

Antidiabetic activity of polyherbomineral formulation: *Chandrakala rasa*

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Abstract

Objective: *Chandrakala rasa* (CKR), a herbomineral formulation is used to treat diabetes mellitus in ayurvedic system of health care. The present study aims to evaluate the antihyperglycemic potential of CKR in normoglycemic and streptozotocin (STZ)-nicotinamide (NAD)-induced Type 2 diabetic rats. **Materials and Methods:** Effects of CKR (100, 200 and 400 mg/kg, p.o.) on hypoglycemia as well as on oral glucose tolerance test (OGTT) were evaluated in normoglycemic rats by measuring the blood glucose concentrations. Similarly, blood glucose level was measured after 7, 14 and 21 days in STZ-NAD-induced diabetic rats treated with CKR. Different biochemical parameters such as total cholesterol, triglyceride, low density lipoprotein-cholesterol, and high density lipoprotein-cholesterol were estimated in a blood sample. *In vivo* antioxidant potential of CKR was measured in isolated liver sample of rats. **Results:** CKR (400 mg/kg, p.o.) did not show any hypoglycemic effect in normoglycemic rats. In OGTT, it significantly reduced the hike in blood glucose levels within 30-60 min after glucose administration without causing any hypoglycemic effect. Administration of CKR significantly reduced the fasting blood glucose levels on 7th, 14th and 21st days in STZ-NAD-induced diabetic rats. Treatment of rats with CKR reversed plasma lipid profile as well as increases liver glycogen level significantly in STZ-NAD-induced diabetic rats. Treatment with CKR in diabetic rats significantly restored the levels of lipid per-oxidation, superoxide dismutase and catalase as compared to negative control rats. **Conclusion:** The present study showed that CKR has antidiabetic activity probably because of antioxidant potential.

Key words: Antioxidant, hypoglycemia, lipid per-oxidation, superoxide dismutase, Type 2 diabetes

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by derangements in carbohydrate, protein and fat metabolism.^[1] It leads to hyperglycemia resulting from a defect in insulin secretion, or insulin resistance in the peripheral tissues or both.^[2] This hyperglycemic state produces classical symptoms *viz.* polyuria, polydipsia, polyphagia and weight loss.^[3] It is assumed that by 2030, the number of diabetic patients will increase to 439 million which was 285 million in 2010.^[4] India has been declared as “Diabetic Capital of the World” by the International Diabetes Federation because 20% of the total diabetic patients in the world found in India. However, among the two major types of diabetes, i.e., Type 1 and Type 2, Type 2 diabetes mellitus is the most common form of diabetes constituting 90-95% of the diabetic population

caused by obesity and an unhealthy lifestyle.^[5] Hyperglycemia is also associated with the generation of reactive oxygen species (ROS) and consequent oxidative stress which leads to^[6] several diabetic complications such as nephropathy, neuropathy, cardiovascular diseases and retinopathy.^[7] The treatment and management of diabetes become a challenge before the medical practitioner globally.^[8] Oral hypoglycemic agents (glyburide, glimepiride, glipizide, metformin, and pioglitazone, etc.) and insulin are basic pharmacotherapies for diabetes mellitus.^[9] Synthetic hypoglycemic agents put

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Received: 29-09-2015

Revised: 23-12-2015

Accepted: 24-12-2015

forth serious side effects especially metallic taste, gastrointestinal discomfort and nausea.^[10]

Furthermore, diabetes has been treated with several types of folklore medicines (herbal and herbomineral preparations) since ancient times. *Bhasmas* are the oldest metallic preparation of nanoparticle range which attracted enormous scientific and technological interest. *Chandrakala rasa* (CKR) is a well-known polyherbometallic preparation, used for the treatment of diabetes in ayurvedic system of medicine.^[11,12] CKR is a combination of six herbs with *Vanga*, *Lauha*, *Abhraka Bhasma*, and *Rasa Sindoor* [Table 1]. It has been reported that metals in the *Bhasmas* are in the nanoparticles range and are taken along with milk, butter, honey, or ghee to enhance the absorption, elimination and to remove the harmful effects.^[13] Moreover, nanoparticles of metals are increasingly gaining attention in the therapeutic area due to their ease of preparation, chemical stability, and unique optical properties.^[14] Hence, the present study was undertaken to investigate the antidiabetic potential of an ayurvedic classical polyherbomineral preparation CKR in experimentally induced diabetes in rats.

MATERIALS AND METHODS

Materials

The CKR was prepared as per the method given in ancient ayurvedic text *Bhaishajya Ratnavali*,^[11] at the Ayurvedic Pharmacy Research Laboratory, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur, Uttar Pradesh, India. Ingredients of CKR were given in Table 1.

