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Efficacy of asiatic acid, a pentacyclic triterpene on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats

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ABSTRACT

Asiatic acid (AA), a triterpenoid derivative of *Centella asiatica*, has shown significant biological effects of antioxidant and anti-inflammatory activities. Aim of this investigation was to evaluate the anti-hyperglycemic effect of AA on the activities of hepatic enzymes of carbohydrate metabolism in streptozotocin (STZ)-induced diabetic rats. To induce diabetes mellitus, rats were injected with streptozotocin intraperitoneally at a single dose of 40 mg/kg b.w. Diabetic rats showed significant ($p < 0.05$) increased in plasma glucose, glycosylated hemoglobin and significant ($p < 0.05$) decreased in circulating insulin and hemoglobin. The altered activities of key enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase of carbohydrate metabolism significantly ($p < 0.05$) increased whereas hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase and glycogen content significantly ($p < 0.05$) decreased in the liver of diabetic rats and also increased activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Oral administration of AA (5, 10 and 20 mg/kg b.w.) and glibenclamide (600 μg/kg b.w.) to diabetic rats for 45 days prevented the above alteration and reverted to near normalcy. Protection of body weight loss of diabetic rats by AA was also observed. No significant effect was observed in normal rats treated with AA (20 mg/kg b.w.). In this search, AA found to be potential bioactive compound to regulate the carbohydrate metabolism by modulating the key regulatory enzymes in diabetic rats. These findings merit further research in this field.

Introduction

Diabetes mellitus is caused by an abnormal carbohydrate metabolism and mainly linked to abnormal blood insulin levels or insensitivity of target organs to insulin (Hahm et al. 2011). According to the International Diabetes Federation (IDF), the global prevalence of diabetes is predicted to grow from 366 million in 2011 to 552 million by 2030 (IDF 2011). With this increasing global prevalence of diabetes, the need for therapeutic measures against the disease has become stronger. Currently, adapting to dietary modification of controlling blood sugar levels, physical exercise, insulin therapy, and oral medications (Sona 2010). Liver plays an important role in glucose homeostasis through glycolysis, glycogenesis, and gluconeogenesis and it is affected severely during diabetes. The net glucose uptake by the liver depends on the activities of glucokinase and glucose-6-phosphatase. The activity of hepatic glucokinase is markedly decreased and activity of glucose-6-phosphatase is almost doubled in diabetic condition (Nordlie et al. 1999). Glucose, taken up by secondary active transporter proteins, is degraded to pyruvate which is then introduced into the citric acid cycle after its decarboxylation to acetyl coenzyme A. The cycle provides NADH for oxidative phosphorylation to generate ATP.

However, several hypoglycemic agents are used for the treatment of diabetes are reported to have adverse effects including liver problems, lactic acidosis, and diarrhea (Rajalakasmi et al. 2009). Thus, use of these drugs may be limited depending on their pharmacokinetic properties, secondary failure rates, and side effect (Donath and Ehses 2006). The developmental process in antidiabetic drug discovery has shifted its focus on plant-derived drugs due to their safety, efficacy, cultural acceptability and lesser side effect (Veerapur et al. 2010). Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents (Tang et al. 2006). Thus, finding natural drugs with hypoglycemic activity has now become the focus of scientists and researchers for the prevention of diabetes mellitus.

Triterpenes are biosynthesized in plants by the cyclization of squalene, and are widely distributed in dietary fruits, green leaves and vegetables, and are major components in many medicinal plants used in Asian countries. Asiatic acid

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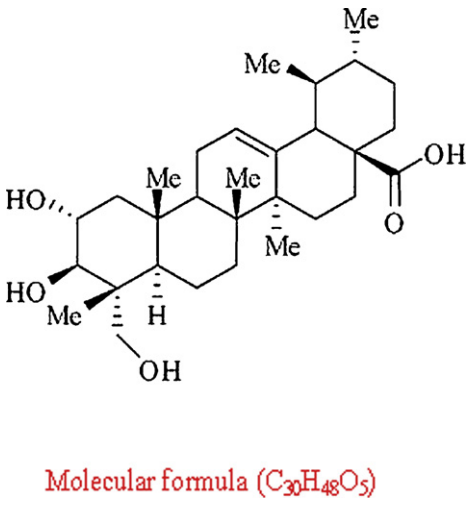

Fig. 1. Structure of asiatic acid.

