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Original Research Article

Antidiabetic, antihyperlipidemic activities and herb–drug interaction of a polyherbal formulation in streptozotocin

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ABSTRACT

Background: Ojamin (OJ), a polyherbal antidiabetic formulation, is extensively used as a food supplement to control diabetes alone or along with synthetic antidiabetic agents. However, it's phytochemical and pharmacological investigations are lacking.
Objective: The present study was undertaken to study antidiabetic and antihyperlipidemic potentials of OJ and its interaction with Metformin in streptozotocin (STZ)-induced diabetic rats.
Materials and methods: Diabetes was induced in Wistar rats by single intraperitoneal (i.p.) injection of streptozotocin (60 mg/kg). Antidiabetic, antihyperlipidemic activities of OJ were evaluated at dose of 0.28 ml/kg by estimating biochemical changes in urine, serum and liver tissue homogenate and histological changes in liver and pancreatic tissues. Metformin (100 mg/kg, p.o.) was used as reference standard drug.
Results: Results indicate that STZ administration caused hyperglycemia, increased serum glycosylated hemoglobin content, altered serum lipid profile, polyuria, decreased liver glycogen content and histological changes in liver and pancreatic tissues. This elevated serum glucose level and urine volume was significantly decreased by OJ. Supplementation with OJ produced significant improvement in serum lipid profile and glycosylated hemoglobin content along with significant increase in the liver glycogen content. OJ treatment also restored histological changes in liver and pancreatic tissue near to the normal. The observed antidiabetic and hypolipidemic effects of OJ were superior to Metformin. Co-treatment of diabetic rats with OJ and Metformin failed to control blood glucose levels.
Conclusion: It is concluded that the OJ possesses significant antidiabetic and antihyperlipidemic activities in rats. However, co-administration of OJ and Metformin is cautioned.

1. Introduction

Diabetes mellitus is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids, carbohydrates, proteins metabolism and it increases the risk of cardiovascular diseases complications [1]. Diabetes mellitus and its associated acute and long-term complications are an important health hazard globally. Its prevalence has now reached pandemic proportions in India [2]. It is reported that the four countries namely China, India, Indonesia and Japan from Asia are center for the global epidemic of diabetes and the number of diabetic patients worldwide has doubled in the past two decades [3]. Herbal drugs have always remained an important source of medicines. As per Indian traditional system of medicine, many medicinal plants have been used for the management of various health disorders including diabetes mellitus. Moreover, some polyherbal formulations, including Ojamin, are recommended as food supplement in diabetes mellitus and these herbal-based formulations are commonly used by the community because they are cost-effective and have fewer side effects than synthetic drugs. Furthermore, there is widespread practice of using such herbal-based drugs with allopathic medicines. Ojamin is Over-the-Counter herbal formulation marketed by M/s Tate Remedies, Pune, India. It contains aqueous extracts of fourteen herbs. The botanical name (family) and quantity of aqueous extract of these plants in 5 ml in formulation are – *Aegle marmelos* (Rutaceae) 66 mg, *Trigonella foenum graecum* (Fabaceae) 66 mg, *Carum carvi* (Umbeliferae) 66 mg, *Emblica officinalis* (Euphorbiaceae) 400 mg,

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Terminalia chebula (Combretaceae) 400 mg, *Terminalia bellirica* (Combretaceae) 400 mg, *Swertia chirata* (Gentianaceae) 66 mg, *Tinospora cordifolia* (Menispermaceae) 66 mg, *Eugenia jambolana* (Myrtaceae) 66 mg, *Picrorhiza kurroa* (Plantaginaceae) 66 mg, *Gymnema sylvestre* (Asclepiadaceae) 66 mg, *Salacia chinensis Linn* (Celastraceae) 76 mg, *Curcuma longa* (Zingiberaceae) 66 mg and *Melia azadirachta* (Meliaceae) 66 mg. However, the rationale behind its usefulness as an antidiabetic has not been established yet, through the systematic pharmacological study. With this background, the present research work was undertaken to standardize and evaluate antidiabetic and antihyperlipidemic activities of a polyherbal formulation, Ojamin, using streptozotocin (STZ)-induced diabetic model in rats. Furthermore, the study investigated effect of simultaneous administration of OJ and Metformin in diabetic rats.

2. Materials and methods

2.1. Standardization of OJ by HPLC

The OJ was standardized for the content of marker compounds, gallic acid (GA) and rutin (RU). The HPLC method was developed in the laboratory and validated as per ICH guidelines (data not given). For analysis, 1 ml of OJ formulation was transferred to 10 ml volumetric flask and diluted to mark with the acetonitrile. The solution was sonicated for 20 min, filtered through 0.45μm membrane filter and 10 μl of the solution was injected into the HPLC system (Waters Inc., Milford, MA) consisted of a binary pump (Model: Waters 515 HPLC pump), auto sampler (Model: 717 plus auto-sampler), column heater (Model: CHM, Sr. No. A08CHM 289M) and photodiode array (PDA) detector (Model 2998). Separation was achieved on Kromasil C-18 column (4.6 mm, 5.0μm) maintained at 40°C using the column oven. Isocratic elution was carried out with acetonitrile:water (50:50) as mobile phase (pH: 3.5 adjusted with o-phosphoric acid). Mobile phase flow rate was maintained at 0.6 ml/min and detection was monitored at 310nm, data collection and analysis was performed using Empower-version 2 software (Waters Inc., Milford, MA).

