



ISSN: 2277- 7695

TPI 2017; 6(3): 37-39

© 2017 TPI

www.thepharmajournal.com

Received: 08-01-2017

Accepted: 09-02-2017

Shaul Ahmed R

Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Sciences and
Animal Husbandry, Junagadh
Agricultural University, Junagadh-
362001, Gujarat, India

Punit R Bhatt

Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Sciences and
Animal Husbandry, Junagadh
Agricultural University, Junagadh-
362001 Gujarat, India

Chirag M Modi

Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Sciences and
Animal Husbandry, Junagadh
Agricultural University, Junagadh-
362001, Gujarat, India

Vipul B Chauhan

Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Sciences and
Animal Husbandry, Junagadh
Agricultural University, Junagadh-
362001, Gujarat, India

Kajal B Pandya

Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Sciences and
Animal Husbandry, Junagadh
Agricultural University, Junagadh-
362001, Gujarat, India

Urvesh D Patel

Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Sciences and
Animal Husbandry, Junagadh
Agricultural University, Junagadh-
362001 Gujarat, India

Harshad B Patel

Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Sciences and
Animal Husbandry, Junagadh
Agricultural University, Junagadh-
362001, Gujarat, India

Correspondence**Urvesh D Patel**

Department of Veterinary
Pharmacology and Toxicology,
College of Veterinary Sciences and
Animal Husbandry, Junagadh
Agricultural University, Junagadh-
362001, Gujarat, India

In-vitro* anti-diabetic activity of different proportions of various extracts from *Glycyrrhiza glabra* and *Tinospora cordifolia

Shaul Ahmed R, Punit R Bhatt, Chirag M Modi, Vipul B Chauhan, Kajal B Pandya, Urvesh D Patel and Harshad B Patel

Abstract

Glycyrrhiza glabra and *Tinospora cordifolia* are traditional medicinal plants which possess anti-spasmodic, anti-inflammatory, anti-cancerous, and antioxidant properties. The present study was carried out to evaluate an *in-vitro* anti-diabetic activity of different extracts of *G. glabra* root and *T. cordifolia* stem in proportions of 1:2 and 2:1 by α -amylase inhibition method. Chloroform, methanol and water extracts of both plants were prepared by soxhlet apparatus. Various concentrations from 10 to 100 μ g/mL were used to determine the activity. Acarbose was used as standard test substance. Water extracts in 1:2 and 2:1 ratio inhibits α -amylase significantly ($p < 0.05$) with 53.69 ± 2.14 and 52.89 ± 1.40 percent, respectively. In case of methanolic extract, 2:1 ratio of both plants inhibits 53.95 ± 0.66 percent ($p < 0.05$), whereas 1:2 ratio of same extract by 48.12 ± 1.40 percent. Chloroform extracts didn't show any inhibition against α -amylase. Presence of triterpenoid in the methanol and water extracts of both plants might be responsible for antidiabetic activity.

Keywords: Diabetes, herbal medicine, phytochemistry, *in-vitro*, α -amylase inhibition.

Abbreviations: GTC- *G. glabra* and *T. cordifolia* Chloroform extracts; GTM- *G. glabra* and *T. cordifolia* Methanol extracts; GTW- *G. glabra* and *T. cordifolia* Water extracts; DMSO- Dimethyl sulphoxide.

1. Introduction

Diabetes is considered as a collective metabolic disorder affecting different organs in the body. The glucose utilisation of the body is severely affected because of improper insulin secretion from β -cell of the pancreas [1]. Not only pancreas but other major organs like kidney and liver are also damaged due to a diabetic condition. The increase in the glycogen catabolism results in low hepatic glycogen level and ultimately hepatic damage. Such condition may result in elevation of liver marker enzymes like transaminase and phosphatase [2]. Pet animals particularly dog and cat are also prone to diabetes mellitus due to obesity and inability to produce insulin [3].

The α -amylase is a key enzyme for the metabolism of starch which converts into maltose, maltotriose, various α -(1-6) and oligo glucans. This reaction ultimately yields glucose. Over activity of the α -amylase raises blood glucose level and finally hyperglycemia [4].

Traditional ethnobotanical and ethnoveterinary practices cover various medicinal plants used in the treatment of diabetes around the world [5]. Herbal remedies are described for the diabetic condition in the dog and cat. Various medicinal plants like Garlic, *Aloe vera* and fenugreek, Dandelion leaf, alfalfa, and calendula are commonly used in the diabetic veterinary patients [6]. *Tinospora cordifolia* (Willd.) Miers. is belonging to Menispermaceae family is distributed throughout tropical Indian subcontinent and China. It's widely used shrub in folk and Ayurveda. It is reported that *T. cordifolia* has anti-spasmodic, anti-inflammatory, anti-allergic, anti-diabetic, and antioxidant properties. *T. cordifolia* is commonly used in traditional medicine as an anti-diabetic plant. The plant is rich in alkaloids like sesquiterpene and diterpenoid, etc [7].

