Antihyperuricemic and diuretic effects of procyanidins extracted from *Crataegus monogyna*.

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Abstract

Background and objectives: Data indicated that procyanidins extracted from grape seeds has uric acid-lowering effects in mice, however the hypouricaemic effect of procyanidins was not accompanied with changes in enzymatic activities of xanthine dehydrogenase and xanthine oxidase. This study was designed to investigate the effect of procyanidins extracted from Crataegus monogyna on serum uric acid, adenosine deaminase (ADA), 5-nucleotidase, xanthine oxidase, and renal function on normal and potassium oxonate induced hyperuricemic rats.

Methods: Thirty female albino rats were divided into three groups. The first group included 18 rats pretreated with the uricase inhibitor potassium oxonate (250 mg/kg, i.p.), served as an animal model for hyperuricemia. The rat models were divided into three subgroups, each subgroup having six rats. The first subgroup served as a normal control. Subgroup 2 received a single daily dose (100 mg/kg p.o) of procyanidins for 7 days. The third subgroup received daily dose (50 mg/kg p.o) of allopurinol for 7 days as positive control. The second group included six rats received only water as a vehicle. The serum uric acid, xanthine oxidase, adenosine deaminase (ADA) and 5-nucleotidase levels were measured and compared to those in normal untreated control group. The Third group included six normal rats received a single dose of Procyanidins (50 mg/kg body weight; i.v.) to study the renal effects of procyanidins..

Results: A single daily dose (100 mg/kg PO) of procyanidins for 7 days significantly reduced serum levels of uric acid, ADA and 5'-nucleotidase, without detectable effects on the level of xanthine oxidase in hyperuricemic rats. Intravenous infusion of a single dose of procyanidins (50 mg/kg i.v) produced marked increases in urinary Na^+ excretion (4.8 folds) and urine flow (2.6 folds) accompanied by insignificant change of potassium excretion in the rats.

Conclusion: The reduction in serum uric acid most probably is due to inhibiting enzymes, ADA and 5-nucleotidase. The antihyperuricemic and diuretic effects of procyanidins recommended it as a good drug for the treatment of gout and renal uric acid calculi.

Key words: Procyanidins, Hypouricemic effect, Uric acid adenosine deaminase, 5-nucleo tidase

Introduction

Gout is a common medical problem. It is a painful condition, characterized by the deposition of uric acid crystals in the joints, which causes episodes of joint inflammation especially of the feet and hands, and arthritic attacks [1]. Gout is often associated with other metabolic disorders such as obesity, diabetes mellitus, and hypertonia, and carries an increased risk of cardiovascular problems [2]. Gout affects men more often than women. It is the most common cause of inflammatory arthritis among men younger than 40 years, and is often encountered among middle-aged to

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elderly men and postmenopausal women [3,4]. The chief purines found in the nucleotides and nucleic acids are adenines and guanines. Uric acid is the final oxidation product (in man) of these purines. Humans convert adenosine and guanosine to uric acid. Adenosine is first converted to inosine by adenosine deaminase (ADA). In mammals other than higher primates, uricase converts uric acid to the water soluble product allantoin. However, since humans lack uricase, the end product of purine catabolism in humans is uric acid [5]. Since only 10% of generated uric acid is excreted in urine without glomerular reabsortion, blood uric acid levels are controlled by not only its production and excretion but also by enzymatic and non enzymatic degradation. The lowering of elevated serum uric acid level is therefore an important step in the treatment of gouty arthritis and in the prevention of uric acid stone formation [6]. In order to lower blood uric acid level of hyperurecaemic and gouty patient's xanthine oxidase inhibitor or several uricosuric agents such as probencid and sulfinepyrazone are frequently utilized. Allopurinol is the only xanthine oxidase inhibitor in that purpose [5]. Although allopurinol is well tolerated by most patients, it shows critical side effects while simultaneously reducing blood uric acid level, such as skin rash, gastrointestinal distress, diarrhea and hepatic toxicity occurs in up to 20% of the treated patients. This necessitates the drug discontinuance in 5% of the patients [7]. Thus, new alternatives with an increased therapeutic activity and less side effects are desired. In China, natural herbs have been used for a long time in the treatment of gout and hyperuricemia-related disorders [8]. Data indicated that procyanidins extracted from grape seeds had uric acid-lowering effects in mice. However, the mechanism by which it reduces serum uric acid is still not understood [9]. This study was designed to investigate the effect of procyanidins extracted from Crataegus monogyna on serum uric acid, ADA, xanthine oxidase, and renal function in potassium oxonate induced hyperuricemic rats.