Preparation of *Vanga Bhashma*

Vanga (tin metal) was melted and poured in *Churnodaka* (lime water) and *Nirgundi swarasa* (*Vitex nigundo* Linn. juice) mixed with *Haridra* (*Curcuma longa* Linn.). The process was called as *Dhalana* and performed in specific apparatus known as *Pithera Yantra*. The purified *Vanga* then fried with *Apamarga panchanga churna* (powder of the whole plant of *Achyranthes aspera* Linn.) to make it in powder form. Then, the *Vanga* powder was triturated with *Kumari swarasa* (*Aloe vera* L. Burm. fresh juice) and made into pellet form. The pellets were heated at 500°C for 1 h and then allowed to cool. This process was repeated 5 times to obtained *Vanga Bhasma*.^[15]

Preparation of *Abhraka Bhashma*

Abhraka (mica) was heated to red hot (at 800-850°C) and immediately quenched into *Triphala kwatha* (decoction of three fruits powder in equal quantity viz. *Terminalia chebula* Retz., *Terminalia bellarica* Roxb., and *Emblica officinale*

Gaertn.). This process was repeated for 7 times. The whole process was called as *Nirvapa*. Fresh *Triphala kwatha* was used every time. After *Nirvapa*, *Abhraka* became brittle and made in the form of powder. The powdered *Abhraka* was mixed with ¼ part *Shali dhanya* (*Oryza sativa* Linn.) to make *Dhanyabhraka*. The *Dhanyabhraka* was triturated with *Kasamarda swarasa* (juice of *Cassia occidentalis* Linn. leaves) and made in the form of pellets. The pellets were heated at 800°C for 1 h. After cooling, the process was repeated for 40 times to obtained *Abhraka Bhasma*.^[16]

Preparation of *Lauha Bhasma*

Tikshna Lauha (iron powder) has been used as the raw material for the preparation of *Lauha Bhasma*. *Tikshna Lauha* was heated to red hot (at 800-850°C), and it was dipped separately into sesame oil, *Takra* (buttermilk), *Gomutra* (cow urine), *Kanji* (sour gruel) and a decoction of horse gram (*Dolichos biflorus* Linn.). The process was repeated for 7 times, taking fresh liquid media every time. After the procedure, *Lauha* became brittle, and it was made in the form of powder. The powdered *Lauha* was subjected to *Bhanu paka* (sun drying) with *Triphala kwatha* and *Sthali paka* (boiling) with *Triphala kwatha*. The powdered material was triturated with *Triphala kwatha* and made pellets. The pellets were heated at 650°C for 1 h. After self-cooling, the process was repeated for 20 times to obtained *Lauha Bhasma*.^[17,18]

Preparation of *Rasa Sindoor*

For the preparation of *Rasa Sindoor*, *Kumari Bhavita Kajjali* (mercuric sulfide, triturated with fresh juice of *A. vera*) was taken in a glass bottle wrapped with seven layers of cloth impregnated with clay. It was successively subjected to heating for 3 h at 250°C, 450°C, and 650°C temperature in

Table 1: Ingredients of CKR

Ingredients	Scientific name	Quantity
<i>Vanga Bhasma</i>	Incinerated tin metal	1 Part
<i>Rasa Sindoor</i>	Red sulphide of mercury	1 Part
<i>Abhraka Bhasma</i>	Incinerated biotite/mica	1 Part
<i>Lauha Bhasma</i>	Incinerated iron	1 Part
Amalaki	<i>E. officinalis</i> Gaertn.	1 Part
<i>Shilajit</i>	<i>Asphaltum</i>	1 Part
Ela	<i>E. cardamomum</i> Maton.	1 Part
Karpoor	<i>C. camphora</i> Nees & Eberm.	1 Part
Shalmali	<i>B. ceiba</i> Linn.	Q. S.
Guduchi juice	<i>Tinospora cordifolia</i> (Willd.) Miers ex Hook.f. & Thoms.	Q. S.

