

Dried flower powder combination of *Clitoria ternatea* and *Punica granatum* demonstrated analogous anti-hyperglycemic potential as compared with standard drug metformin: *In vivo* study in Sprague Dawley rats

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ABSTRACT

The diabetes mellitus type-2 (DMT-2) is among the highly escalating disease that is expected to affect nearly 400 million people across the globe by the end of 2030. In DMT-2, the control of post-prandial surge is the most imperative requirement for the management of metabolic disturbances. The inclined trend toward herbal-based medicine among the developing nations owing to their safety and reduced side-effects has compelled the researchers to explore the hidden potentials of herbs and shrubs of Mother Nature. The increasing interest of herbal products among the patient population has also revolutionized the traditional ethnopharmacological aspects. While processing toward the direction, the research involved yet unreported anti-hyperglycemic activity of two well-known Indian plants *Clitoria ternatea* (Family: Fabaceae) and *Punica granatum* (Family: Punicaceae). In the present study, the dried flower powder of *C. ternatea* and *P. granatum* was screened individually and in combination for their anti-hyperglycemic activity in alloxan-induced diabetic rat model employing the standard drug metformin hydrochloride. The research demonstrated that the combination of dried flower powder of *C. ternatea* and *P. granatum* exhibited a significant anti-hyperglycemic effect after 5 hours of oral administration. The phytoconstituents present in the plant material; primarily, the flavonoids and alkaloids play a crucial role in mediating the pharmacological activity. This study will open new perspectives of future research in developing novel herbal formulations for the management of diabetes mellitus at the clinical level.

INTRODUCTION

The diabetes mellitus type-2 (DMT-2) is among the highly escalating disease that is expected to affect nearly 400 million people across the globe by the end of 2030 (Mahapatra *et al.*, 2015). In DMT-2, the control of post-prandial surge is the most imperative requirement for the management of metabolic disturbances (Mahapatra and Bharti, 2018). The current pharmacotherapeutic strategy for DMT-2 involves managing the uncontrolled pace of disease progression every year

(Mahapatra and Bharti, 2017). At present, several classes of anti-diabetic drugs of synthetic origin are available like biguanides (metformin), sulfonylureas (glipizide, glyburide, etc.), metaglinide (repaglinide, nateglinide, etc.), thiazolidinedione (pioglitazone, rosiglitazone, etc.), cellulose-type (acarbose, miglitol, etc.) and so on, which work by various pharmacological mechanisms like protein tyrosine phosphatase 1B, peroxisome proliferator-activated receptor- γ , dipeptidyl peptidase-4, aldose reductase, and α -glucosidase (Chhajed *et al.*, 2017; Godbole *et al.*, 2017). However, the inclined trend toward herbal-based anti-diabetic agents among the developing nations owing to their safety and reduced side-effects has compelled the researchers to explore the hidden potentials of herbs and shrubs of Mother Nature (Mahapatra and Bharti, 2016). The increasing interest of herbal

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products among the patient population has also revolutionized the traditional ethnopharmacological aspects (Kamble *et al.*, 2017). At present, the plant extracts or pure phytoconstituents are studied for diverse characteristics of effective control of blood glucose level, increased peripheral uptake of carbohydrates, offer repression of hyperglycemic raise, reduction or maintenance of body weight (obesity-related issues), and effectual management of serum triglyceride levels (Askary-Ashtiani *et al.*, 2016; Mahapatra *et al.*, 2017).

While processing toward the direction, it has been observed in various clinical database that the flowers and seeds of *Clitoria ternatea* (Family: Fabaceae) (Daisy and Rajathi, 2009; Daisy *et al.*, 2009; Verma *et al.*, 2013; Kalyan *et al.*, 2011; Kavitha and Premalakshmi, 2012; Sharma and Majumder, 1990) and *Punica granatum* (Family: Punicaceae) (Bagri *et al.*, 2009; Das and Barman, 2012; Das *et al.*, 2001; Huang *et al.*, 2005; Jafri *et al.*, 2000; Khalil, 2004; Li *et al.*, 2005; Radhika *et al.*, 2001) have been reported to possess adequate anti-hyperglycemic activity, individually. However, the role of both the dried plant flower extract powder in combination was never explored in exhibiting the hypoglycemic effect. In the present study, dried flower powder of *C. ternatea* and *P. granatum* was screened in combination for their anti-hyperglycemic activity in an alloxan-induced diabetic rat model, employing the standard drug metformin hydrochloride.

