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Antidiabetic activity of *Chandraprabha vati* – A classical Ayurvedic formulationManish M. Wanjari ^{a,*}, Sujata Mishra ^b, Yadu Nandan Dey ^a, Deepti Sharma ^a,
Sudesh N. Gaidhani ^c, Ankush D. Jadhav ^a^a National Research Institute for Ayurveda-Siddha Human Resource Development, Gwalior, 474009, Madhya Pradesh, India^b School of Studies in Biochemistry, Jiwaji University, Gwalior, 474011, Madhya Pradesh, India^c Central Council for Research in Ayurvedic Sciences, New Delhi, 110058, India

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ABSTRACT

Background: *Chandraprabha vati* is a classical Ayurvedic formulation, markedly used for mitigation of *Prameha*, which correlates in many ways with obesity, metabolic syndrome and diabetes mellitus.**Objective:** The present study was aimed to investigate effect of *Chandraprabha vati* in experimentally-induced hyperglycemia and lipid profile alterations.**Materials and methods:** Antidiabetic effect of *Chandraprabha vati* was studied in fifty five Wistar rats. Graded doses of *Chandraprabha vati* (50, 100 and 200 mg/kg) were administered orally for 7 days to normal and alloxan-hyperglycemic rats (65 mg/kg, intravenously), and to glucose loaded normal rats for oral glucose tolerance test (OGTT). Fasting plasma glucose levels were assessed on different time intervals along with plasma cholesterol and triglycerides. Metformin (500 mg/kg, orally) was used as standard drug. **Results:** *Chandraprabha vati* did not cause any significant reduction in plasma glucose levels of normal rats ($p > 0.05$) but normalized the impaired glucose tolerance at 60 and 120 min ($p < 0.05$ – $p < 0.001$) in OGTT when compared to vehicle control. In alloxan-hyperglycemic rats, administration of *Chandraprabha vati* (200 mg/kg) significantly reduced plasma glucose at 3 h, 12 h, 3rd day and 7th day ($p < 0.01$ – $p < 0.001$) along with reduction in cholesterol and triglycerides levels ($p < 0.01$ – $p < 0.001$) when compared to diabetic control group. The effects were comparable with metformin.**Conclusions:** *Chandraprabha vati* exhibited anti-hyperglycemic effect and attenuated alterations in lipid profile. The results support the use of *Chandraprabha vati* for correction of *Prameha* in clinical practice.© 2016 Transdisciplinary University, Bangalore and World Ayurveda Foundation. Publishing Services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Diabetes mellitus is categorized as a metabolic disease, characterized by hyperglycemia which results from defects in insulin secretion, insulin action or both. The hyperglycemia in turn damages many of the body's systems leading to diabetic complications, which further exacerbate the diabetic condition and affect the quality of life. The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and USA will have the largest number of people with diabetes [1]. Despite appreciable progress made in the management of diabetes mellitus using conventional

antidiabetic management strategies, the search for products of natural origin for control of diabetes mellitus continues. The World Health Organization (WHO) has also long back recommended that this practice should be encouraged, especially in countries where access to conventional treatment of diabetes mellitus is not adequate [2]. Many herbal formulations are widely used in treatment of diabetes [3] and many more are being evaluated for their effectiveness in controlling diabetes. Ayurveda and other traditional systems of medicines describe number of plants/minerals and/or their formulations for treatment of diabetes.

Chandraprabha vati (CPV) is an Ayurvedic formulation available in classical vati form. It is used in Ayurvedic system of medicine for various indications [4] such as *Vibandha* (Constipation), *Anaha* (Distension of abdomen due to obstruction to passage of urine and stools), *Shula* (Colicky Pain), *Granthi* (Cyst), *Pandu* (Anaemia),

* Corresponding author.

E-mail address: manish.nriashrd@gmail.com (M.M. Wanjari).

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Kamala (Jaundice), *Mutrakricchra* (Dysuria), *Ashmari* (Calculus), *Arsha* (Hemorrhoids), *Arbuda* (Tumor), *Mutraghata* (Urinary obstruction), *Antra-Vrddhi* (Hernia), *Kati shula* (Lower backache), *Kustha* (Diseases of skin), *Kandu* (Itching), *Plihodara* (Disorder of spleen, ascites associated with splenomegaly), *Bhagandara* (Fistula-in-ano), *Dantaroga* (Dental disease), *Netraroga* (Eye disorder), *Aru-chi* (Tastelessness), *Mandagni* (Impaired digestive fire), *Striroga* (Gynaecological disorders), *Artava Ruja* (Dysmenorrhoea), *Shukra Dosh*a (Vitiation of semen), *Daurbalya* (Weakness) and *Prameha*. *Chandraprabha vati* has got very remarkable effect in mitigation of *Prameha* which correlates in many ways with obesity, metabolic syndrome and diabetes mellitus (*Madhumeha*) [5]. It contains 37 herbomineral ingredients (Table 1) [4]. Most of these ingredients exhibited both glucose and lipid lowering activities in experimental studies. The ingredients like *Acorus calamus* [6,7], *Cyperus rotundus* [8], *Phyllanthus niruri* [9], *Tinospora cordifolia* [10], *Curcuma longa* [11], *Berberis aristata* [12], *Piper longum* [13,21], *Coriandrum sativum* [14], *Terminalia chebula* [15], *Terminalia bellerica* [16], *Embelica officinalis* [17], *Embelia ribes* [18], *Zingiber officinale* [19], *Piper nigrum* [20], *Hordeum vulgare* [22], *Ipomoea turpethum* [23], *Cinnamomum zeylanicum* [24] and *Asphaltum punjabianum* [25] showed remarkable antidiabetic effects in several studies. Most of the important plant constituents of this formulation [15,21,24,25] including *Commiphora wightii* (Guggulu) [26] have demonstrated hypolipidemic effect. In experimental studies, Suresh et al. [27] demonstrated curative effect of *Chandraprabha vati* on streptozotocin-induced diabetes. Despite the long history of use of *Chandraprabha vati* in diabetes and antidiabetic and lipid lowering effect of constituent plants, the systematic scientific studies are still lacking to delineate and validate its therapeutic utility in controlling diabetes. Hence, the present study demonstrates the effect of *Chandraprabha vati* on alloxan-induced hyperglycemia and alterations of lipid profile in rats.

