**International Journal of Pharmaceutical Science and Research**
**ISSN: 2455-4685**
**Impact Factor: RJIF 5.28**
**www.pharmacyjournal.net**
**Volume 2; Issue 5; September 2017; Page No. 04-07**

# Combined flower decoction of *Clitoria ternatea* and *Punica granatum* expressed comparable hypoglycemic activity with that of metformin: *In vivo* study

**[*1] Sachin P Borikar, [2] Nilesh G Kallewar, [3] Debarshi Kar Mahapatra, [4] Nitin G Dumore, [5] Kishor R Danao**

[1, 4] Department of Pharmacology, Dadasaheb Balpande College of Pharmacy, Nagpur, Maharashtra, India

[2] Department of Pharmacology, Shrimati Kishoritai Bhoyar College of Pharmacy, Nagpur, Maharashtra, India

[3, 5] Department of Pharmaceutical Chemistry, Dadasaheb Balpande College of Pharmacy, Nagpur, Maharashtra, India

**Abstract**
For achieving effect hypoglycemic control, mainly five classes of therapeutic drugs are used commonly which acts by different mechanism(s), however producing numerous complications. The effectiveness of these marketed products for the management of diabetes mellitus is also under question. In order to develop potential drug principles having both efficacy and good safety profile, Mother Nature remained the best source for finding the leads that have the capability to control glucose levels, decrease the uptake of carbohydrates, provide suppression of hyperglycemic lift, reduction of body weight, and serum triglyceride level. Heading towards the road of research, unreported activity of two well known Indian plants *Clitoria ternatea* (Family: Fabaceae) and *Punica granatum* (Family: Punicaceae) were selected and evaluated accordingly. In the present study, flower decoctions of *C. ternatea* and *P. granatum* were screened for their hypoglycemic potential in alloxan induced diabetic rat model utilizing metformin hydrochloride as the standard drug. The work indicates preliminary phytochemical investigations which largely helped in understanding the role of bioactive molecules in mediating the pharmacological effect. The results of the present investigation clearly indicate that the combination of decoction of *C. ternatea* and *P. granatum* flowers have significant antihyperglycemic effect in alloxan-induced diabetic rats and holds the perspective to be used in clinical practices based on the toxicity profiles available. However, further research in the development of formulations based on above two principles may open new avenues in the management of diabetes mellitus.

**Keywords:** diabetes, hypoglycemic, *clitoria ternatea*, *punica granatum*, decoction, alloxan

## 1. Introduction
Insulin is one of the most important hormones responsible for maintaining the homeostasis of glucose, triglycerides, amino acids, fatty acids, translocation of vital material, glycogen formation, and synthesis of biomolecules [1]. Diabetes mellitus type-II arises due to the inability of the body to use the produced insulin to catalyze the biochemical function. The dietary carbohydrates, glycoproteins, and glycolipids obtained from food are processed which subsequently results in raising the glucose bulk in the body, causing postprandial hyperglycemia [2]. In the majority of the type-II-DM cases, the postprandial surge is the root cause for provocation of metabolic disturbance. The obese population is primarily affected by postprandial hyperglycemic conditions which often aggravate DM like conditions [3]. For achieving effect control, mainly five classes of therapeutic drugs (protein tyrosine phosphatase 1B, peroxisome proliferator activated receptor-γ, dipeptidyl peptidase-4, aldose reductase, and α-glucosidase) are used commonly for combating hyperglycemia which acts by different mechanism(s), however producing numerous complications [4]. The effectiveness of the molecules in the DM management of these five classes is also under question, based on the reported class studies. In order to develop potential drug principles having both efficacy and good safety profile, Mother Nature remained the best source for finding the leads [5]. An ideal drug would have been such which will afford effectual glucose level control, decrease the uptake of carbohydrates, provide suppression of hyperglycemia lift, reduction of body weight, and serum triglyceride level [6]. Heading towards the road of research, unreported activity of two well known Indian plants *Clitoria ternatea* (Family: Fabaceae) and *Punica granatum* (Family: Punicaceae) were selected and evaluated accordingly.

In the present study, flower decoctions of *C. ternatea* and *P. granatum* were screened for their hypoglycemic potential in alloxan induced diabetic rat model utilizing metformin hydrochloride as the standard drug. The work indicates preliminary phytochemical investigations which largely helped in understanding the role of bioactive molecules in mediating the pharmacological effect.

## 2. Materials and Methods
### 2.1 Chemicals
The alloxan monohydrate was purchased from HiMedia Ltd., India. Metformin hydrochloride was obtained as a generous gift from Zim Laboratories Ltd., Nagpur. All other miscellaneous analytical grade chemicals used for the experiment were procured from HiMedia.

