Journal of Ethnopharmacology 206 (2017) 19–30

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Chromane isolated from leaves of *Dillenia indica* improves the neuronal dysfunction in STZ-induced diabetic neuropathy

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ARTICLE INFO

Keywords:
Advanced glycation end-products
Dillenia indica
Nitrosative stress
Oxidative stress

ABSTRACT

Ethnopharmacological relevance: According to the Indian traditional medicine, *Dillenia indica* L. has shown therapeutic efficacy in various diseases. Fruits and leaves of the plant possess anti-oxidant and anti-inflammatory properties. Reactive oxygen species, formation of advanced glycation end products (AGEs) and apoptosis are implicated in the pathogenesis of diabetic neuropathy.

Aim of the study: The aim of the present study was to explore the effect of *D. indica* and its isolate, chromane (CR), on thermal and mechanical hyperalgesia, allodynia, MNCV and oxidative-nitrosative stress in streptozotocin-induced experimental diabetes.

Material and methods: Diabetes was induced by intraperitoneal administration of Streptozotocin (STZ; 65 mg/kg) for the development of diabetic neuropathy. Chronic treatment with DAE (100, 200 and 400 mg/kg, *p.o.*) and CR (5 and 10 mg/kg, *p.o.*) for 30 days was started from the 60th day of STZ administration. Development of neuropathy was evident from a marked hyperalgesia and allodynia; reduced MNCV associated with increased formation of AGEs and reactive oxygen species.

Results: significantly attenuated behavioral and biochemical changes associated with diabetic neuropathy. Present study suggested that DAE and CR ameliorated hyperglycemia and diabetic neuropathic pain *via* modulation of oxidative-nitrosative stress and reduction in AGEs formation in the diabetic rats.

Conclusion: Thus *D. indica* might be beneficial in chronic diabetics, ameliorate the progression of diabetic neuropathy and may also find application in diabetic neuropathic pain.

1. Introduction

Diabetic peripheral neuropathy (DPN) is one of the most frequent long-complication of diabetes mellitus and frequently results in clinically significant morbidity. This complication occurs in about one-quarter of diabetic patients (Kandhare et al., 2012). Painful diabetic neuropathy is associated with symptoms and signs such as burning, tingling, or lancing type of spontaneous pain, allodynia, and hyperalgesia (Morani et al., 2012; Kaur et al., 2014). Thus, novel therapeutic targets are required for the satisfactory treatment of diabetic neuropathic pain (Tesfaye and Selvarajah, 2012; Raafat et al., 2014). Pathogenesis of diabetic neuropathy (DN) remains controversial due to multifactorial causes of the disease. Chronic hyperglycemia remains the main culprit in the initiation of biochemical changes that contributes to the development of DN (Singh et al., 2014). Abnormal free radicals' high levels cause membrane damage leading to decline of antioxidant defence mechanisms causing cell and tissue damage (Maritim et al., 2003). Researchers have evidenced that the strategy for alleviating the oxidative damage in diabetes mellitus is based on supplementation with certain dietary antioxidants such as vitamins E and C, and flavonoids (Rahimi et al., 2005; Matough et al., 2012). Diabetic neuropathy is precipitated due to an array of factors including elevated hexosamine shunt, aldose reductase activation, decrease in the nerve myoinositol content, an impaired neurotrophic support, activation of protein kinase C (PKC), activation of poly (ADP-ribose) polymerase (PARP), impaired insulin/C peptide action, and formation of advanced glycation end products (AGEs) which modulate various intertwining biochemical pathways to orchestrate autooxidative glycosylation and polyol pathways leading to structural and functional aberration of peripheral neurons, spinal glial cells and nerve fibers. Various cytokines and excitatory neurotransmitters (NT) also contribute to down regulation of pain threshold of the neurons (Singh et al., 2014). One mechanism that has been recognized to play a significant role in the pathogenesis of sensory neuron damage, is the process of reactive dicarbonyls forming AGEs due to hyperglycemia (Jack and Wright, 2012). Collectively these pathways cause an imbalance in the

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http://dx.doi.org/10.1016/j.jep.2017.05.018
Received 23 January 2017; Received in revised form 17 April 2017; Accepted 9 May 2017
Available online 13 May 2017

mitochondrial redox state of the cell and lead to excessive formation of reactive oxygen species (ROS).

Oxidative stress also plays a key role in the pathogenesis of diabetes and diabetic complications. The excessive free radicals' generation occurs in diabetes due to glucose oxidation, non-enzymatic glycation of proteins etc. These high levels of free radicals can lead to lipid peroxidation, damage of enzyme system and cellular organelles. It can also lead to insulin resistance (Kaur et al., 2016b; Kishore et al., 2016c; Lin et al., 2016). These consequences of oxidative stress can uphold the development of complications of diabetes mellitus (Singh et al., 2013). Isolated bioactive moieties from the class of flavonoids are being recognized as promising free radical scavengers playing pivotal role in amelioration of various diseases (Bors et al., 1996). The hydrogen donating substituent (hydroxyl groups) attached to the aromatic ring structures of flavonoids, which enable the flavonoids to undergo a redox reaction enabling them to scavenge free radicals easily (Rice-Evans et al., 1997). Thus it's a scientific challenge for researchers and clinicians worldwide, to identify the mechanisms involved in the pathogenesis of DPN and explore new treatment options that would slow or prevent the progression of DPN.

