS

Urine volume

Table 1 shows the urine volume data. The hydrochlorothiazide (HCTZ) group, showed the highest output of urine during the first hour, as well as the maximum total urine volume. The positive control group (HCTZ), with a diuretic index of 1.90 showed the highest diuretic activity.

Table 1: Urine volume observed over a period of 4 h (mean ± SEM)

Treatment	Urine volume (ml/100g body weight)					Diuretic index[1]
	1h	*2h*	*3h*	*4h*	*Total*	
Distilled water	0.76	0.26	0.21	0.07	1.30±0.19	1.00
HCTZ (10mg/kg)	1.43*	0.08	0.61	0.34*	2.50±0.30**	1.90
TM (2g/kg)	0.73	0.11	0.36	0.10	1.30±0.29	1.00
FM (2g/kg)	0.83	0.32	0.03	0.44*	1.62±0.28	1.25
RM (2g/kg)	0.52	0.58	0.69*	0.54*	2.32±0.12**	1.69
TW (2g/kg)	0.79	0.21	0.19	0.16	1.34±0.27	1.03
FW (2g/kg)	1.04	0.28	0.21	0.12	1.64±0.43	1.26
RW (2g/kg)	0.66	0.23	0.16	0.30	1.35±0.23	1.04

[1]*Diuretic index = total urine volume of treated group/total urine volume of control group; *p < 0.05 compared with the control group; **p < 0.01 compared with the control group; distilled water = control group*

Table 2: Urine electrolyte levels (mean ± SEM) observed over a period of 4h

Treatment	Na+（mmol/L）				K+（mmol/L）			
	1h	2h	3h	4h	1h	2h	3h	4h
Control	131.7±9.9	140.1±33.5	124.7±12.3	129.0±9.3	84.4±4.8	58.6±14.2	66.0±17.9	92.7±13.7
HCTZ 10mg	188.6±24.5**	180.8±41.1*	167.8±19.9**	141.5±13.0	112.4±19.1*	109.9±35.4**	162.0±31.8*	135.9±37.8*
TM 2g	120.7±29.5	147.0±11.6	130.8±32.7	126.3±11.6	70.0±24.4	91.4±22.3	79.9±14.2	114.1±28.3
FM 2g	135.2±23.1	138.9±15.8	172.5±38.8*	143.5±25.2	90.6±25.2	64.2±21.9	109.7±11.3*	96.8±35.6
RM 2g	139.2±5.8	191.5±31.3**	178.6±15.5**	192.3±33.4*	69.8±6.4	39.1±10.3	59.4±28.2	57.0±12.1**
TW 2g	120.7±37.4	127.2±26.7	143.4±19.7	152.9±37.6	93.8±17.5	90.5±22.7*	62.8±5.0	68.3±23.3*
FW 2g	149.2±41.4	178.0±39.8	140.1±12.3	124.5±15.8	91.5±11.0	65.3±16.3	84.4±31.1	71.5±13.9
RW 2g	128.2±46.1	133.3±22.0	161.8±41.8	129.1±41.0	102.2±35.8	66.4±28.7	76.2±14.5	68.5±23.4*

*Control = distilled water (10 ml/kg BW); HCTZ = hydrochlorothiazide; TM, FM and RM = methanol extracts of the three different raspberries fruits used; TW, FW, RW = hot-water extract of the three different raspberries used; *p < 0.05 compared with the control group, **p < 0.01 compared with the control group*

The total urine volume of animals treated by raspberry methanol extract (RM) was also high (2.32±0.12 ml/100 g BW) was statistically higher than the volume for the control group. Similarly, the diuretic index of RM was 1.69, which is the highest of all the raspberry extracts. It can be also seen from Table 1 that neither of the two kinds of the cultivated raspberry fruits (T or F) caused a significant increase in total urine volume.

Urine electrolytes

The electrolyte data - sodium and potassium – for the rats are shown in Table 2.

For the positive control group treated with HCTZ, the excretion of sodium and potassium were markedly increased almost throughout the experiment, compared with the control group. However, the urinary electrolyte pattern of the three groups treated with aqueous raspberry extracts (TW, FW and RW) were similar to that of the control group. Urine sodium in the animals treated with RM was substantially higher than in the control group throughout the duration of the test, just as was observed for the positive group (HCTZ). However, no increase in potassium urine level of treated animals with RM was found.

DISCUSSION

As expected, hydrochlorothiazide (HCTZ) produced a marked diuretic effect at a dose of 10 mg/kg as indicated by a significant increase in urine output.

According to Cetin Cekic [16], the chemical properties of raspberry fruits probably differ between the wild species and those cultivated. The degree of maturity may also influence the chemical composition of the fruits. The results of the present study show that neither of the two kinds of the cultivated raspberry fruits (T or F) caused a significant increase in total urine volume. In contrast, the wild species collected from China showed significant diuretic effect in experimental rats, indicating that wild raspberry fruits might contain more diuretic active components than those cultivated fruits.

Two kinds of solvents, hot water and methanol (99.5 %), were used to obtain the raspberry fruit extracts. No significant increase in urine volume was observed in the group treated with the aqueous extracts. A probable reason is either that the active ingredient in raspberry fruits is not water-soluble or that it is degraded in hot water.

There appears to be some correlation between in the urine volume and the electrolyte data in that the methanol extract not only increased urine volume but also enhanced sodium excretion to a higher level than the aqueous extract. The difference in Na^+ excretion between the extracts increased progressively from 8.5 % in the first hour to 43.6 % in the second hour, and 54.3 % in the fourth hour. Interestingly, sodium excretion by the methanol extract was even higher than by HCTZ, except in the first hour. It is noteworthy that there was no increase in potassium excretion following administration of the methanol extract. This suggests that this extract has potassium-sparing properties, unlike HCTZ. Thus, the diuretic action of methanol raspberry extract may be by inhibition of aldosterone action or the inhibition epithelial sodium channels, unlike HCTZ, which inhibits sodium and chloride re-absorption in the distal tubules.

CONCLUSION

The results obtained in this study indicate that *Rubus idaeus L.* (raspberry) fruit does possess some diuretic effect in experimental rats. Thus, the findings provide some justification for the use of raspberry as a diuretic in traditional Chinese medicine. Another notable finding is that the methanol extract exhibit potassium-sparing diuretic. Further studies are required to elucidate the extract's active components that are responsible diuretic activity as well as the mechanism of diuresis.

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