### 2.2 Instruments
The Ultraviolet-Visible spectroscopy was performed using double-beam Shimadzu® UV-Vis Spectrophotometer of Model UV-1800, Japan. The weighing of compounds was performed using a Shimadzu® electronic balance of Model AUW220D, Japan. The Glucose strips (One Touch™) were purchased from the local pharmacy.

### 2.3 Animals
For the hypoglycemic potential screening of the compounds, Sprague Dawley rat of age 5-6 weeks, having an average weight of 150-200 g were employed after prior approval from the CPCSEA (853/AC/04/CPCSEA/2009). The 6 rats were given free access to water, fed with standard rodent pellets, and housed in proper hygienic conditions in clean polypropylene cage under controlled temperature rooms (25–26ºC, humidity 50–55%, 12 hr light and 12 hr dark).

### 2.4 Collection and authentication of plant material
The flowers of *C. ternatea* and *P. granatum* were collected freshly in the month of September from the medicinal plant garden of the Institution situated in Nagpur city, Maharashtra state, India. The plants and their parts were identified and authenticated by Dr. Dongarwar, Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India.

### 2.5 Extraction protocol
The *C. ternatea* and *P. granatum* flowers were dried for two weeks and grounded to obtain a fine powder. 200 g of powder was macerated with 500 mL of methanol for 15 days. The solvent was evaporated to dryness using a rotator vacuum evaporator. The % yield for *C. ternatea* and *P. granatum* were found to be 8.4 and 9.6, respectively.

### 2.6 Phytochemical estimation
#### 2.6.1 Preliminary screening
The preliminary phytochemical screening of the decoction was carried out for the determination of essential primary metabolites such as sugars, steroids, proteins; and secondary metabolites like alkaloids, terpenes, flavonoids, glycosides, and tannins as per the given standard test procedures [7].

#### 2.6.2 Determination of total flavonoid content
The aluminium chloride method was used for the determination of the total flavonoid content of the sample extracts. Initially, 50 mg of dried extract of each sample was taken in a volumetric flask and the volume was made up with 3 mL methanol. Afterward, 0.1 mL $AlCl_3$ (10%), 0.1 mL Na-K tartarate, and 2.8 mL distilled water were added sequentially. The test solution was vigorously shaken and the absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated using known concentrations of quercetin at 415 nm. The concentration of flavonoid in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent per gram of sample [8].

### 2.7 Pharmacological screening
#### 2.7.1 Oral acute toxicity studies
The aim to perform acute toxicity studies were for establishing the therapeutic index of a particular drug and to ensure the safety *in vivo*. An acute toxicity study was generally carried out for the determination of $LD_{50}$ value in experimental animals. The $LD_{50}$ determination of test samples was performed in Sprague Dawley rats, according to the OECD guideline 423 [9]. The selection of the dose was made based upon the minimum concentration of drug required for therapeutic action which will be economically fruitful for further research and formulation. For the study, dose was initially selected from 5 mg/kg and gradually increased up to 5000 mg/kg.

#### 2.7.2 Anti-hyperglycemic activity screening
Diabetes mellitus was induced in a batch of normoglycemic rats starved for 16 hr by injecting intraperitoneally 150 mg/kg body weight of alloxan monohydrate dissolved in physiological saline. Afterwards, the rats were treated with 20% glucose solution intraperitoneally after 6 hr. For the next 24 hr, the rats were kept on 5% glucose solution in their cages to prevent hypoglycemia. After 48 hours of alloxan injection, rats with a blood glucose level more than 250 mg/dl were considered as diabetic and included in the study. They were divided into different groups, with 6 rats in each group. The hydroalcoholic flower decoction of *C. ternatea* and *P. granatum*, of dose 100 and 200 mg/kg body weight, based on the $ED_{50}$ value were administered to the animals and blood glucose was estimated at the end of 1 hr, 3 hr, and 5 hr after the oral administration of the decoction, using distilled water as the control. The lowest dose that brought about the maximum antihyperglycemic effect for each plant was given through oral intubation for the repeated administration. The potential of decoction in reducing the blood glucose level was calculated as % anti-hyperglycemic activity, according to the AUC method [10].

### 2.8 Statistical analysis
The experiments were executed in a triplicate mode. All the results were expressed for 6 animals in each group. The acquired data were statistically evaluated via Prism application version 5.0 and expressed as mean ± SEM. The P-values $< 0.05$ were considered to indicate statistical significance. The hypothesis testing method included two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests to compare the replicate means.

## 3. Results and Discussion
### 3.1 Phytochemical investigations
#### 3.1.1 Preliminary phytochemical screening
The decoction of *C. ternatea* dried flowers demonstrated the presence of carbohydrates, flavonoids, saponins, and glycosides whereas the decoction of *P. granatum* dried flowers displayed the presence of carbohydrates, sterols, flavonoids, saponins, tannins, alkaloids, and glycosides. Table 1 represents the preliminary phytochemical screening of both the plant decoctions.