MATERIALS AND METHODS

Chemicals

The standard drug metformin hydrochloride was obtained in the form of generous gift sample from Zim Laboratories Ltd., Nagpur. The alloxan monohydrate was procured from HiMedia Ltd., India. The other chemicals used during the experiment were of analytical grade and purchased from HiMedia Ltd., India.

Instruments

Shimadzu® electronic balance (Model: AUW220D, Japan) was employed in weighing purpose. The Glucose strips (One Touch™), obtained from the local Pharmacy, were used for monitoring the blood glucose level.

Animals

The potentials of the dried flower powder in reducing blood glucose level were screened on Sprague Dawley rats, having an average weight of 150–200 g, aged 5–6 weeks, kept in clean polypropylene cage under controlled environment (25°C–26°C, humidity 50%–55%, and 12 hours light/dark cycle) in animal house, and given free access to water and fed them with standard rodent pellets. The Departmental Ethical Committee permission and CPCSEA approval (853/AC/04/CPCSEA/2009) were obtained in carrying out this experiment.

Collection and authentication of plant material

The *C. ternatea* and *P. granatum* flowers were freshly gathered in the month of September from the Institute's Medicinal Plant Garden. The obtained plants and their flowers were authenticated (Voucher No: 9260, 9261) by Dr. Dongarwar, Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India.

Extraction protocol

The flowers of *C. ternatea* and *P. granatum* were dried for the duration of 2 weeks and then coarse powder was attained by grinding. About 200 g of content was macerated thoroughly using water-free methanol for 15 days. Utilizing a rotator vacuum evaporator, the solvent was evaporated to dryness and the solid dark violet color mass (yield: 8.4%), in the case of *C. ternatea*, and solid dark brown color mass (yield: 9.6%) were obtained for *P. granatum*. The solid mass was further grounded and converted into fine powder.

Pharmacological screening

Oral acute toxicity studies

The acute toxicity studies were performed in the plant dried flower powder to determine the minimum concentration of drug required for therapeutic action and maximum *in vivo* safety limit. In the Sprague Dawley rats, the dose was administrated with rapidly escalating doses from 5 to 5,000 mg/kg. According to the OECD guideline 423, the LD₅₀ values were determined to find out the therapeutic index (Kanhed *et al.*, 2016).

Anti-hyperglycemic activity screening

The hypoglycemic potentials of the dried flower powder were evaluated using alloxan-induced diabetes mellitus rats. The normoglycemic rats were starved for 16 hours by injecting alloxan monohydrate (150 mg/kg bw) via the intraperitoneal route after dissolving in physiological saline. Subsequently, the rats were treated with 20% glucose solution after 6 hours via the oral route. The rats were kept on 5% glucose solution for the next 24 hours to prevent hypoglycemia. After 48 hours of alloxan administration, the rats presenting the blood glucose level >250 mg/dl were considered as hyperglycemic and included in the study (six in number/group). The dried flower powder of *C. ternatea* and *P. granatum* in the dose of 100 and 200 mg/kg bw and the blood glucose level was verified at the end of 1, 3, and 5 hours after the oral administration. According to the AUC method, the potential of the dried flower powder in reducing the blood glucose level was computed as % anti-hyperglycemic activity (Kuhite *et al.*, 2017).

Statistical analysis

The procedure was performed in a triplicate manner. The results were obtained from six animals in each group and expressed as mean ± SEM. The obtained results were statistically evaluated using Prism v5.0. Two-way analysis of variance followed by Bonferroni *post-hoc* tests to compare the data. The *p*-value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Determination of LD₅₀ values

As per the OECD guidelines, the minimum dose that can bring about the maximum anti-hyperglycemic effect for each plant dried flower powder through oral route for repetitive administration was determined. In the dose range of 5 to 5,000 mg/kg of both the plant dried powder, two doses 100 and 200 mg/kg bw were selected for screening the *in vivo* anti-diabetic potentials in Sprague Dawley rats, where no toxic signs or symptoms were detected along with mortality.