2. Materials and methods

2.1. Drugs and chemicals

Chandraprabha vati was procured from Indian Medicines Pharmaceutical Corporation Ltd., Ramnagar, Uttarakhand, India. Alloxan monohydrate was procured from CDH Chemicals, India while metformin was gift sample from ZIM Laboratories Ltd., Nagpur, India. Glucose, cholesterol and triglycerides estimation kits (ERBA-

Mannheim) were procured from Transasia Bio-Medicals Pvt. Ltd., Mumbai, India. Quercetin was procured from Sigma Aldrich, USA. Tannic acid and all other reagents used in the experiments were of analytical grade and procured from Qualigens Fine Chemicals Ltd., Mumbai, India.

2.2. Standardization of *Chandraprabha vati*

Chandraprabha vati was first standardized as per standard procedures/guidelines [28,29]. Various physicochemical parameters viz. total ash, acid-soluble and -insoluble ash, water-soluble and -insoluble ash, alcohol and water-soluble extractive values, loss on drying and pH were determined accordingly.

2.3. Phytochemical screening and quantitative estimation of phytoconstituents

Chandraprabha vati (10 g powder formulation) was macerated with 100 ml water for 48 h and filtered through Whatman filter paper no. 1. The filtrate was used for preliminary phytochemical screening [29] and quantitative estimation of phytoconstituents. The total phenolic content of aqueous extract of CPV was determined by Folin and Ciocalteu's reagent colorimetric assay spectrometrically [30] and expressed as gram of tannic acid equivalents per 100 g of powder drug while total flavonoid content was measured by aluminium chloride colourimetric assay [31] and expressed as gram of quercetin equivalents per 100 g of powder drug.

2.4. Animals

Healthy adult Wistar albino rats (200–250 g) of either sex between 2 and 3 months of age were used for the investigations. They were housed in group in polypropylene cages, maintained under standard conditions (12 h light and dark cycle; temperature 25 ± 1 °C; humidity 40–60%) and fed with standard rat pellet diet (Ashirwad brand, Chandigarh, India) and purified water *ad libitum*. Experiments were performed at pharmacology division of National Research Institute for Ayurveda-Siddha Human Resource Development (NRIASHRD), Gwalior, India in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, and Climate change, New Delhi after seeking approval from

Table 1
Composition of *Chandraprabha vati* [4].

1. Chandraprabha (Karpura) (Sub. Ext.) 3 g	20. Maricha (Fr.) 3 g
2. Vacha (Rz.) 3 g	21. Pippali (Fr.) 3 g
3. Musta (Rz.) 3 g	22. Makshika dhatu bhasma (Makshika) 3 g
4. Bhunimba (Kiratiktika) (Pl.) 3 g	23. Yava kshara (Yava) (Pl.) 3 g
5. Amruta (Guduchi) (St.) 3 g	24. Sarji Kshara (Svarjiksara) 3 g
6. Daruka (Devadaru) (Ht. Wd.) 3 g	25. Saindhava lavana 3 g
7. Haridra (Rz.) 3 g	26. Sauvarchala lavana 3 g
8. Ativisha (Rt. Tr.) 3 g	27. Vida lavana 3 g
9. Darvi (Daruharidra) (St.) 3 g	28. Trivrit (Rt.) 12 g
10. Pippalimula (Pippali) (Rt.) 3 g	29. Danti (Rt.) 12 g
11. Chitraka (Rt.) 3 g	30. Patraka (Tejapatra) (Lf.) 12 g
12. Dhanyaka (Fr.) 3 g	31. Tvak (St.Bk.) 12 g
13. Haritaki (P.) 3 g	32. Ela (Sukamaila) (Sd.) 12 g
14. Bibhitaka (P.) 3 g	33. Vamshalochana (Vamsa) (S.C.) 12 g
15. Amalaki (P.) 3 g	34. Lauha bhasma 24 g
16. Chavya (St.) 3 g	35. Sita 48 g
17. Vidanga (Fr.) 3 g	36. Silajatu 96 g
18. Gajapippali (Fr.) 3 g	37. Guggulu (Exd.) 96 g
19. Sunthi (Rz.) 3 g	

Sub. Ext. – Sublimed extract, Rz. – Rhizome, Pl. – Plant (Whole), St. – Stem, Ht. Wd. – Heart wood, Rt. Tr. – Root trunk, St. – Stem, Rt. – Root, Fr. – Fruit, P. – Pericarp, Lf. – Leaf, St. Bk. – Stem bark, Sd. – Seed, S.C. – Silicacious Concretion, Exd. – Exudate.

the Institutional Animals Ethics Committee (IAEC) of NRIASHRD, Gwalior (Proposal No. CRI-GWL/IAEC/2010/11).

