



ANTIDIABETIC EFFECT OF CHANDRAPRABHA VATI – A CLASSICAL AYURVEDIC FORMULATION

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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 fiftyfive Wistar rat. Graded doses of Chandraprabha vati (50,100 and 200mg/kg, orally) 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 chandraprabhavati 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.

KEYWORDS:

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. 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. Many herbal formulations 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 such as Vibandha (constipation), Anaha (Distension of abdomen due to obstruction to passage of urine and stools), Shula (Colicky Pain), Granthi (Cyst), Pandu (Anaemia), Kamala (Jaundice), Mutrakricchra (Dysuria), Ashmari (Calculus), Arsha (Hemorrhoids), Arbuda (Tumor), Mutraghata (Urinary obstruction), Antra-Vridhi (Hernia), Kati shula (Lower backache), Kustha (Diseases of skin), Kandu (Itching), Plihodara (Disorder of spleen, ascites associated with splenomegaly), Bhagandara (Fistula in ano), Dantoroga (Dental disease), Netra roga (Eye disorder), Aruchi (Tastelessness), Mandagni (Impaired digestive fire), Striroga (Gynaecological disorders), Artava Ruja (Dysmenorrhoea), Shukra Dosha (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 diabetic

mellitus (Madhumeha). It contains 37 herbomineral ingredients exhibited both glucose and lowering activities in experimental studies. The ingredients like *Acorus calamus*, *Cyperus rotundus*, *Phyllanthus niruri*, *Tinospora cordifolia*, *Curcuma longa*, *Berberis aristata*, *Piper longum*, *Coriandrum sativum*, *Terminalia chebula*, *Terminalia belerica*, *Embelica officinalis*, *Embelica ribes*, *Zingiber officinale*, *Piper nigrum*, *Hordeum vulgare*, *Ipomoea turpethum*, *Cinnamomum zeylanicum* and *Asphaltum punjabianum* show remarkable antidiabetic effects in several studies. Most of the important plant constituents of this formulation including *Commiphora wightii* (Guggulu) have demonstrated hypolipidemic effect. In experimental studies, Suresh *et al.* 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. MATERIAL 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 CDDH Chemicals, India while metformin was gift sample from ZIM Laboratories Ltd. Nagpur, India. Glucose, cholesterol and triglycerides 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. 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. The filtrate was used for preliminary phytochemical screening and quantitative estimation of phytochemical screening 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 and expressed as gram of tannic acid equivalents per 100 g of powder drug while total

flavonoid content was measured by aluminium chloride colorimetric assay 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 investigations.

2.5 Determination of human equivalent dose of Chandraprabha vati

For administration purpose, human equivalent dose of CPV for rats were determined on basis of surface area ratio as described in standard textbook. The calculated doses were approximately 50 and 100 mg/kg body weights were tested to plot complete dose response curve in the present study. As per Ayurvedic literature, the suggested vehicle/adjuvant (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 Pharmacological evaluation

2.6.1 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 treating group received metformin (500mg/kg orally). The treatments were given for 7 days. Blood was withdrawn from fasted rats (10h) through retro orbital sinus 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 were estimated using Glucose oxidase-peroxidase glucose estimation kit using semiautomated clinical chemistry analyser.

2.6.2 Effect of CPV on oral glucose tolerance test (OGTT)

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

2.6.3 Effect of CPV on alloxan-induced hyperglycemia and lipid profile

2.6.4.1. 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. Immediately after preparation, it was administered intravenously through tail vein at a dose of 65 mg/kg. 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.

2.6.3.2. 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. Test drug treated groups received metformin (500mg). The vehicle or drug treatments were given orally for 7 days. Blood was withdrawn from fasted rats (10 h) on 0, 1, 3, 12h, 72h (3rd 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.

3 RESULTS

3.1 Standardization of formulation

Table 1: Physico - chemical evaluation of formulation of CPV.

Standardization parameters	Value
Ash analysis(% w/w)	
1. Ash content	23.39 ± 0.32
2. Ash 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)	0.106±0.002
pH (1% aqueous solution)	5.47±0.04

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 of CPV was found to be 6.19 g tannic acid equivalents/100g of powdered drug while its total flavonoid content was found to be 3.28 g of quercetin equivalents/100g 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 found to be more than 2000mg/kg. No lethality or any toxic reactions or moribund state were observed up to the end of the study period

Table 2: Effect of CPV on plasma glucose in euglycemic rats.