Hypoglycemic potential

The oral administration of dried flowers powder of *C. ternatea* and *P. granatum* combination facilitated anti-hyperglycemic effect in alloxan-induced diabetic rats [$F_{(5,72)} = 17.35, p < 0.0001$]. In Group 1, the control rats demonstrated a substantial rise in the blood glucose level from 349.5 to 478.9 mg/dl over the period of 5 hours. The administration of alloxan monohydrate results in a decreased insulin release from the β cells due to the massive destruction in the islets of Langerhans. In Group 2, the standard drug metformin at 120 mg/kg dose significantly suppressed the hyperglycemic rise in the Sprague Dawley rats from 396.8 to 102.8 mg/dl. The oral administration of the dried flower powders of *C. ternatea* and *P. granatum* represented an impressive decrease in the alloxan-induced elevated blood glucose level in rats. In the Group 3, *C. ternatea* (200 mg/kg) and *P. granatum* (200 mg/kg) presented a time-dependent reduction of plasma glucose level from 401.7 to 198.2 mg/dl, which signifies the potential of the combination of plant extracts. The combination exhibited a comparable anti-diabetic perspective than that of metformin, the standard anti-hyperglycemic drug. In the Group 4, *C. ternatea* (100 mg/kg) and *P. granatum* (100 mg/kg) displayed

a comparable decrease in the blood plasma glucose level from 399.8 to 207.1 mg/dl (Table 1). However, the study highlighted that doubling the dose of *C. ternatea* resulted in a marginal enhancement in the glucose-lowering potential. The hypoglycemic activity may be exerted due to the flavonoid principles and the alkaloids present in the plant materials (Borikar et al., 2018). It might be predicted that the anti-hyperglycemic mechanism involves potentiating the insulin secretion from the β -cell or by enhancing the transport of blood glucose from plasma to the peripheral tissues (Borikar et al., 2017). The *post-hoc* Bonferroni multiple comparison revealed that metformin, *C. ternatea* (100 and 200 mg/kg), *P. granatum* (100 mg/kg) significantly decreased the blood glucose level after 3 hours ($p < 0.001$) and 5 hours ($p < 0.001$). The *P. granatum* (200 mg/kg) significantly decreased the blood glucose level after 1 hour ($p < 0.01$), 3 hours ($p < 0.001$), and 5 hours ($p < 0.001$) (Fig. 1).

In the reported studies, the hypoglycemic screening of *P. granatum* flower hydroalcoholic extract in fasted Zucker diabetic fatty rats showed a potent α -glucosidase inhibition (IC_{50} of 1.8 μ g/ml), peroxisome proliferator-activated receptor- γ activation, and improvement in glucose sensitivity at peripheral milieu which effectively cause reduction in the blood glucose level from 360 to

Table 1. Anti-hyperglycemic potential of combined dried flower powder of *C. ternatea* and *P. granatum*.

Groups	Dose (mg/kg)	Blood glucose level (mg/dl)			
		0 hour	1 hour	3 hours	5 hours
Saline	1 ml	349.5 \pm 11.74	372.3 \pm 13.41	340.6.3 \pm 12.64	478.9 \pm 10.56
Metformin HCl	120	396.8 \pm 4.23	314.4 \pm 7.39	189.3 \pm 5.58	102.8 \pm 6.81
Combined dried flower powder of <i>C. ternatea</i> and <i>P. granatum</i>	400	401.7 \pm 8.44	379.7 \pm 6.67	209.6 \pm 9.82	198.2 \pm 7.13
Combined dried flower powder of <i>C. ternatea</i> and <i>P. granatum</i>	200	399.8 \pm 7.66	378.9 \pm 4.51	268.1 \pm 5.96	207.1 \pm 5.87

$n = 6$; ED_{50} values were found to be 100, 200, and 400 mg/kg bw; $p < 0.05$.