2.5. Determination of human equivalent dose of Chandraprabha vati

For administration purpose, human equivalent dose of CPV for rats was determined on basis of surface area ratio as described in standard textbook [32]. The calculated doses were approximately 50 and 100 mg/kg body weight of rats. So the three doses of CPV i.e. 50, 100 and 200 mg/kg body weight were tested to plot complete dose response curve in the present study. As per Ayurvedic literature, the suggested vehicle/adjutant (anupana) for CPV is water, milk, gungily powder. Hence, the powdered drug was suspended in distilled water using 4% gum acacia and suspension was administered orally to animals by intragastric feeding needle.

2.6. Acute toxicity study

Acute oral toxicity study was carried out to determine the safe dose by acute toxic class method as per Organization for Economic Co-operation and Development (OECD) 423 guidelines [33]. The overnight fasted rats ($n = 3$) were orally administered CPV in the limit test dose of 2000 mg/kg and observed continuously for behavioural, neurological and autonomic profiles for 2 h and after a period of 24, 72 h and thereafter up to 14 days for any lethality, moribund state or death. The limit test was repeated in another group of rats ($n = 3$) for confirmation and toxic class of LD₅₀ determination.

2.7. Pharmacological evaluation

2.7.1. Effect of CPV on plasma glucose in healthy control rats

Rats were divided in five different groups (5 rats/group). Vehicle treated group received 4% gum acacia in distilled water while CPV was administered in dose of 50, 100 and 200 mg/kg, orally. Reference standard treated group received metformin (500 mg/kg, orally). The treatments were given for 7 days. Blood was withdrawn from fasted rats (10 h) through retro orbital sinus [34] on 0, 1, 3, 12 h, 72 h (3rd day) and 168 h (7th day) and clear plasma was obtained after centrifugation at 3000 rpm for 10 min. Fasting plasma glucose levels were estimated using a Glucose Oxidase–Peroxidase glucose estimation kit using semiautomated clinical chemistry analyser (Microlab 300, Vital Scientific-Merck).

2.7.2. Effect of CPV on oral glucose tolerance test (OGTT)

On day 7, the oral glucose tolerance test [35,36] was performed in the same groups of above-mentioned euglycemic rats. Glucose (4 g/kg) was fed orally, 1 h after the administration of drugs/vehicle. Blood was withdrawn at 0, 30, 60 and 120 min of glucose administration and fasting plasma glucose levels were estimated as mentioned above.

2.7.3. Effect of CPV on alloxan-induced hyperglycemia and lipid profile

2.7.3.1. Induction of hyperglycemia. The hyperglycemia was induced by single dose of alloxan monohydrate. It was prepared freshly in normal saline which was acidified to increase stability of alloxan [37]. Immediately after preparation, it was administered intravenously through tail vein at a dose of 65 mg/kg [38,39]. Glucose solution (5% w/v), 1–2 ml per rat, was immediately administered intragastrically to alloxan treated rats in order to prevent transient hypoglycemia. Plasma glucose was estimated in fasted (10 h) rats 48 h after the administration of alloxan as mentioned above.

The rats exhibiting fasting plasma glucose levels more than 250 mg/dl were considered hyperglycemic (diabetic) and continued for further investigations.

2.7.3.2. Experimental design. Rats were divided in six different groups (5 rats/group). Non-diabetic healthy control group received normal saline intravenously while diabetic vehicle control group received alloxan and vehicle of the CPV (4% gum acacia in distilled water, 5 ml/kg). Test drug treated groups received CPV (50, 100 and 200 mg/kg) while standard treated group received metformin (500 mg/kg). The vehicle or drug treatments were given daily orally for 7 days. Blood was withdrawn from fasted rats (10 h) on 0, 1, 3, 12 h, 72 h (3rd day) and 168 h (7th day) and fasting plasma glucose levels were estimated at all intervals while plasma levels of total cholesterol and triglycerides were also estimated at 168 h (7th day) by commercially available kits.

2.8. Statistical analysis

The data were analyzed with one-way ANOVA and two-way ANOVA, wherever applicable followed by Bonferroni multiple comparison post hoc test. A statistical difference of $p < 0.05$ was considered significant in all cases.

3. Results

3.1. Standardization of formulation

The physico-chemical characterization of CPV is mentioned in Table 2.

3.2. Qualitative Phytochemical screening

Qualitative phytochemical tests of CPV showed the presence of steroids, flavonoids, alkaloids, tannins and phenolic compounds, while carbohydrates, proteins, amino acids, and glycosides were absent.

3.3. Quantitative estimation of phytoconstituents

The total phenolic content of CPV was found to be 6.19 g tannic acid equivalents/100 g of powdered drug while its total flavonoid content was found to be 3.28 g quercetin equivalents/100 g of powdered drug.

3.4. Acute toxicity study

Acute oral toxicity studies revealed that the CPV was safe up to a dose level of 2000 mg/kg of body weight (limit test) and LD₅₀ was

Table 2
Physico-chemical evaluation of formulation of Chandraprabha vati.