Glycyrrhiza glabra (Linn.) is another medicinal plant which has been used by human beings for at least 4000 years. Its root has demulcent, antacid, anti-ulcer, anti-inflammatory, expectorant, diuretic, laxative and sedative properties [8]. *G. glabra* contains two major phytochemicals; glycyrrhizin and its aglycone, 18 β -glycyrrhetic acid. These two chemicals exhibited extensive pharmacological activities including anti-diabetic action. Apart from these,

more than 300 flavonoids have been isolated from the *Glycyrrhiza* species which proved as a strong antioxidant [9]. The objective of the present study was to evaluate the *in-vitro* anti-diabetic properties of different extracts of *T. cordifolia* stem and of *G. glabra* root in various proportions (1:2 and 2:1) by α -amylase inhibition method.

2. Materials and Methods

2.1 Collection of plant material

T. cordifolia stem and *G. glabra* root powders were purchased from reliable local suppliers of Junagadh region (Gujarat) India.

2.2 Chemicals and reagent

Various chemicals and solvents like sodium hydroxide, dinitro salicylic acid, sodium potassium tartrate etc, were procured from SD fine chemical Pvt. Ltd. Alpha-amylase was procured from HiMedia Pvt. Ltd. Acarbose was purchased from Sigma-Aldrich, USA.

2.3 Preparation of extract and phytochemical screening

Twenty-five gramme of each powder was extracted by soxhletion with 250 mL of chloroform, methanol and water for 48 hours. After 48 hours the content was filtered with Whatman filter paper no.1 and solvents were evaporated under *vacuum*.

The extracts thus obtained were stored at 4°C until use. The qualitative phytochemical screening was performed to check the presence of various phytochemicals like alkaloid, glycoside, saponin, flavonoid, tannins etc [10].

One milligramme per mL solution was prepared by dissolving 30 mg extract in 30 mL Milli-Q water. Methanol and Chloroform extracts were dissolved by incorporating DMSO as a solvent. Each extract solution was mixed in the proportion of 1:2 and 2:1 with respective extracts.

2.4 In vitro α -amylase inhibition activity by DNS method

The *in-vitro* anti-diabetic assay was performed as per the method, given by Dey *et al.*, (2015) [11] with minor modifications. Briefly, about 500 μ L test or standard solution

(Acarbose 1 mg/mL in distilled water) was prepared in sodium phosphate buffer solution (pH-6.7), then added 500 μ L α -amylase (1U/mL) followed by 1 mL of 1 % starch solution. The reaction mixture was then put in an incubator for 5 to 10 minutes at 37 °C. Simultaneously, water was heated up to 90 °C. Finally 1 mL DNS (3, 5-dinitrosalicylic acid) reagent (prepared by mixing 438 mg dinitro salicylic acid in 20 mL distilled water and 12 gm Sodium potassium tartrate in 8 mL 2 M NaOH, after mixing both solutions 12 mL distilled water was added to obtain bright orange color) was added into the reaction mixture & immediately whole test-tubes were kept in boiled water for 3 minutes. After heating, the test-tubes were cooled at room temperature and 6 mL of distilled water was added in each tube & the solution was measured at 540 nm in UV spectrophotometer (FusionTek, UV-2900). Control solution was prepared by adding all the reagents except test or standard. Percent inhibition was calculated by following formula;

$$\% \alpha \text{ amylase inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

Where A0 is the absorbance of blank and As is the absorbance of test samples

2.5 Statistical analysis

All data were expressed in Mean \pm Standard Error (n=3) and were analysed by one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) to compare the difference in means.

3. Results and discussion

Results showed that water extracts in 1:2 and 2:1 ratio inhibited α -amylase significantly ($p < 0.05$) with 53.69 \pm 2.14 and 52.89 \pm 1.40 percent, respectively. In the case of methanol extract 2:1 ratio of both plants produced significant inhibition 53.95 \pm 0.66 percent ($p < 0.05$), whereas 1:2 ratio of the same extract inhibited α -amylase by 48.12 \pm 1.40 percent at 100 μ g/mL concentration. At higher concentrations (75 and 100) μ g/mL, inhibition of α -amylase was more significant.