Methods

The animals

A total of 30 female rats (Wistar Albino) were used in the present study. Their weights ranged from 175-260 grams (Mean±SE 1.44±0.05). The rats were kept in the animal house of the College of Medicine, Hawler Medical University in a room temperature of 25C^{o}. They were maintained on palatable food containing all essential components of rodent's diet.

Experimental design:

The experimental rats were divided into three groups. The first group pretreated with the uricase inhibitor potassium oxonate (250 mg/kg, i.p.) and served as an animal model for hyperuricemia. The rat models were divided into three subgroups, each group having six rats. The first subgroup was considered as a control (kept on distilled water only). Rats in subgroup 2 and 3 received a single daily dose of (100 mg/kg p.o) of procyanidins and single daily dose of allopurinol (50 mg/kg p.o) respectively for 7 days after one day of IP administration of potassium oxonate. The second group included six rats received only water as a vehicle. The serum uric acid, xanthine oxidase, adenosine deaminase and 5-nucleotidase levels were measured. The Third group included six rats received a single dose of procyanidins (50 mg/kg body weight; i.v.) to study the renal effects of procyanidins..

Extraction and identification of Procyanidins

The method of Dizaye et al [10] was used to extraxt procyanidins. A sample of (50g.) of powdered herb material was suspended in (1000 ml) distilled water in a glass beaker, mixed well, sonicated for 2.5 hr., in an ultrasonic machine bath (Decon FS 200 Frequency Sweep) at a constant temperature of 25 C°. Extract was separated by simple filtration. The filtrate was treated with n-hexane. Then the hydrophilic phase extracted with chloroform and dried under Freez-drying.

The powder was extracted with ethanol 90%, the solvent was concentrated and separated with sephadex LH chromotography (elution with methanol). The procyanidins was identified according to British herbal pharmacopia (1996) [11, 12].

Anesthesia:

Rats were anaesthetized by intraperitoneal (ip) injection of a combination of ketamine (ketamine hydrochloride, Rotex media GMBH, TRITTAU, Germany) in a dose of 35 mg/Kg body weight with xylazine (2%, Ceva sante aninale-laBallastiere, 33501 Libourne Cedex-france) in a dose of 10 mg/Kg body weight [13]. This combination provided perfect surgical anesthesia, and supplementary small doses of the combination were given as necessary to maintain the level of anesthesia.

Blood sampling

At the end of the experiment, rats were sacrificed, blood samples were collected and sera were separated from whole blood samples immediately by centrifugation.

Serum analysis

The assay of ADA was based on its hydrolytic action on adenosine; the ammonia formed being measured by the berthelot reaction. The substrate used was 0.675 mM adenosine in 0.2 M phosphate buffer at a pH of 7.05. After incubation at 37^{o}C for one hour, the ammonia liberated was estimated by measuring the blue colour produced with the phenol-hypochlorite reagent at 640 nm, in a spectrophotometer. Serum 5-nucleotidase was estimated using 5' AMP as the substrate and the Pi liberated was determined by the Fiske and Subbarow method [14]. Serum uric acid was determined by phosphotangstic acid method [15].

Urine collection:

In female rats, the urinary bladder was catheterized via the urethra with PP 25 polythene tubing. A mark was made on the urethral cannula, about 2cm from its tip, when this mark is at the edge of the urethral opening, the tip of the cannula lies just inside the bladder. This helps to prevent damage to, and the consequent bleeding of the bladder during urine collection. The urethral cannula was inserted directly through the urethral opening by careful manipulation. The urethral cannula was held in place by a ligature tied around the external orifice of the urinary tract. Urine was collected in sample cups by applying gentle pressure to the lower abdomen above the bladder. Urine was analyzed for sodium and potassium by flame photometry (Jenway, PFP7).

Statistical analysis

All data are expressed as means± standard error means (M±SEM) and statistical analysis was carried out using statistically available software (SPSS Version 11.5). Data analysis was made using one-way analysis of variable (ANOVA). The comparison between groups were done using Duncan test. $P<0.05$ was considered as statistical significance.

