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Potential pharmacodynamic and pharmacokinetic interaction of pomegranate juice and nateglinide against diabetis induced complications in rats

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ABSTRACT

Objective: Pomegranate can inhibit CYP2C9 activity which is majorly responsible for metabolism of nateglinide. The present study has been undertaken to evaluate pharmacokinetic and pharmacodynamic interactions of pomegranate and nateglinide against diabetic induced complications.
Methods: Diabetes was induced by administration of alloxan (150 mg/kg, i.p). Rats (n = 8) were treated with pomegranate juice (PJ) (3 ml/animal, p.o.), nateglinide (NAT) (20 mg/kg, p.o.) and the combination of both for 4 weeks. Twenty four hours after the last treatment pharmacodynamic interaction of PJ and NAT were evaluated by antinociceptive activity, electrocardiographic parameters, serum glucose, biomarkers and lipid profile values. Influence of PJ on the pharmacokinetics of NAT were studied by HPLC method.
Results: The treatment of rats with PJ and NAT resulted in improvements in parameters indicating diabetic complications. The combination group was found to be the best protected group. Significant improvements of the antinociceptive activity, a restoration of electrocardiographic parameters, serum glucose, biomarkers and lipid profiles compared to the NAT treated group were observed. Results of the pharmacokinetic parameters revealed that the addition of PJ increased the bioavailability and half-life of NAT, along with a decrease in the clearance and elimination rate of NAT.
Conclusion: In comparison to NAT alone, the combination of PJ and NAT improved alloxan induced parameters of diabetic complications probably due to a higher bioavailability of NAT. This phenomenon deservers further investigations.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder associated with macro and micro vascular complications. Pathological conditions associated with diabetes are responsible for the impairment of numerous organs and functions of the organism resulting in chronic deterioration of the organs and premature morbidity and mortality [1].

From the ancient times, in many societies, herbs and herb-based therapy played an important role the treatment of different diseases and in the improvement of the quality-of-life. Herb-drug interaction studies are important as the interactions may influence the pharmacokinetic and pharmacodynamic profile of each other, and they can mimic, magnify, or oppose the action of each other [2].

Punica granatum, popularly known as pomegranate, is a member of Lythraceae family, which is a large deciduous shrub or small tree, and has a rich history of traditional use in medicine. Pomegranate juice has been shown to exert significant anti-cancer, anti-inflammatory, anti-diabetic, anti-microbial, anti-oxidant, anti-atherosclerotic, anti-hypertensive effects [3,4]. Some of the studies have been indicated significant anti-diabetic effect associated with pomegranate may be due to the presence of oleanolic, ursolic, and gallic acids as chief chemical constituent. No attempts have been undertaken to evaluate potential beneficial effects of pomegranate in diabetes induced secondary complications [5,6].

Nateglinide, a maglitinide analogue which is an insulin secretagogue, is used very frequently in the treatment of diabetes.

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Nateglinide is extensively metabolized in the liver, primarily by the cytochrome P450 (CYP) 2C9 (70%) and CYP3A4 (30%) isoenzymes. Metabolites have a poor anti-diabetic properties and nateglinide has a relatively small volume of distribution [7].

One of the interesting findings regarding pomegranate juice is its ability to inhibit CYP2C9 activity by which it can potentiate the effect of those drugs which are metabolized by the same pathway. It has been reported that pomegranate fruit juice with a dose of 3ml p.o. is responsible for increased potency of tolbutamide by inhibiting CYP2C9 activity. Pomegranate is also responsible for the inhibition of the uptake of the solute carrier transporters which are membrane proteins responsible for cellular influx of various substances including drugs and xenobiotics [8,9].

The present study was designed to evaluate the effect of pomegranate fruit juice alone and in combination with nateglinide against alloxan induced diabetic complications.

2. Methods

2.1. Chemicals

All chemicals used were of analytical grade and purchased from standard companies such as R L Fine Chem, Bengaluru and Rankem, Mumbai. Biochemical kits were procured from Crest Biosystems (Goa, India). Pure sample of nateglinide was gifted by the Bangalore Test House (Bangalore, India).