Dillenia indica L. (Dilleniaceae), commonly known as Elephant apple has been used traditionally to cure various ailments. Traditionally, the juice of leaves, bark and leaves are mixed and given orally for the treatment of cancer and diarrhea in the tribal areas of Mizoram, India (Sharma et al., 2001). The leaves and bark are used as a laxative and astringent. The leaves extract have also shown antioxidant activity *in vitro* (Kirtikar and Basu, 2003). Chemical investigation of the plant revealed pentacyclic triterpene lactone, (Banerji et al., 1975) dihydro-isorhamnetin, dillenetin, (Haque et al., 2008) lupeol, betulinaldehyde, betulinic acid and stigmasterol (Parvin et al., 2009). *Dillenia indica* is a medicinal plant used for treating diabetes mellitus and related symptoms (Tarak et al., 2011). Taking into consideration the promising constituents present in *D. indica* and its ameliorating effect on diabetes and oxidative stress, the present study involves the evaluation of *D. indica* and its isolate chromane (CR) on diabetic neuropathy.

2. Material and methods

2.1. Chemicals

Streptozotocin (STZ) was obtained from Sigma Aldrich, USA and Nicotinamide (NAD) from Finar India Ltd. and diagnostic kits for the biochemical estimations were obtained from Reckon Diagnostics Pvt. Ltd., India. All the other chemicals used were of analytical grade.

2.2. Collection of plant material

Leaves of *Dillenia indica* (L.) (Dilleniaceae) were procured Kurukshetra University, Kurukshetra and identified by Dr. Sunita Garg, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/Consult/2013/2352-132-2) was deposited in the herbarium of NISCAIR, New Delhi for future reference. Botanical name of the plant was verified from published literature and database (The Plant List, 2015).

2.3. Preparation of extract

The leaves were dried in the shade, powdered and then used for the extraction of potential antidiabetic constituents into different solvents (petroleum ether, chloroform, ethanol and hydroalcohol). Leaves were sequentially extracted with solvents in order of increasing polarity i.e. Petroleum Ether 60–80 °C, Chloroform, Ethanol and Hydro-alcohol (40%) by soxhlet extraction method (Raaman, 2006). The extracts were distilled and concentrated under reduced pressure and finally freeze dried.

2.4. Phytochemical screening

Phytochemical analysis was carried out to identify various chemical constituents (for e.g. alkaloids, carbohydrates, fixed oils and fats, terpenoids, phenolic compounds, tannins, glycosides, saponins, proteins, amino-acids and flavonoids) present in the extracts. Phytochemical analysis was carried out in accordance with the methods mentioned in Trease and Evans, and Harborne (Trease and Evans, 1989; Harbourne, 1998).

2.5. Determination of total phenol content

According to the Folin–Ciocalteau method, the total phenolic (soluble) content was estimated by using the Folin–Ciocalteau reagent. This method based on the oxidation reaction. Gallic acid was used as standard reagent in this procedure Liu et al. (2013). DAE (1.0 g/ml) was taken in the flask and then dilution of extract was made up to 46 ml with distilled water. After dilution, Folin–Ciocalteau reagent (1 ml) was added and mixed. After proper mixing, the solution was stand for 3 min. Further sodium carbonate was mixed into the above mixture solution and allowed to stand for 180 min by occasional shaking. Blue color developed was then noted at 760 nm. Phenolic compounds in DAE were determined as µg of gallic acid equivalent (Liu et al., 2013).

2.6. Quantitative analysis

2.6.1. Estimation of total flavonoids

Plant material (10 g) was extracted in triplicate with 80% aqueous methanol (100 ml) at room temperature. The whole solution was filtered using Whatman filter paper no. 42. The filtrate was transferred to a crucible and evaporated to dryness and weighed to a constant weight (Boham and Kocipal-Abyazan, 1974; Kaur and Kishore, 2012).

2.6.2. Terpenoid extraction and TLC analysis

Plant material (50 g) was macerated with methanol and water (4:1) for 24 h at 37 °C and filtered with Whatman filter paper. Filtrate was concentrated at 40 °C and concentrate was then acidified with 2 M sulphuric acid and the mixture was then extracted with chloroform. Non-aqueous layer was separated and evaporated to dryness. The dried fraction contained terpenoids and further confirmed using thin layer chromatography (TLC). TLC was performed using chloroform (100%) and spots were visualized with conc. sulphuric acid and the plates were heated for 10 min at 100 °C. Based on the color, spots were identified (Harborne, 1984).

2.7. Isolation of the active compound

Chromane (CR) was isolated as per the procedure given in our previous study. The dried alcoholic extract (5 g) was then subjected to column chromatography (silica gel packed column, Molychem 100–200 mesh, 160 g) by pre-adsorbing with silica gel 10 g extract was eluted using chloroform (100%) and the mixture of chloroform and methanol up to 5%. The fractions (100 ml each) obtained from the column were collected and combined on monitoring TLC. Seventy-five fractions were obtained. Fraction 65–71 yielded a compound, 3,5,7, -trihydroxy-2-(4-hydroxybenzyl)-chroman-4-one (CR) (Kaur et al., 2016a).

2.8. AGE's inhibitory assay

In-vitro advanced glycated end products (AGEs) were assessed by using bovine serum albumin (BSA) glycation method (Kaur et al., 2017). In this method, the solution of BSA (10 mg/ml) was mixed with fructose (1.1 M), phosphate buffer solution (0.1 M, pH 7.4) and sodium azide (0.02%). After mixing all the above reagents, the final solution