### 3.1.2 Total flavonoid content

The flavonoids and phenols are one of the most diverse and widespread component present in the natural compounds. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Using the standard plot of quercetin ($R^2$ = 0.9911), the flavonoid contents of *C. ternatea* and *P. Granatum* flowers decoction was found to be 41.25 and 65.66 mg quercetin equivalent/g of dry sample, respectively.

## 3.2 Pharmacological screening

### 3.2.1 Determination of $LD_{50}$

The oral acute toxicity, as per OECD guidelines helped selecting the minimum concentration of dose required for exhibiting therapeutic action without mortality. The study demonstrated that on increasing the dose from 5 mg/kg to 5000 mg/kg of both the plant decoctions, no toxic signs or symptoms were observed along with mortality. For the *in vivo* anti-diabetic screening, two doses 100 mg/kg and 200 mg/kg body weight of Sprague Dawley rat were employed for the plant decoctions.

### 3.2.2 Hypoglycemic potential

The oral administration of dried flower decoction of *C. ternatea* and *P. granatum* facilitated antihyperglycemic effect in alloxan induced diabetic rats [F (5,72) = 16.05, P <0.0001]. The post hoc Bonferroni multiple comparison revealed that metformin HCl (120 mg/kg), *C. ternatea* (100 mg/kg), and *P. granatum* (100 mg/ kg) significantly decreased the blood glucose level after 3 hours (P <0.001) and 5 hours (P <0.001) (Figure 1). *P. granatum* and *C. ternatea* (200 mg/kg) significantly decreased the blood glucose level after 1 hr (P <0.01), 3 hr (P <0.001) and 5 hr (P <0.001). Table 2 depicts the pattern of hypoglycemic activity of combined plant decoctions.

The alloxan causes a massive reduction in insulin release by the destruction of β cells of the islets of Langerhans and thereby induces hyperglycemia. The oral administration of flower decoction of *C. ternatea* and *P. granatum* resulted in a decrease in blood glucose level in alloxan-induced diabetic rats. The possible antihyperglycemic mechanism of these may be through potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose in the peripheral tissues. The flavonoid principles present in the decoction were believed to play pivotal role in mediating the hypoglycemic activity. Other phytoconstituents like glycoside or any alkaloid may be believed to impart the glucose lowering attribute. In this study, the diabetic rats have higher levels of blood glucose level, the significant decrease of blood glucose level after 5 hours of oral administration.

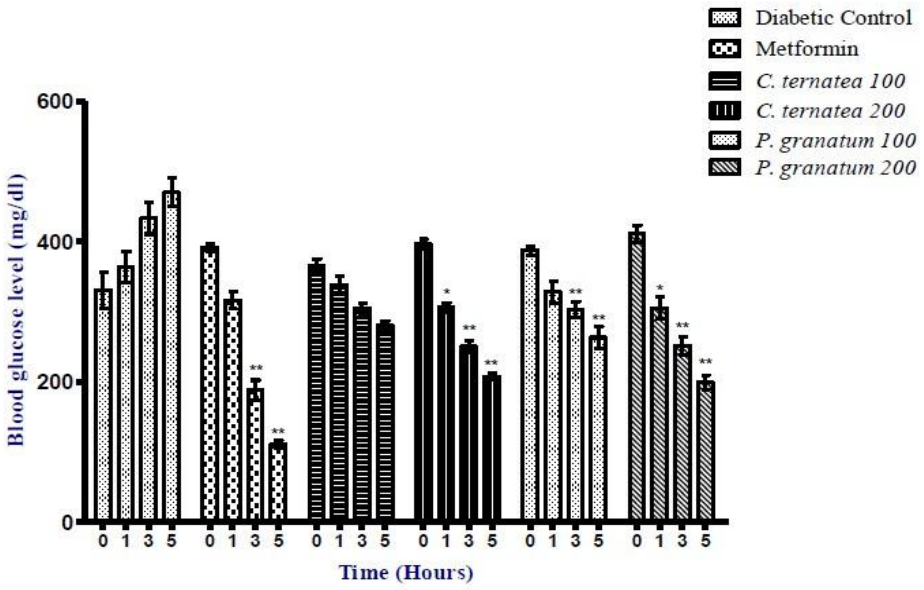


**Fig 1:** Effects of *C. ternatea* and *P. granatum* dried flowers decoction in alloxan induced diabetic rats.

**Table 1:** Preliminary phytochemical screening of decoction of *C. ternatea* and *P. granatum*.

| Component | Tests | Decoction of *C. ternatea* | Decoction of *P. granatum* |
|---|---|---|---|
| Sterol | Salkowaski test | - | - |
| | Liebermann's test | - | + |
| | Liebermann-Burchard test | - | - |
| Sugar | Molisch's test | + | + |
| | Fehling's test | + | + |
| | Barford test | + | - |
| Flavonoid | Shinoda test | + | + |
| Saponin | Froth test | + | + |