2.2. Experimental animals

Healthy adult Wistar albino rats of either sex weighing 175–250 g were housed in polypropylene cages, maintained under standardized conditions (12h light: Dark cycles, $25^{\circ} \pm 5\,^{\circ}C$) with paddy husk bedding at the Central Animal House, of our institute, were provided with standard pellet food and had free access to purified drinking water. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study (SDCP/IAEC-06/2013-14).

2.3. Plant material

Fresh pomegranate fruits were collected from Malabar region of India in the month of June 2015. The authentication was performed by Dr. Neoline J. Pinto, H.O.D., Department of Botany,St. Agnes College, Mangalore (SAC/MNG/SMP/Drug/2015-06/55). The seeds were separated from the pomegranate fruits and fruits were ground to obtain juice.

2.4. Phytochemical estimations

Pomegranate juice (PJ) was subjected to qualitative analysis to investigate the presence of various phytochemical constituents like alkaloids, glycosides, flavonoids, tannins, saponins, carbohydrates, proteins, phytosterols [10,11].

2.4.1. Determination of total phenolic compound

PJ was subjected for total phenolic compound based on Folin-Ciocalteu reagent assay. Total phenolics content was expressed as mg Gallic acid Equivalents (GAE) [12].

2.4.2. Determinationof total flavonoids

Total flavonoid content of PJ was measured by the aluminum chloride colorimetric assay. The total flavonoid content was expressed as mg quercetin equivalents (QE) [13].

2.4.3. Determination of the ascorbic acid content

The 2,6-dichlorophenolindophenol method was adopted to determine ascorbic acid present in PJ [14].

2.5. Dose selection

Based on earlier literature review, therapeutic dose of nateglinide (NAT) is 50 mg/kg [15]. It was found that pomegranate juice (PJ) with a dose of 3ml/ rat was able to inhibit CYP2C9 enzyme. So the same dose is used here for the experimental protocol [8].

2.6. Experimental protocol

The animals were divided into five different treatment groups of eight animals each. Group I and Group II received saline were termed as normal control and diabetic control, respectively; Group III & IV received NAT (50mg/kg) and PJ (3ml/ rat); Group V received combination of NAT and PJ. All the treatments were given for 4 weeks through oral route. Apart from normal control group for all other groups diabetic rats were used.

2.6.1. Induction of diabetes

Diabetes was induced by intra peritoneal injection of a freshly prepared aqueous solution of alloxan monohydrate (150mg/kg body weight) [6] in normal Saline (Group II-IV). 72 h after alloxan administration blood was withdrawn from the overnight fasted rats through the tail vein for glucose analysis and rats with fasting glucose ranging from 210 to 220 mg/dl, showing clear signs of polyuria, polyphagia and polydipsia were considered as diabetic. Animals with fasting blood glucose less than 200 mg/dl were not used for experimentation.

2.6.2. Estimation of serum glucose level

Twenty four hours after the last treatment serum glucose level was estimated by commercial kits with the help of a semi-autoanalyzer [6].

2.6.3. Effect on fasting insulin level

Twenty four hour after the last treatment blood was collected and serum was separated by centrifugation. Insulin levels were measured using rat insulin enzyme-linked immunosorbent assay kit (Mercodia, Sweden) in serum [16].

2.6.4. Anti- nociceptive activity

Twenty four hours after the last treatment antinociceptive activity was carried out by hot plate and tail immersion tests [15].

2.6.5. Electrocardiographic studies

Twenty four hours after the last treatment, the animals were anesthetized with the combination of ketamine (75 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Leads were attached to the dermal layer of both the front paws and hind legs and recordings were made with the help of a digital physiograph (model no-DI-2, INCO, Ambala City, India). The changes in heart rate, QRS interval, QT interval, and RR interval were noted [17].

2.6.6. Serum biomarkers levels

Twenty four hours after the last treatment, blood was collected by retro-orbital puncture and the serum was separated by centrifugation. Isolated serum was analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), creatine kinase MB (CKMB), creatine kinase NAC (CKNAC), lactate dehydrogenase (LDH), creatinine and albumin. Estimation of different biomarkers was estimated by commercial kits with the help of a semiautoanalyzer [17,18].