Extract/ ratio	Concentrations (μ g/mL)				
	10	25	50	75	100
Acarbose	37.64 \pm 0.61 ^a	39.61 \pm 0.17 ^a	40.63 \pm 0.47 ^a	43.62 \pm 0.69 ^b	46.43 \pm 0.26 ^b
1:2 GTW	42.68 \pm 1.22 ^a	45.33 \pm 1.87 ^a	47.98 \pm 1.74 ^a	50.11 \pm 1.46 ^{ab}	53.69 \pm 2.14 ^{ab}
2:1 GTW	40.95 \pm 1.16 ^a	44.13 \pm .93 ^a	46.66 \pm 1.80 ^a	50.37 \pm 1.53 ^{ab}	52.89 \pm 1.40 ^{ab}
1:2 GTM	37.23 \pm 1.27 ^a	39.62 \pm 0.70 ^a	42.54 \pm 1.63 ^a	46.13 \pm 1.13 ^{ab}	48.12 \pm 1.40 ^{ab}
2:1 GTM	34.45 \pm 10.02 ^a	35.51 \pm 9.96 ^a	42.81 \pm 8.24 ^a	51.57 \pm 0.93 ^a	53.95 \pm 0.66 ^a

Data is expressed in mean \pm SEM (n=3). Same superscripts in each column show the non-significant difference at $p < 0.05$.

Both plants contain flavonoids and triterpenoids along with alkaloids in the *T. cordifolia* and a large number of flavonoids in the *G. glabra* [7, 9]. The higher inhibition by water and methanol extracts can be correlated with a solubility of these phytochemicals in these solvents and their α -amylase inhibition up to 53.95% in 2:1 ratio of *G. glabra* and *T. cordifolia* methanol extract. Also 1:2 ratio of these plants exhibited inhibition up to 53.69%. The lowest inhibition was found to be 48.12 \pm 1.4 percent in the case of 1:2 ratio of *G. glabra* and *T. cordifolia* methanol extracts. Though individually both plants are anti-diabetic but in combination, both plants might show synergistic action against the α -amylase. This synergism may be due to pharmacokinetic or pharmacodynamic [12].

Whereas chloroform extracts (GTC) of both plants doesn't

shows any inhibition against α -amylase in either of the combinations.

3.1 Ethical matter

This research work does not use any laboratory animals.

4. References

- Jarald E, Joshi S, Jain D. Diabetes and Herbal Medicines. Iranian Journal of Pharmacology & Therapeutics 2008; 7:97-106.
- Amarapurkar D, Das H. Chronic liver disease in diabetes mellitus. Tropical gastroenterology: official journal of the Digestive Diseases Foundation. [cited 2017 Feb 8]; 2002; 23(1):3-5. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/12170918>.

3. University of Illinois College of Veterinary Medicine. Diabetes risks and treatment in pets - veterinary medicine at Illinois, 2017 [cited 2017 Feb 8]. Available from: http://vetmed.illinois.edu/pet_column/diabetes-risks-treatment-pets/.
4. Zinjarde PSSS, Bhargava SY, Kumar AR. Potent α -amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complementary and Alternative Medicine, 2011; 20(1):11.
5. Hsu YJ, Lee TH, Chang CLT, Huang YT, Yang WC. Anti-hyperglycemic effects and mechanism of *Bidens pilosa* water extract. Journal of Ethnopharmacology 2009; 122(2):379-83.
6. Natural Dog Health Remedies. Diabetes in dogs | how natural remedies may help, 2008, [cited 2017 Feb 8]. Available from: <http://www.natural-dog-health-remedies.com/diabetes-in-dogs.html>.
7. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. *Tinospora cordifolia*: Chemistry and medicinal properties chemistry and medicinal properties of *Tinospora cordifolia* (GUDUCHI). Indian Journal of Pharmacology (Educational Forum). 2003; 35(2):83-91.
8. Sharma V, Agrawal RC. *Glycyrrhiza glabra*-a plant for the future. Mintage journal of Pharmaceutical & Medical Sciences. 2013; 2(3):15-20.
9. http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-HMPC_assessment_report/2012/08/WC500131285.pdf (Cited on 8th February 2017).
10. Mamta S, Jyoti S. Phytochemical screening of *Acorus calamus* and *Lantana camara*. International Research Journal of Pharmacy. 2012; 3(5):324-326.
11. Dey P, Saha MR, Chowdhuri SR, Sen A, Sarkar MP, Haldar B et al. Assessment of anti-diabetic activity of an ethnopharmacological plant *Nerium oleander* through alloxan induced diabetes in mice. Journal of Ethnopharmacology. 2015; 161:128-37.
12. Ma XH, Zheng CJ, Han LY, Xie B, Jia J, Cao ZW et al. Synergistic therapeutic actions of herbal ingredients and their mechanisms from molecular interaction and network perspectives. Drug Discovery Today. 2009; 14(11-12):579-88.