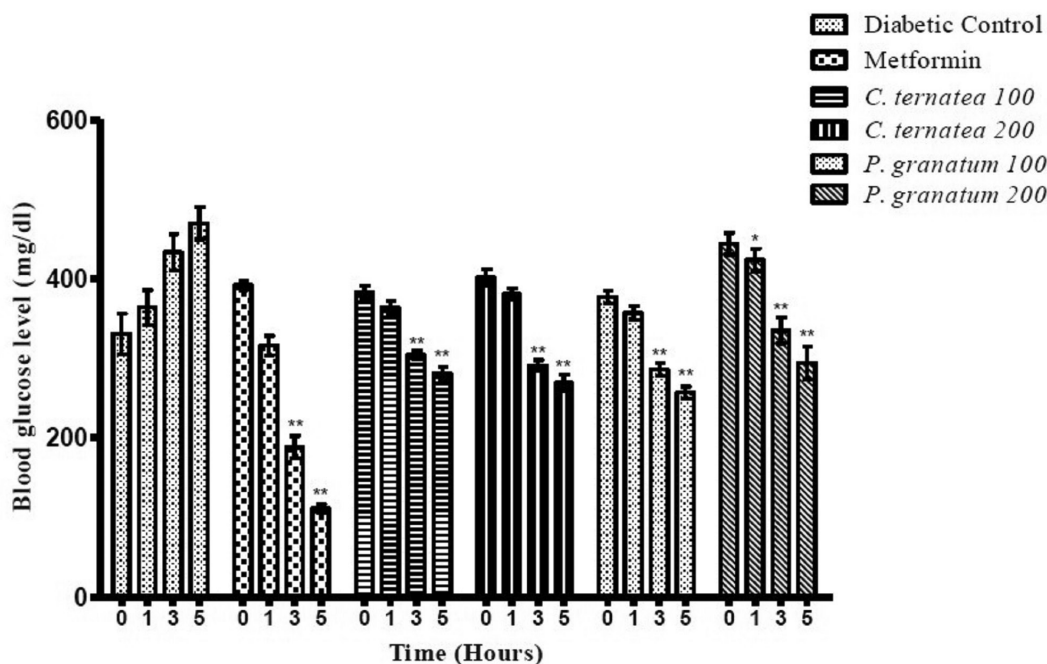


Figure 1. Effects of *C. ternatea* and *P. granatum* dried flowers powder in alloxan-induced diabetic rats.

240 mg/dl (Huang *et al.*, 2005; Jafri *et al.*, 2000; Li *et al.*, 2005). The methanolic combined extracts of *C. ternatea* and *P. granatum* demonstrated a swift decrease in the blood glucose level from 400 to 200 mg/dl than the single plant extract, which justified the synergistic potential of the therapeutics. In addition to it, the extract expressed an effectual modulation of glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase, and glutathione-S-transferase which gave emphasis toward the hyperlipidemic, pancreatic cells lipid peroxidation, and antioxidant activities which accentuate anti-diabetic prospects along with the probable mechanism(s) (Bagri *et al.*, 2009). In the same way, the anti-diabetic screening of the methanolic extract of *P. granatum* seeds in streptozotocin-induced diabetic rats reflected 52% reduction (135 mg/dl) in blood glucose level at 600 mg/kg dose after 12 hours time (Das *et al.*, 2001). A conclusion may be drawn from this study that the methanolic extracts presented a far better anti-hyperglycemic action than that of the reported hydroalcoholic extracts.

The ethanolic, chloroform, and butanolic extract of *C. ternatea* flower and leaves displayed potential pancreatic regeneration activity, anti-hyperlipidemic activity, and anti-diabetic activity by valuable increase in the HDL-cholesterol, glucokinase, glycolytic enzyme, liver and skeletal muscle glycogen, and glucose-6-phosphatase; significantly modulating glycosylated hemoglobin, total cholesterol, triglycerides, urea, creatinine, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase levels; prevents renal damage; and considerable reduction in the serum glucose level from 550 to 220 mg/dl in streptozotocin-induced diabetic rats for 14 days treatment (Daisy and Rajathi, 2009; Daisy *et al.*, 2009; Kalyan *et al.*, 2011; Talpate *et al.*, 2013; Verma *et al.*, 2013). The alkaloids, carbohydrates, glycosides, flavonoids, tannins, and saponins present in the various extract forms played an imperative role in exhibiting anti-hyperlipidemic and anti-diabetic activity. The methanolic combined extracts of *C. ternatea* and *P. granatum* produced a profound decrease in the blood glucose level within 6 hours of duration as compared to single extract which exhibited analogous results in 12 hours.

CONCLUSION

The research demonstrated that the combination of dried flower powders of *C. ternatea* and *P. granatum* exhibited a significant anti-hyperglycemic effect after 5 hours of oral administration. The phytoconstituents present in the plant material; primarily the flavonoids and alkaloids play a crucial role in mediating the pharmacological activity. This study will open new perspectives of future research in developing novel herbal formulations for the management of diabetes mellitus at the clinical level.

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