Results

The effects of procyanidins on serum uric acid and xanthine oxidase of hyperuraecemic rats. A single daily dose of 100 mg/kg PO of procyanidins for 7 days significantly reduced serum level of uric acid of rats pretreated with potassium oxonate (250 mg/kg, i.p.). Whereas no significant change was noticed in serum xanthine oxidase of hyperuraecemic rats (Table 1). Allopurinol (50 mg/kg) significantly lowered serum levels of both xanthine oxidase and uric acid in the potassium oxonate pretreated rats. The same dose of procyanidins induced a significant reduction in the serum levels of uric acid, ADA and 5-nucleotidase as shown in (Figures 1 & 2).

The effects of procyanidins on urine flow and urinary excretion rates of Sodium and Potassium of rats. Intravenous injection of 50mg/kg of procyanidins in the rat produced significant increase in the urine flow and sodium excretion rate of the rats. The observed urinary potassium excretion turned out to be statistically insignificant (Table2).

Table 1: The effects of procyanidins (100 mg/kg PO) and allopurinol (50 mg/kg) on serum level of xanthine oxidase and uric acid of rats pretreated with potassium oxonate (250 mg/kg, i.p.).

Parameters	Control	Hyperurecemic control	Procyanidins	Allopurinol
Serum Uric acid mg/dl	1.6±0.07[a]	3.6±0.16 [b]	2.1±0.08[a]	1.4±0.6[a]
Serum Xanthine oxidase (IU/L)	5.01±0.17[a]	5.04±0.11[a]	4.3±0.09[a]	2.26±0.08[b]

The different letters indicate there is significant difference at $P < 0.05$

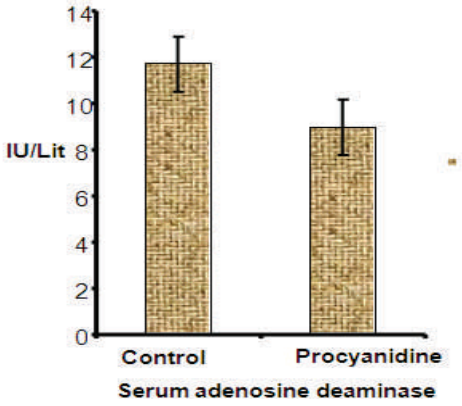

Figure 1: The inhibitory effects of procyanidins (100 mg/kg PO) on serum adenosine deaminase (ADA) of rats pretreated with potassium oxonate (250 mg/kg, i.p.).

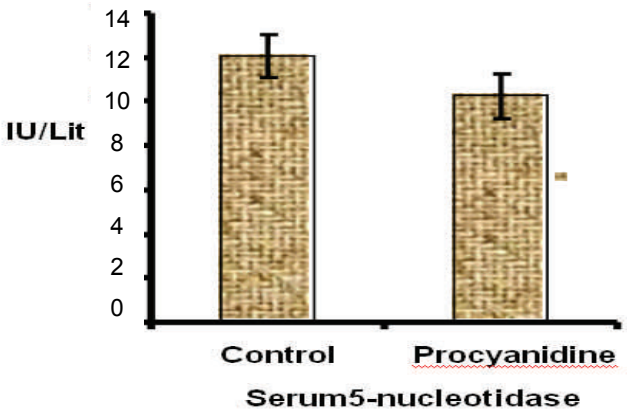

Figure 2: The inhibitory effects of procyanidins (100 mg/kg PO) on serum 5-nucleotidase of rats pretreated with potassium oxonate (250 mg/kg, i.p.)

Table 2: The effects of intravenous injection of 50 mg/kg of procyanidins on urine flow and urinary excretion rates of sodium and potassium in rats. (n=6)

Parameters	Control	Procyanidins
Urine flow µl/min./100g	8.55 ± 1.54	16.46±2.4*
Na^+ Exc. rate µEq/min./100g	1.236±0.5	6.33±2.44*
K^+ Exc. rate µEq/min./100g	0.906±0.2	1.164±0.25