Standardization parameters	Value
Ash analysis (% w/w)	
1. Ash content (Total ash)	23.39 ± 0.32
2. Acid soluble ash	18.55 ± 0.17
3. Acid insoluble ash	4.84 ± 0.20
4. Water soluble ash	18.54 ± 0.17
5. Water insoluble ash	13.33 ± 1.06
Extractive value (Maceration process)	
1. Water soluble (% w/w)	39.57 ± 0.79
2. Alcohol soluble (% w/w)	22.77 ± 0.48
Moisture content (Loss on drying) (% w/w)	0.106 ± 0.002
pH (1% aqueous solution)	5.47 ± 0.04

Values are expressed as mean ± SEM; $n = 3$.

Table 3
Effect of CPV on plasma glucose in euglycemic rats.

Groups	Plasma glucose (mg/dl)					
	0 h	1 h	3 h	12 h	Day 3	Day 7
Vehicle	105 ± 3.79	111 ± 4.93	107 ± 6.31	113 ± 2.82	117 ± 3.63	111 ± 5.57
CPV 50	116 ± 2.66 ^{ns}	113 ± 2.48 ^{ns}	110 ± 3.94 ^{ns}	111 ± 4.37 ^{ns}	110 ± 4.28 ^{ns}	105 ± 5.14 ^{ns}
CPV 100	119 ± 6.38 ^{ns}	122 ± 9.46 ^{ns}	111 ± 6.37 ^{ns}	110 ± 4.36 ^{ns}	108 ± 4.06 ^{ns}	95 ± 8.86 ^{ns}
CPV 200	99 ± 6.87 ^{ns}	94 ± 7.84 ^{ns}	96 ± 7.63 ^{ns}	106 ± 5.51 ^{ns}	123 ± 9.63 ^{ns}	93 ± 4.55 ^{ns}
MET 500	122 ± 9.47 ^{ns}	114 ± 6.15 ^{ns}	103 ± 6.24 ^{ns}	125 ± 3.31 ^{ns}	118 ± 4.18 ^{ns}	101 ± 4.21 ^{ns}

Values are expressed as mean ± SEM, (n = 5); Doses are expressed as mg/kg body weight.

ns – non significant.

CPV – *Chandraprabha vati*; MET – Metformin.

found to be more than 2000 mg/kg. No lethality or any toxic reactions or moribund state were observed up to the end of the study period.

3.5. Effect of CPV on plasma glucose in healthy control rats

Two-way ANOVA did not show any significant effect of CPV on plasma glucose levels in healthy control rats ($p > 0.05$). Post hoc test indicated that CPV at 50, 100 and 200 mg/kg did not exhibit significant reduction in the plasma glucose in healthy control rats (Table 3). The standard drug metformin ($p > 0.05$) also did not influence plasma glucose level.

3.6. Effect of CPV on oral glucose tolerance test (OGTT)

Two-way ANOVA showed that CPV showed significant influence on OGTT after glucose load administration (Table 4) and CPV (50, 100 and 200 mg/kg) normalized the impaired glucose tolerance and showed observable reduction in serum glucose levels from 60 to 120 min after glucose load. The standard drug, metformin significantly normalized the impaired glucose tolerance compared to control (Table 4) and showed reduction in the plasma glucose from 60 min onwards.

3.7. Effect of CPV on hyperglycemia, cholesterol and triglycerides

Two-way ANOVA showed that alloxan administration caused significant ($p < 0.001$) hyperglycemia in rats at 0, 1, 3, 12 h, 3rd and 7th day after confirmation of hyperglycemia when compared to euglycemic rats. Post hoc test indicated that administration of CPV at 50 and 100 mg/kg significantly ($p < 0.01$ – $p < 0.001$, wherever applicable) reduced the plasma glucose levels in alloxan-induced hyperglycemic animals only at day 3 and 7 while dose of 200 mg/kg significantly ($p < 0.01$ – $p < 0.001$, wherever applicable) reduced the plasma glucose levels in alloxan-induced hyperglycemic animals at 3 h, 12 h, day 3 and day 7. The standard drug, metformin (500 mg/kg) also showed significant reduction in the plasma glucose levels in alloxan-induced hyperglycemic rats 3 h onwards

Table 4
Effect of CPV on oral glucose tolerance test (OGTT).

Groups	Plasma glucose (mg/dl)			
	0 min	30 min	60 min	120 min
Vehicle	105 ± 3.79	175 ± 4.92	196 ± 6.74	162 ± 5.29
CPV 50	109 ± 3.36 ^{ns}	171 ± 2.48 ^{ns}	178 ± 3.22 [*]	143 ± 2.89 [*]
CPV100	103 ± 6.01 ^{ns}	166 ± 2.48 ^{ns}	174 ± 4.35 [@]	140 ± 6.25 [@]
CPV 200	105 ± 3.48 ^{ns}	161 ± 4.09 ^{ns}	163 ± 2.92 [#]	129 ± 3.15 [#]
MET 500	101 ± 4.21 ^{ns}	160 ± 4.18 ^{ns}	160 ± 4.84 [#]	112 ± 5.49 [#]

Values are mean ± SEM, (n = 5); Doses are expressed in mg/kg body weight.

ns – non significant, * $p < 0.05$, @ $p < 0.01$, # $p < 0.001$ compared to vehicle.

CPV – *Chandraprabha vati*; MET – Metformin.

(Table 5). The effect of metformin was comparable to higher dose of CPV ($p > 0.05$).