| | | | |
|---|---|---|---|
| Tannin | Lead acetate test | - | - |
| | Bromine water test | - | - |
| | $FeCl_3$ test | - | + |
| | Potassium dichromate test | - | - |
| Glycoside | Borntrager's test | + | + |
| | Keller-Killiani test | - | + |
| | Legal test | - | - |
| | Modified Borntrager's test | - | + |
| Amino acids | Biuret test | - | - |
| | Million's test | - | - |
| | Xanthoproteic test | - | - |
| | Ninhydrin test | - | - |
| Alkaloid | Wagner's test | - | + |
| | Dragendroff test | - | - |
| | Mayer's test | - | + |
| | Hager's test | - | - |

**Table 2:** Hypoglycemic potential of combined decoction of *C. ternatea* and *P. granatum*.

| Groups | Dose (mg/kg) | Blood glucose level (mg/dl) | | | |
|---|---|---|---|---|---|
| | | 0 hr | 1 hr | 3 hr | 5 hr |
| Saline | 1 mL | 330.6 ± 25.19 | 364.6 ± 21.92 | 398.3 ± 25.97 | 433.4 ± 22.8 |
| Metformin HCl | 120 | 391.8 ± 5.86 | 316.5 ± 12.20 | 250.2 ± 13.66 | 188.2 ± 14.29 |
| Decoction of *C. ternatea* | 100 | 365.2 ± 10.24 | 355.6 ± 12.03 | 338.1 ± 12.34 | 304.4 ± 7.97 |
| | 200 | 396.6 ± 6.72 | 362.2 ± 7.94 | 306.8 ± 6.45 | 250.6 ± 8.58 |
| Decoction of *P. granatum* | 100 | 387.4 ± 6.51 | 377.4 ± 9.69 | 328.2 ± 15.23 | 303.4 ± 11.50 |
| | 200 | 411.2 ± 12.07 | 361.6 ± 14.35 | 305.9 ± 15.65 | 251.2 ± 13.30 |
| Combined decoction of *C. ternatea* and *P. granatum* | 400 | 403.7 ± 12.44 | 359.4 ± 7.96 | 278.6 ± 10.49 | 216.8 ± 10.59 |

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

## 4. Conclusion

The results of the present investigation clearly indicate that the combination of decoction of *C. ternatea* and *P. granatum* flowers have significant antihyperglycemic effect in alloxan-induced diabetic rats and holds the perspective to be used in clinical practices based on the toxicity profiles available. However, further research in the development of formulations based on above two principles may open new avenues in the management of diabetes mellitus.

## 5. References


1. Mahapatra DK, Bharti SK. Drug Design. New Delhi: Tara Publications Private Limited, First Edition, 2016.
2. Mahapatra DK, Bharti SK. Handbook of Research on Medicinal Chemistry. New Jersey: Apple Academic Press, First Edition. 2016.
3. Mahapatra DK, Chhajed SS, Shivhare RS. Development of Murrayanine-Chalcone hybrids: An effort to combine two privilege scaffolds for enhancing hypoglycemic activity. Int J Pharm Chem Anal. 2017; 4(2):30-34.
4. Chhajed SS, Chaskar S, Kshirsagar SK, Haldar GA, Mahapatra DK. Rational design and synthesis of some PPAR-γ agonists: Substituted benzylideneamino-benzylidene-thiazolidine-2, 4-diones. Comp Biol Chem, 2017; 67:260-5.
5. Godbole MD, Mahapatra DK, Khode PD. Fabrication and Characterization of Edible Jelly Formulation of Stevioside: A Nutraceutical or OTC Aid for the Diabetic Patients. Inventi Nutraceut, 2017; 2017(2):1-9.
6. Mahapatra DK, Asati V, Bharti SK. Chalcones and their therapeutic targets for the management of diabetes: structural and pharmacological perspectives. Eur J Med Chem. 2015; 92:839-65.
7. Kamble MA, Mahapatra DK, Dhabarde DM, Ingole AR. Pharmacognostic and pharmacological studies of *Bombax ceiba* thorn extract. J Pharm Pharmacog Res. 2017; 5(1):40-54.
8. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. J Taibah Univ Sci. 2015; 9(4):449-54.
9. Onwusonye JC, Uwakwe AA, Iwuanyanwu P, Iheagwam U. Oral acute toxicity ($LD_{50}$) study of methanol extract of Annona senegalensis leaf in albino mice. Sky J Biochem Res. 2014; 3(5):46-8.
10. Al-Lami MQ. The Hypoglycemic and Hypolipidemic Activity of Aqueous Green Tea Extract in Normal and Alloxan-Induced Diabetic Male Albino Rats. Iraq J Biotechnol. 2015; 14(2):249-61.