2.2. Antidiabetic and antihyperlipidemic activities of Ojamin

2.2.1. Animals

Male Wistar albino rats weighing between 150 and 250 g supplied by National Institute of Biosciences, Pune, India were used for the study. The animals were acclimatized for ten days under standard conditions in an animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The animals were given a standard diet supplied by Nutrivet Life Sciences, Pune, India. The study protocol was approved by the Institutional Animal Ethics Committee (Ref. No.: MIP/IAEC/2014-15/M1/Apr/007) of Maharashtra Institute of Pharmacy, Pune, India.

2.2.2. Chemicals and instruments

The Ojamin (OJ) was obtained as a gift sample from M/s Tate Remedies, Pune, India for the study. Gallic acid (Thomas Beaker Chemicals Pvt. Ltd, India), Rutin (Sigma–Aldrich, India) and streptozotocin (Himedia Lab Pvt. Ltd, India) were purchased from the local market. Glucose estimation kit, total cholesterol estimation kit, HDL-cholesterol estimation kit and triglyceride estimation kit manufactured by Benesphera Diagnostics, India were used for the study. All other chemicals and reagents used were of analytical grade and procured from approved vendors. Instruments such as analytical balance (Schimadzu, AUW 220D), UV–visible spectrophotometer (Varian-Cary 100), cold centrifuge (Bioera, BL-165-D), homogenizer (Biolab, B244), ulrasonicator (Ultrasonics, 5.5L-150H), pH meter (Equip-Tronics, EQ-621), UV-semiautoanalyzer (Benesphera, C61) were used for the study.

2.2.3. Selection of OJ dose

Antidiabetic and hypolipidemic activities of OJ were evaluated at the dose of 0.28 ml/kg twice daily which was extrapolated from the adult dose (20 ml, twice daily) commonly used in humans [4].

2.2.4. Induction of diabetes

Diabetes was induced in overnight-fasted rats by administering single intraperitoneal (i.p.) injection of freshly prepared streptozotocin 60 mg/kg bw in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg bw. The induction of diabetes was confirmed by measuring fasting blood glucose level on the fifth day of STZ administration. Rats with fasting blood glucose level of more than 200 mg/dl were considered as diabetics and used for the experiment [5].

2.2.5. Experimental design

Animals were randomly divided into five groups as Groups I, II, III, IV and V containing six animals in each. Group I served as a normal control and was maintained on regular rat food and drinking water *ad libitum*. All remaining groups i.e. Groups II–V received diabetes induction treatment. Group II served diabetic control group. Group III served as a reference standard treatment group and received Metformin at a dose of 100 mg/kg for 21 days. Group IV served as OJ-treatment group and received OJ at dose of 0.28 ml/kg twice daily for 21 days. Group V served as OJ-Metformin treated group and received OJ (0.28 ml/kg, twice daily) and Metformin (100 mg/kg) for 21 days.

2.2.6. Body weight and serum analysis

All animals were monitored for body weight during the treatment period. Blood was collected on 0th, 7th, 14th and 21st day of the treatment from the retro-orbital sinus under ether anesthesia condition. The serum was separated by centrifugation at 10,000 g for 10 min and analyzed for glucose level by using diagnostic kits. The serum collected on 21st day of the treatment period was also analyzed for total cholesterol (TC), high density lipoprotein cholesterol (HDLc) and triglyceride (TG) levels by using diagnostic kits. The low density lipoprotein cholesterol (LDLc) and very low density lipoprotein-cholesterol (VLDLc) were also calculated by using Friedwald's formula [2].

2.2.7. Urine volume analysis

After blood collection, all animals were kept in individual metabolic cages for the collection of 5 h urine samples and urine volume was measured.

2.2.8. Glycosylated hemoglobin, liver glycogen and histopathological analysis

At the end of treatment period, blood was also collected in EDTA tubes from the retro-orbital sinus under ether anesthesia and analyzed for glycosylated hemoglobin content. After blood collection, animals were sacrificed by cervical dislocation under ether anesthesia; liver and pancreas were isolated and subjected to histopathological analysis. Glycogen content of part of the isolated liver tissue was estimated by the method reported earlier [6].

2.2.9. Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). The statistical significance between diabetic control group and normal control group was calculated by Student's t-test, whereas one-way analysis of variance (ANOVA) followed by

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