One-way ANOVA showed that alloxan administration significantly influenced plasma levels of total cholesterol and triglycerides at 168 h (7th day) as compared to healthy control rats (Fig. 1). Post hoc test indicated that alloxan-hyperglycemic rats exhibited raised cholesterol and triglycerides levels ($p < 0.001$) compared to healthy control rats. CPV at 100 and 200 mg/kg significantly ($p < 0.01$ – $p < 0.001$) prevented rise in cholesterol and triglycerides levels while lower dose CPV (50 mg/kg) did not have significant influence on triglycerides and cholesterol levels (Fig. 1).

4. Discussion

The present study was designed and conducted to evaluate the influence of CPV on blood glucose profile and lipid profile in alloxan-induced hyperglycemic rats and to scientifically validate its traditional use in diabetes.

The determined physico-chemical constants of the formulation were as per the previous studies [40]. The toxicity study of CPV in the limit test dose of 2000 mg/kg revealed neither toxicity of any nature nor moribund stage. This indicated that the CPV has no toxicity at such high dose and considered to be safe for administration. The calculated human equivalent dose of CPV for rats is approximately 50–100 mg/kg which is fairly lower (20 times) than that of the limit test dose of 2000 mg/kg at which there was no toxicity or moribund state. Hence, the study was conducted with initial human equivalent dose of 100 mg/kg. Based on the results the doses lower (50 mg/kg) and higher (200 mg/kg) than initial dose were used for further evaluation.

In order to study the influence on blood glucose in hyperglycemic conditions, the effect of CPV was first tested for its hypoglycemic effect in euglycemic animals. The results of the investigations in euglycemic rats revealed that treatment with CPV for seven days did not show any significant decrease in the basal glucose levels in healthy control rats. Similarly, metformin, a standard anti-hyperglycemic agent, did not affect the basal plasma glucose levels. This is in accordance with the reports which demonstrated that metformin does not produce hypoglycemia in non-diabetic state [41]. These investigations suggest that CPV, *per se*, has no hypoglycemic effect. However, in the previous study [27], it is mentioned that CPV showed some hypoglycemic effect at 6 h of administration in euglycemic rats. It is possible that the doses used in the present study (50–200 mg/kg) are very less than that of the previous study which used nearly 10 times higher doses (0.5–1.5 g/kg).

In glucose loaded normal rats, significant suppression of plasma glucose was observed at 120 min after administration of the CPV. This indicates that CPV was able to normalize the impaired glucose tolerance in glucose loaded normal rats. Similar effect was also observed for metformin.

Table 5
Effect of CPV on plasma glucose in alloxan-induced hyperglycemia.

Groups	0 h	1 h	3 h	12 h	Day 3	Day 7
Healthy control	115.6 ± 4.11	111.4 ± 4.94	107.8 ± 6.31	113.6 ± 2.82	117 ± 3.63	111.8 ± 5.57
Vehicle	417.6 ± 10.07*	396 ± 7.27*	381.4 ± 10.15*	356.2 ± 4.04*	345.4 ± 9.01*	310.6 ± 8.41*
CPV 50	414.4 ± 6.65 ^{ns}	378.4 ± 4.80 ^{ns}	380.2 ± 4.5 ^{ns}	354 ± 4.40 ^{ns}	305.2 ± 7.95 [@]	233 ± 10.46 [#]
CPV 100	406.6 ± 6.52 ^{ns}	372.4 ± 8.04 ^{ns}	366.6 ± 5.73 ^{ns}	348.6 ± 6.82 ^{ns}	283.8 ± 6.72 [#]	164 ± 7.51 [#]
CPV 200	418 ± 11.84 ^{ns}	370.6 ± 12.07 ^{ns}	340.8 ± 6.41 [@]	318.2 ± 6.45 [@]	246 ± 10.54 [#]	142.2 ± 5.90 [#]
MET 500	402.6 ± 8.91 ^{ns}	367 ± 14.72 ^{ns}	336 ± 4.88 [#]	312.6 ± 6.37 [#]	243.2 ± 10.75 [#]	132.2 ± 11.11 [#]

Values are expressed as mean ± SEM, (n = 5); Doses are expressed in mg/kg body weight.
ns – nonsignificant, *p < 0.001 compared to healthy control, @p < 0.01, #p < 0.001 compared to vehicle.
CPV – *Chandraprabha vati*; MET – Metformin.

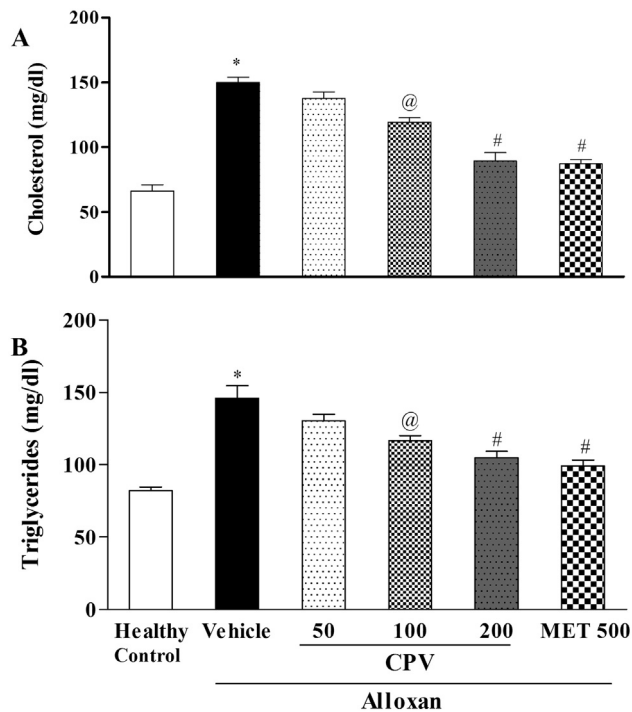


Fig. 1. Effects of CPV on lipid profile (A), Cholesterol (B) Triglycerides. Results are expressed as mean ± SEM, (n = 5); Doses are expressed in mg/kg body weight. *p < 0.001 compared to healthy control, @p < 0.01, #p < 0.001 compared to vehicle. CPV – *Chandraprabha vati*; MET – Metformin