Groups Plasma glucose (mg/dl)					
0 h 1h 12h Day 3 Day 7					
Vehicle	105±3.7	111±4.9	113±2.8	117±3.6	111±5.5
CPV 50	116±2.6	113±2.4	111±4.3	110±4.2	105±5.1
CPV 100	119±6.3	122±9.4	110±4.3	108±4.0	95±8.86
CPV 200	99±6.8	94±7.8	106±5.5	123±9.6	93±4.5
MET 500	122±9.4	114±6.1	125±3.3	118±4.1	101±4.2

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

Two-way ANOVA showed that CPV showed 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 200mg/kg did not exhibit significant reduction in the plasma glucose in healthy control rats (Table 2). 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 observed reduction in serum glucose levels from 60 to 120 min after min after glucose load. The standard drug, metformin significantly normalized the impaired glucose compared to control (Table 3) and showed reduction in the plasma glucose from 60 min onwards.

Table 3: Effect of CPV on oral glucose tolerance test (OGTT).

Groups Plasma glucose (mg/dl)					
0 min 30min 60min 120min					
Vehicle	105±3.7	175±4.9	196±6.7	162±5.2	
CPV 50	109±3.3	171±2.4	178±3.2	143±2.8	
CPV 100	103±6.0	166±2.4	174±4.3	140±6.2	
CPV 200	105±3.4	161±4.0	163±2.9	129±3.1	
MET 500	101±4.2	160±4.1	160±4.8	112±5.4	

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 12h, 3rd and 7th day after conformation of hyperglycemia when compared to euglycemic rats. Post hoc test indicated that administration of CPV at 50 and 100 mg/kg ($p < 0.01$ - $p < 0.001$) reduced the plasma glucose levels in alloxan-induced hyperglycemic animals at 3h, 12h, 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). The effect of metformin was comparable to higher dose of CPV ($p > 0.05$).

Table 4: Effect of CPV on Plasma Glucose in alloxan-induced hyperglycemia.

Groups	0h	1h	12h	Day 3	Day 7
Healthy control	115±4.1	114±4.9	113±2.8	117±3.6	118±5.5
Vehicle	417±10.0	396±7.2	356±4.0	345±9.0	310±8.4
CPV 50	414±6.6	378±4.8	354±4.4	305±7.9	233±10.4
CPV100	406±6.5	372±8.0	348±6.8	283±6.7	164±7.51
CPV 200	418±11.8	370±12	318±6.4	246±10	142±5.9
MET500	402±8.9	367±14	312±6.3	243±10	132±11.1

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. The toxicity study of CPV in the limit test dose of 2000mg/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. Based on the results the doses lower (50 mg/kg) than initial dose were used for further evaluation.

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.

It was observed that single intravenous injection of alloxan exhibited significant hyperglycemia. Alloxan-induced hyperglycemia stimulates to type 1 diabetes. 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 accumulate of alloxan by the beta cells. Excessive hepatic glycogenolysis and gluconeogenesis associated with decrease utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state. As the hyperglycemia induced by alloxan falls under category of reversible diabetes and may reverse after a week, the effect of the CPV in hyperglycemic rats was studied during 7 days treatment. The result 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 investigations,

it appeared that the effect of CPV is dependent on the dose. Low doses of CPV (50 and 100mg/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-glycemic 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, utilization and metabolism of glucose, provided some endogenous insulin is present. It is not clear as to how CPV caused the reduction in hyperglycemia. It is not possible that CPV, like metformin, might be improving insulin action at the cellular level or enhancing the actions 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 or regeneration of pancreatic beta cells, antioxidant action or influencing insulin resistance or by altering the carbohydrate mechanism or insulin like effect via peripheral glucose utilization. Thus, the antihyperglycemic effect of the CPV may be attributed to the cumulative effect of these constituent plants.

Many times diabetes is associated with hyperlipidemia. It is well documented that there is elevation of plasma lipid concentration in diabetics. In the present investigation, alloxan induced hyperglycemic rats also exhibited rise in the total cholesterol and triglycerides levels when compared to untreated diabetic group. CPV treated groups showed decrease in cholesterol and triglycerides. This may be due to the presence of hypocholesterolemics 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. The reduction in triglycerides levels indicates that CPV might be enhancing the action of insulin and there by normalizing the action of lipoprotein lipase. The present study adds antihyperlipidemic effect of CPV in diabetic rats compared to previous study and suggests effectiveness of CPV in controlling diabetes along with lipid abnormalities.

Further, Phytochemical investigation of CPV revealed the presence of steroids, flavonoids, alkaloids, tannin 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. 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 drug like chandrababha vati may provide promising effects against type2 diabetes. Hence, further studies are needed for assessment in chronic models of type 2 diabetes viz. Streptozotocin-Nicotinamide induced diabetes or fructose induced diabetes.

5 CONCLUSIONS

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

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