*P<0.05

Ddiscussion

Prolonged hyperuricemia carries a risk for gouty arthritis and renal stone formation. Recent epidemiologic data indicated that the incidence of primary gout has increased by 2-fold over the past 20 years [4].
Dietary control of purine intake reduces the mean serum urate concentration by only 1.0 mg/dl and decreases the urinary uric acid excretion by 200-400 mg/ day. When hyperuricemia persists in spite of dietary control, medication is indicated [16].
Antihyperuricemic agents are commonly employed for the treatment of chronic gouty arthritis [17]. Current antihyperuricemic agents in use include uri-cosuric agents, xanthine oxidase inhibitors, and the enzyme urate oxidase[18]. Uricosuric agents such as probenecid, sulfinpyrazone, and benzbromarone act on the urate anion transport pathway and inhibit renal proximal tubular urate reabsorption. Xanthine oxidase inhibitors such as allopurinol interfere with the conversion of hypoxanthine to xanthine and then to uric acid. Urate oxidase oxidizes urate to the more soluble allantoin, which is more readily excreted through the kidney. Although a number of antihyperuricemic agents are available, their utilization is sometimes limited by the associated undesirable side effects. In general, allopurinol is the drug of choice. However, about 5% of patients are unable to tolerate its adverse side effects, which include gastrointestinal irritation, bone marrow suppression, and hypersensitivity syndromes ranging from simple skin rash to life-threatening conditions in which the patients develop toxic epidermal necrolysis, fever, hepatitis, eosinophilia, and worsening renal function [19-21]. Therefore current attempts are made to find safe hypouricemic agents as alternative to allopurinol from natural sources.
Plants rich in procyanidine (grape seeds) were found to lower serum uric acid & decrease hepatic xanthine oxidase & dehydrogenase activity in experimentally-induced hyperuricemic in rats [9].
Since the effects of procyanidine from Crataegus monogyna on the activity of ADA and 5-nucluotidase enzymes which are also known to be involved in the biosynthesis of uric acid have not been investigated yet, therefore in the present in vivo study, the effects of procyanidines isolated from Crataegus monogyna in oxonate-induced hyperuricemic rats on serum uric acid, xanthine oxidase, ADA and

5-nucluotidase were investigated as a biomarker for their hypouricemic effects.

In the present study, potassium oxonate (250 mg/kg ip) successfully elevated serum uric acid level in the rat model through partial blockade of conversion of uric acid to allantoin and hence artificially serum uric acid level was increased to provide a hyperuricemic animal model [18].

A single daily dose of (100 mg/kg PO) of procyanidins significantly reduced serum level of uric acid of hyperuricemic rats induced by potassium oxonate.) The hypouricaemic effect of procyanidins is not like allopurinol as it is less potent and unlike allopurinol it did not inhibit serum level of xanthine oxidase significantly.

The hypouricemic effect of procyanidins is in agreement with the finding of Wang et al [9] who found that, oral administration of procyanidins, to the oxonate-pretreated hyperuricaemic mice, was able to elicit a dose-dependent hypouricaemic effect. Furthermore, these workers observed that the hypouricaemic effects in the experimental animals did not seem to parallel the changes in xanthine dehydrogenase and xanthine oxidase activities, implying that the procyanidins might be acting via other mechanisms apart from simple inhibition of enzyme activities [9].

In the present study procyanidins induced a significant reduction in the serum levels of uric acid, ADA and 5-nucleotidase. This antihyperuricaemic effect of procyanidins could be attributed to inhibition of the both enzymes. Other possible mechanisms such as enhanced urate clearance through promoting the renal tubular secretion of uric acid need further investigations.

Results of this study showed that intravenous infusion of procyanidins induced significant increase in urine flow, and urinary sodium excretion rate ($P<0.05$) which were accompanied by insignificant change in urinary potassium excretion. This result is in agreement with Dizaye etal [10] who reported that the diuretic effects of procyanidins extracted from Crataegus azarolus could be attributed to its antagonisting action on adenosine A1-receptor. These properties make this drug superior to other diuretic drugs as thiazides, loop diuretics and potassium sparing diuretic which can affect potassium excretion significantly [22]. It is important and encouraging to find a new diuretic drug which does not cause hypokalaemic or hyperkalaemic effects. Therefore, potassium supplements or using potassium sparing diuretics may not be required with procyanidins.

Conclusion

Procyanidins significantly reduced serum levels of uric acid, 5-nucleotidase and ADA, without detectable effects on the level of xanthine oxidase. The hypourecemic effect of procyanidins is attributed to inhibition of ADA and Nucleoltidase. Procyanidins has good diuretic and natriuretic effects without any significant effects on the excretion of Potassium. These dual (hypouricaemic and diuretic) effects of procyanidins recommended it as a good drug for the treatment of gout and renal uric acid calculi.

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