It was observed that single intravenous injection of alloxan exhibited significant hyperglycemia. Alloxan-induced hyperglycemia simulates to type 1 diabetes (insulin dependent diabetes mellitus). It has two distinct pathological effects: selective inhibition of glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell; and generation of free radicals through redox cycling between alloxan and its reduction product dialuric acid, resulting in the selective necrosis of beta cells. These two effects can be attributed to selective cellular uptake and accumulation of alloxan by the beta cell [37,42]. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state [43]. As the hyperglycemia induced by alloxan falls under category of reversible diabetes and may reverse after a week [44], the effect of the CPV in hyperglycemic rats was studied during 7 days treatment. The results of the investigations in alloxan-induced hyperglycemic rats showed long term administration of CPV was effective to decrease plasma glucose levels. The standard drug, metformin also showed anti-hyperglycemic effect. This indicated that CPV has

antihyperglycemic effect. From the investigations, it appeared that the effect of CPV is dependent on the dose. Low doses of CPV (50 and 100 mg/kg) showed significant decrease in glucose levels from 3rd day onwards while higher dose of 200 mg/kg exhibited decrease within 3 h of administration similar to metformin, the standard drug, which also showed anti-hyperglycemic effect. As a biguanide, metformin reduces both fasting and postprandial glucose levels mainly by inhibiting hepatic gluconeogenesis and glucose output. Metformin also facilitates peripheral uptake, utilisation and metabolism of glucose, provided some endogenous insulin is present. Thus, metformin reduces both fasting and postprandial hyperglycaemia by promoting insulin-mediated peripheral glucose utilisation and metabolism in adipose tissues and skeletal muscles through up-regulation of glucose transporters [45,46]. It is not clear as to how CPV caused the reduction in hyperglycemia. It is possible that CPV, like metformin, might be improving insulin action at the cellular level or enhancing the action of insulin or by increasing the glucose metabolism or glucose homeostasis in the diabetic animals. Therefore, the mechanism underlying this effect needs to be studied. As stated earlier CPV contains various ingredients which have shown antidiabetic property in various experimental studies. These plant ingredients showed antidiabetic activity by various mechanisms viz. insulin sensitizing activity [6] or regeneration of pancreatic beta cells [12], antioxidant action [8,18] or influencing insulin resistance [11] or by altering the carbohydrate metabolism [10] or insulin-like effect via peripheral glucose utilization. Thus, the antihyperglycaemic effect of the CPV may be attributed to the cumulative effect of these constituent plants.

Many times diabetes is associated with hyperlipidemia [47]. It is well documented that there is elevation of plasma lipid concentration in diabetics [48]. In the present investigation, alloxan-induced hyperglycemic rats also exhibited rise in the total cholesterol and triglycerides levels when compared to untreated diabetic group. It is well known that under normal circumstances insulin activates the enzyme lipoprotein lipase which hydrolyses the triglycerides. Insulin deficiency results in the failure to activate the enzymes thereby resulting in hypertriglyceridemia. CPV treated groups showed decrease in cholesterol and triglycerides. This may be due to the presence of hypocholesterolemic plants constituents and minerals like guggulu (*Commiphora wightii*), musta (*C. rotundus*), shilajit (*A. punjabianum*), etc. present in CPV that may act as inhibitors for some enzymes such as hydroxyl methyl glutaryl CoA reductase, which participates in cholesterol synthesis or reduces the absorption of cholesterol from intestine [49]. The reduction in the triglycerides levels indicates that CPV might be enhancing the action of insulin and thereby normalizing the action of lipoprotein lipase. The present study adds antihyperlipidemic effect of CPV in diabetic rats compared to previous study [27] and suggests effectiveness of CPV in controlling diabetes along with lipid abnormalities.

Further, phytochemical investigation of CPV revealed the presence of steroids, flavonoids, alkaloids, tannins and phenolic compounds. The quantitative phytochemical studies showed a fair amount of phenolic and flavonoids in the CPV. Various phenolic compounds were reported to have anti-hyperglycemic activity and antidiabetic activities [50,51]. So, the presence of the active moieties in the individual plants might be contributing important role in attenuation of hyperglycemia and hyperlipidemia.

The present study still has certain limitations like lack of estimation of insulin levels and histopathology of pancreas. The results of the present investigations in fact suggest that the drugs like *Chandraprabha vati* may provide promising effects against type II diabetes. Hence, further studies are needed for assessment in chronic models of type II diabetes viz. Streptozotocin-Nicotinamide induced diabetes or fructose induced diabetes.

5. Conclusions

The present investigation suggest *Chandraprabha vati* exhibits the anti-hyperglycemic effect and attenuates the glycation associated elevation in the lipid profile. This further supports the use of *Chandraprabha vati* for correction of *Prameha* in clinical practice.