E. officinalis: *Emblica officinalis* Gaertn., *E. cardemonum*: *Elettaria cardemonum* Maton., *C. camphora*: *Cinnamomum camphora* Nees & Eberm., *B. ceiba*: *Bombax ceiba* Linn., *T. cordifolia*: *Tinospora cordifolia* (Willd.) Miers ex Hook.f. & Thoms., CKR: *Chandrakala rasa*

a muffle furnace. *Rasa Sindoor* was collected in the form of sublimate at the neck of the bottle.^[19]

Preparation of CKR

For the preparation of CKR, all the ingredients (*Vanga Bhasma*, *Rasa Sindoor*, *Abhraka Bhasma*, *Lauha Bhasma*, *Shilajit*, *E. officinalis*, *Elettaria Cardemonum*, and *Cinnamomum camphora*) were taken in equal quantities, triturated 8 times separately with *Tinospora cordifolia* juice and *Bombax ceiba* juice until dry powder obtained. CKR was stored in an airtight container for the further experiment.

Drugs and Chemicals

Glibenclamide (GC), streptozotocin (STZ), nicotinamide (NAD) (Sigma–Aldrich Co. LLC., New Delhi, India) adenine dinucleotide, nitro blue tetrazolium (NBT) (Sisco Research Laboratories Pvt. Ltd., Mumbai) were used for the experimental purpose. All other reagent and solvents used in the experiment were of the analytical grade.

In Vivo Antidiabetic Activity

Experimental animals

Adult Charles foster albino rats (140 ± 20 g) of either sex were procured from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University; Varanasi. The animals were kept in the laboratory at controlled temperature (22 ± 2°C) and humidity (55 ± 10%) and 12 h light/12 h dark cycle. The animals were provided with standard pelleted feed (Amrut Pvt. Ltd. Pune, India) and water *ad libitum*. Rats were kept in a standard laboratory environment for at least 1 week before the commencement of the experiment. The protocols for the study have been approved by the Institutional Animal Ethical Committee of Banaras Hindu University, Varanasi (Ref. No. Dean/13-14/CAEC/212).

Acute oral toxicity study

The acute oral toxicity study of CKR was performed according to the Organization for Economic Co-operation and Development-425 guidelines.^[20] A single dose of CKR 2000 mg/kg, p.o. was administered in 24 h fasted rats ($n = 5$) and observed at 0, 30, 60, 120, 180, and 240 min and then once a day for next 14 days for any signs or symptoms of toxicity or abnormalities. The number of rats that survived at the end of the study period was recorded.

Oral Glucose Tolerance Test (OGTT)

30 normoglycemic rats were used for the experiment. The animals were allowed free access of water *ad libidum* for 18 h before the experiment. The animals were divided into five groups *viz.* control group, reference drug GC (10 mg/kg,

p.o., in 0.5% carboxymethylcellulose [CMC]) treated group, and three test drug (CKR 100, 200, and 400 mg/kg, p.o.) treated groups. The control group received only vehicle (0.5%, CMC) by the oral route. 10 min after treatment with reference and test drugs, glucose (2 g/kg, 10% solution in water) was administered orally to each rat in all the groups. The glucose level in the blood samples collected from tail vein was determined by glucometer (Accu-Chek Meters, Roche Diagnostics India, Pvt. Ltd.) based on enzymatic glucose-oxidase method at 0 (before glucose administration), 30, 60, 90, and 120 min after glucose administration.^[21]

Induction of Diabetes in Rats

The rats were divided into six groups (Group I-VI) with six rats in each group. Group I received only water (10 ml/kg, p.o.). Remaining Groups (II-VI) were administered with STZ (60 mg/kg, i.p., in 0.1 M cold citrate buffer, pH 4.5) 15 min after the administration of NAD 100 mg/kg, i.p.) to induce diabetes.^[22] Glucose solution (20% in water) was given to the STZ-NAD-treated rats for 24 h to avoid initial hypoglycemic mortality induced by STZ-NAD. After 96 h of the administration of STZ-NAD, blood samples of all the animals groups were taken from tail vein for the estimation of blood glucose levels. Diabetes was confirmed when blood glucose level found above 250 mg/dL. After the induction of diabetes, Group III was treated with standard drug GC (10 mg/kg, p.o., in 0.5% CMC) and Groups IV-VI were treated with the test drug CKR 100, 200, and 400 mg/kg, p.o., respectively, for 21 days. Body weights and blood glucose level estimation were done weekly in overnight fasted animals. On the next day, blood samples were collected from a retro-orbital vein from the overnight fasted animals for the estimation of various biochemical estimations. After the experiment, all the animals were sacrificed for removal of the liver.

Biochemical Analysis

Biochemical estimation kits (Span Diagnostic, Surat, Gujarat, India) were used for the estimation of total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), and high density lipoprotein-cholesterol (HDL-C) estimation.