(AA; 2α, 3β, 23-trihydroxyurs-12-en-28-oic acid; molecular formula $C_{30}H_{48}O_5$; Fig. 1) is one of the triterpenoid components found in *Centella asiatica* (Schaneberg et al. 2003) which grows in India and is widely spread in Asian countries too. AA exhibits several pharmacological activities such as antioxidant (Lee et al. 2003), hepatoprotective (Ma Zhang et al. 2009), anticancer (Liu et al. 2006), antiinflammatory (Huang et al. 2011), neuroprotective (Jew et al. 2000) and antialzheimers (Singh and Agarwal 2007) properties. AA has also reported to lower blood glucose level in type 1 diabetic rats (Liu et al. 2010). Hence, the present study, evaluated the effect of AA on blood glucose, plasma insulin, total hemoglobin, glycosylated hemoglobin and carbohydrate metabolic enzymes and glycogen in streptozotocin-induced diabetic rats.

Materials and methods

Chemicals

Streptozotocin and asiatic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade obtained from E. Merck and Himedia, India.

Animals

Adult Male albino Wistar rats (9 weeks old; 180–200 g) were obtained from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained at a constant temperature (25 ± 1 °C) on a 12 h light/12 h dark cycle with feeds (Pranav Agro Industries Ltd., Pune, Maharashtra, India) and water were provided ad libitum. The experimental protocol was approved by the Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA, Proposal No.: 848), Annamalai University, Annamalainagar.

Induction of diabetes mellitus

The animals were rendered diabetic by a single intraperitoneal injection of 40 mg/kg b.w. streptozotocin (STZ) diluted in 0.1 M sodium citrate buffer (pH 4.5) solution. STZ-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemia. STZ-injected animals exhibited hyperglycemia within a few days. Diabetic condition rats were confirmed by measuring the elevated plasma glucose (by glucose oxidase method) 72 h STZ after injection. The rats with blood glucose above 235 mg/dL were considered to be diabetic and used for the experiment.

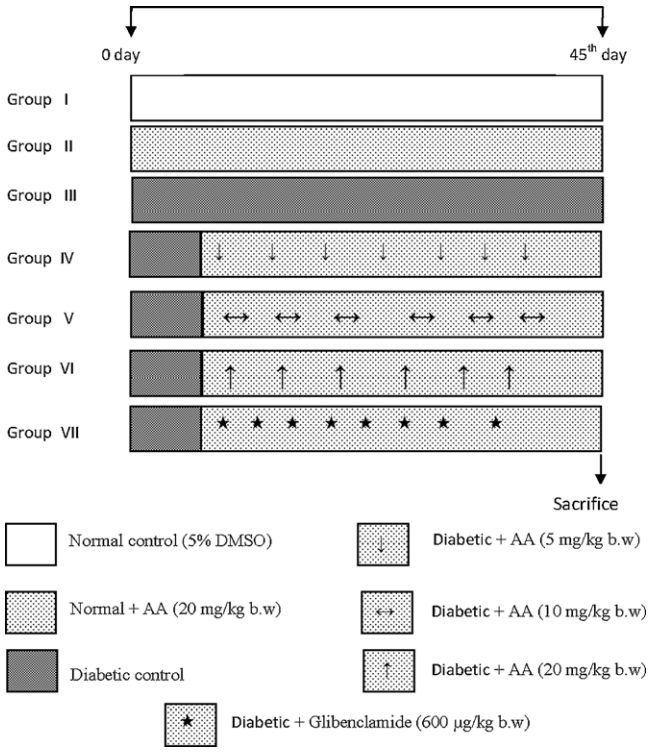

Fig. 2. Experimental protocol.

Experimental protocol

The rats were divided into seven groups each comprising a minimum of six rats each. AA were dissolved in 5% DMSO and glibenclamide was diluted in water and administered orally to experimental groups using intragastric tube daily for a period of 45 days (Fig. 2).

The initial and final body weights of each rat in various groups were recorded. At the end of the experimental period, all the animals were fasted overnight, anesthetized using ketamine (24 mg/kg b.w. intramuscular injection), and sacrificed by cervical decapitation. Blood samples were collected in tubes containing potassium oxalate and sodium fluoride (3:1) mixture for the estimation of plasma glucose and insulin. Hb and HbA1c levels were estimated in whole blood samples. Tissue was immediately dissected, washed in ice-cold saline, homogenized in Tris–HCl buffer (0.1 M, pH 7.5). The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical analysis

The estimation of glucose, Hb and HbA1c were done by the methods of Trinder using a commercial kit (1969), Drabkin and Austin (1932) and Sudhakar and Pattabiraman (1981), respectively. Plasma insulin was estimated using a commercial kit by enzyme-linked immunosorbent assay by the method of Burgi et al. (1988). The activities of hexokinase, glucose 6-phosphatase, fructose 1,6-bisphosphatase, glucose 6-phosphate dehydrogenase, pyruvate kinase and glycogen were assayed by the methods of Brandstrup et al. (1957), Koide and Oda (1959), Gancedo and Gancedo (1971), Bergmeyer (1984), Valentine and Tanaka (1966)

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