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References

- [1] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004;27:1047–53.
- [2] WHO. Technical report series. World Health Organization Expert Committee on diabetes mellitus. Second report, vol. 646; 1980. Geneva.
- [3] Mishra R, Shuaib M, Shrivani, Mishra PS. A review on herbal antidiabetic drugs. *J Appl Pharm Sci* 2011;1:235–7.
- [4] Ayurvedic Formulary of India. Part 1. Government of India Ministry of Health and Family Welfare. New Delhi: Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy; 2003. p. 512–5.
- [5] Sharma H, Chandola HM. Prameha in Ayurveda: correlation with obesity, metabolic syndrome, and diabetes mellitus. Part 1-etiology, classification, and pathogenesis. *J Altern Complement Med* 2011;17:491–6.
- [6] Wu HS, Zhu DF, Zhou CX, Feng CR, Lou YJ, Yang B, et al. Insulin sensitizing activity of ethyl acetate fraction of *Acorus calamus* L. *in vitro* and *in vivo*. *J Ethnopharmacol* 2009;23(2):288–92.
- [7] Prisilla DH, Balamurugan R, Shah HR. Antidiabetic activity of methanol extract of *Acorus calamus* in STZ induced diabetic rats. *Asian Pac J Trop Biomed* 2012;2:S941–6.
- [8] Raut NA, Gaikwad NJ. Antidiabetic activity of hydro-ethanolic extract of *Cyperus rotundus* in alloxan induced diabetes in rats. *Fitoterapia* 2006;77:585–8.
- [9] Okoli CO, Ibiem AF, Ezike AC, Akah PA, Okoye TC. Evaluation of antidiabetic potentials of *Phyllanthus niruri* in alloxan diabetic rats. *Afr J Biotech* 2010;9:248–59.
- [10] Stanely M, Prince P, Menon VP. Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan induced diabetic rats. *J Ethnopharmacol* 2000;70:9–15.
- [11] Kuroda M, Mimaki Y, Nishiyama T, Mae T, Kishida H, Tsukagawa M, et al. Hypoglycemic effects of turmeric (*Curcuma longa* L. rhizomes) on genetically diabetic KK-Ay mice. *Biol Pharm Bull* 2005;28:937–9.
- [12] Semwal BC, Gupta J, Singh S, Kumar Y, Giri M. Antihyperglycemic activity of root of *Berberis aristata* D.C. in alloxan-induced diabetic rats. *Int J Green Pharm* 2009;3:259–62.
- [13] Manoharan S, Silvan S, Vasudevan K, Balkrishnan S. Antihyperglycemic and antilipidperoxidative effects of *Piper longum* (Linn.) dried fruits in alloxan induced diabetic rat. *J Biol Sci* 2007;7:161–8.
- [14] Eidi M, Eidi A, Saeidi A, Molanaei S, Sadeghipour A, Bahar M, et al. Effect of coriander seed (*Coriandrum sativum* L.) ethanol extract on insulin release from pancreatic beta cells in streptozotocin-induced diabetic rats. *Phytother Res* 2009;23:404–6.
- [15] Murali YK, Anand P, Tandon V, Singh R, Chandra R, Murthy PS. Long-term effects of *Terminalia chebula* Retz. on hyperglycemia and associated hyperlipidemia, tissue glycogen content and *in vitro* release of insulin in streptozotocin induced diabetic rats. *Exp Clin Endocrinol Diabetes* 2007;115:641–6.
- [16] Sabu MC, Kuttan R. Antidiabetic and antioxidant activity of *Terminalia bellerica* Roxb. *Indian J Expt Biol* 2009;47:270–5.
- [17] Tigrar PR, Shah KV, Patel VP, Desai TR, Goyal RK. Investigation into mechanism of action of anti-diabetic activity of *Embelia officinalis* on streptozotocin induced type I diabetic rat. *Res J Pharm Biol Chem Sci* 2010;1:672–82.
- [18] Bhandari U, Ansari MN. Antihyperglycaemic activity of aqueous extract of *Embelia ribes* Burm in streptozotocin induced diabetic rats. *Indian J Expt Biol* 2008;46:607–13.
- [19] Asha B, Krishnamurthy KH, Siddappa D. Evaluation of anti hyperglycaemic activity of *Zingiber officinale* (Ginger) in albino rats. *J Chem Pharm Res* 2011;3(1):452–6.
- [20] Kaleem M, Sheema, Sarmad H, Bano B. Protective effects of *Piper nigrum* and *Vinca rosea* in alloxan induced diabetic rats. *Indian J Physiol Pharmacol* 2005;49:65–71.
- [21] Nabi SA, Kasetti RB, Sirasanagandla S, Tilak TK, Kumar MV, Rao CA. Antidiabetic and antihyperlipidemic activity of *Piper longum* root aqueous extract in STZ induced diabetic rats. *BMC Complement Altern Med* 2013;13:37.
- [22] Naseri M, Khalaj SZ, Ghavami B, Kamalinezhad M, Naderi GA, Faghizadeh S. Effect of barley (*Hordeum vulgare* L.) seed extract on fasting serum glucose level in streptozotocin induced diabetic rats. *J Med Plants* 2010;9:57–66.
- [23] Raut NA, Kasliwal AR, Gaikwad NJ. Evaluation of antidiabetic potential of *Ipomoea turpethum* R.Br. and *Ipomoea batata* L. (Convolvulaceae) in alloxan induced diabetes in rats: a comparative study. *Res J Pharm Biol Chem Sci* 2014;5:137–41.
- [24] Khan A, Safdar M, Ali Khan MM, Khattak KN, Anderson RA. Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes Care* 2003;26:3215–8.
- [25] Trivedi NA, Mazumdar B, Bhatt JD, Hemavathi KG. Effect of shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. *Indian J Pharmacol* 2004;36:373–6.
- [26] Verma SK, Bordia A. Effects of *Commiphora mukul* (gum guggulu) in patients of hyperlipidemia with special reference to HDL cholesterol. *Indian J Med Res* 1988;7:356–60.
- [27] Suresh P, Dixit SK, Gode KD, Joshi D. Anti-Diabetic effect of *Chandraprabha vati*-a reappraisal (experimental study). *Sachitra Ayurved* 1995;48:395–9.
- [28] WHO. Quality control methods for herbal materials. Updated edition. 1998. p. 29.
- [29] Khandelwal KR. Practical pharmacognosy. 15th ed. Pune: Nirali Prakashan; 2006. p. 149–59.
- [30] Singleton VL, Rossi Jr JA. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *Am J Enol Vitic* 1965;16:144–58.
- [31] Marinova D, Ribarova F, Atanasova M. Total phenolics and flavonoids in Bulgarian fruits and vegetables. *J Univ Chem Technol Metall* 2005;40:255–60.
- [32] Paget GE, Barnes JM. Evaluation of drug activities pharmacometries. In: Laurence DR, Bacharach AL, editors. Toxicity tests, vol. 1. London and New York: Academic Press; 1964. p. 135–66.
- [33] Organization for Economic Cooperation and Development (OECD). Guideline for the testing of chemicals. Revised draft guideline 423. Document on acute oral toxicity and acute toxicity class method. 2001. Available from: www.oecd.org.
- [34] Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 2010;1:87–93.
- [35] Prakasam A, Sethupathy S, Pugalendi KV. Effect of *Casaria esculenta* root extract on blood glucose and plasma antioxidant status in streptozotocin diabetic rats. *Pol J Pharmacol* 2003;55:43–9.
- [36] Zanatta L, de Sousa E, Cazarolli LH, Junior AC, Pizzolatti MG, Szpoganicz B, et al. Effect of crude extract and fractions from *Vitex megapotamica* leaves on hyperglycemia in alloxan-diabetic rats. *J Ethnopharmacol* 2007;109:151–5.
- [37] Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 2008;51:216–26.
- [38] Frode TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. *J Ethnopharmacol* 2008;115:173–83.
- [39] Kumar P, Baraiya S, Gaidhani SN, Gupta MD, Wanjari MM. Antidiabetic activity of stem bark of *Bauhinia variegata* in alloxan-induced hyperglycemic rats. *J Pharmacol Pharmacother* 2012;3:64–6.
- [40] Weerasekera KR, Wijayasiriwardhena C, Dhammarathana I, Tissera MHA, Ariyawansa HAS. Establishment quality and purity of “*Chandraprabha vati*” using sensory characteristics, physicochemical parameters, qualitative screening and TLC fingerprinting. *Int J Herb Med* 2014;2:26–9.
- [41] Ferner RE, Rawlins MD, Alberti KG. Impaired beta-cell responses improve when fasting blood glucose concentration is reduced in non-insulin-dependent diabetes. *Q J Med* 1988;66:137–46.
- [42] Szkudelski T. The mechanism of alloxan and streptozotocin action in β -cells of the rat pancreas. *Physiol Res* 2001;50:537–46.
- [43] Latner A. Clinical biochemistry; Carbohydrate metabolism: abnormalities of post absorptive blood sugar level. 2nd ed. Philadelphia: WB Saunders Co; 1958. p. 48.
- [44] Jain DK, Arya RK. Anomalies in alloxan-induced diabetic model: it is better to standardize it first. *Indian J Pharmacol* 2011;43:91.
- [45] Davis SN, Granner DK. Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In: Brunton LI, Lazo JS, Parker KL, editors.

- Goodman and Gilman's: the pharmacological basis of therapeutics. 11th ed. New York: McGraw Hill; 2006.
- [46] Nolte MS, Karam JH. Pancreatic hormones and antidiabetic drugs. In: Katzung BG, editor. Basic and clinical pharmacology. 9th ed. New York: McGraw-Hill; 2004. p. 693–715.
- [47] Maiti R, Das UK, Ghosh D. Attenuation of hyperglycemia and hyperlipidemia in streptozotocin induced diabetic rats by aqueous extract of seed of *Tamarindus indica*. Biol Pharm Bull 2005;28:1172–6.
- [48] Chase HP, Glasgow AM. Juvenile diabetes mellitus and serum lipids and lipoprotein levels. Am J Dis Child 1976;130:1113–7.
- [49] Sharma SB, Nasir A, Prabhu KM, Murthy PS, Dev G. Hypoglycemic and hypolipidemic effect of ethanolic extracts of seeds of *Eugenia jambolana* in alloxan-induced diabetic model of rabbits. J Ethnopharmacol 2003;85:201–6.
- [50] Urizar NL, Liverman AB, Dodds DT, Silva FV, Ordentlich P, Yan Y, et al. A natural product that lowers cholesterol as an antagonist ligand for FXR. Science 2002;296:1703–6.
- [51] Nabavi SF, Thiagarajan R, Rastrelli L, Daglia M, Sobarzo-Sanchez E, Alinezhad H. Curcumin: a natural product for diabetes and its complications. Curr Top Med Chem 2015;15:2445–55.