Tissue Preparation

The liver was carefully removed, weighed, and washed with ice-cold saline to remove the traces of blood. The liver tissue was sliced into pieces and homogenized (Glass Teflon homogenizer, Thomas Scientific, Swedesboro, USA) in Tris–HCl buffer (0.025 M, pH 7.5). The liver homogenate was centrifuged at 10,000 g for 10 min at 41°C. The supernatant was separated and used for the estimations of various antioxidant enzymes.

Assay of Antioxidant Activity

The level of lipid per-oxidation (LPO) was estimated and expressed in terms of malondialdehyde as per the method of Ohkawa *et al.*, 1979.^[23] The activity of superoxide dismutase (SOD) was estimated by following the procedure of Kakkar *et al.*, 1984^[24] based on the reduction of NBT to blue colored formazan in the presence of phenazine methosulfate. The level of catalase (CAT) was estimated as per the method of Sinha, 1972.^[25]

Statistical Analysis

All the values of the experimental results were expressed as mean \pm standard error of mean. Two-ways ANOVA followed by Bonferroni post-test was used to access effect on normoglycemic, OGTT, and STZ-NAD-induced diabetic rats. One-way ANOVA followed by Tukey's multiple comparison tests was performed for the statistical analysis of the rest of parameters. Both the statistical analysis were performed using GraphPad Prism, Version 5.0.1., Software.

RESULTS

Acute Oral Toxicity Study

Acute oral toxicity study of CKR did not show any toxicity or behavioral changes at a dose level of 2000 mg/kg. This finding suggests that CKR was safe or non-toxic to rats up to 2000 mg/kg. The doses of CKR 100, 200, and 400 mg/kg, b.w. were selected on the basis of pilot study for the *in vivo* antidiabetic study.

Antidiabetic Study of CKR

Effect on blood glucose levels in fasted normal rats

Figure 1 illustrates the effect of CKR on overnight fasted rats. Two-way ANOVA revealed that there was a significant difference between control group and treatment groups. GC at the dose of 10 mg/kg, p.o., significantly reduced the blood glucose level in rats when compared to normal control (NC) group. However, CKR in all the doses tested did not show any hypoglycemic effect on normal rats.

Effect on OGTT

The effect of CKR (100, 200, and 400 mg/kg, p.o.) on OGTT is depicted in Figure 2. Two-way ANOVA indicates that there were significant differences between experimental groups after treatment. Animals treated with CKR (400 mg/kg), and GC showed a significant decrease in blood glucose level when compared to NC animals. The administration of CKR significantly prevented the increase in blood glucose levels without causing any hypoglycemic effect. The maximum

effect of CKR was observed at 30 and 60 min after the oral glucose administration.

Effect on Fasting Blood Glucose Level of STZ-NAD-Induced Diabetic Rats

Figure 3 indicates the effect of CKR (100, 200, and 400 mg/kg, p.o.) on the STZ-NAD-induced diabetic rats. Two-way ANOVA reveals that there were significant differences in the experimental groups. A significant increase in the level of blood glucose was observed in STZ-NAD treated rats when compared to NC rats ($P < 0.05$). Administration of CKR significantly reduced the fasting blood glucose levels on 7th, 14th, and 21st days as compared to diabetic control (DC). Treatment of diabetic rats with GC also significantly reduced the increased blood glucose level.

Effect on plasma lipid profile

Effect of CKR on plasma lipid profile, i.e., TC, TGs, and lipoproteins are shown in Table 2. The levels of plasma TC, TGs, and LDL-C were significantly increased ($P < 0.05$), whereas level of HDL-C was significantly decreased ($P < 0.05$), in diabetic rats as compared to NC rats. The treatment of

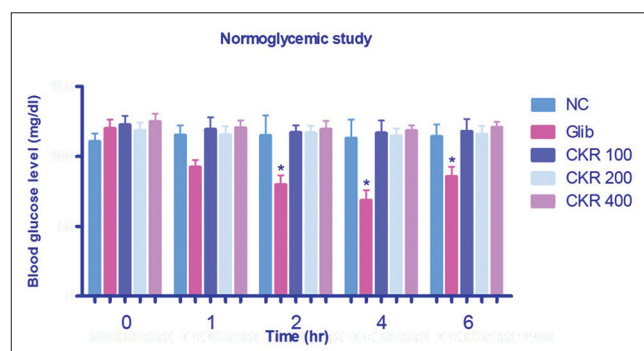


Figure 1: Effect of *Chandrakala rasa* on normal rats. Two-way ANOVA followed by Bonferroni post-test revealed that there was a non-significant difference between control group and treatment group * $P < 0.05$ compared to normal control (NC: Normal control, Glib: Glibenclamide, CKR: *Chandrakala rasa*)

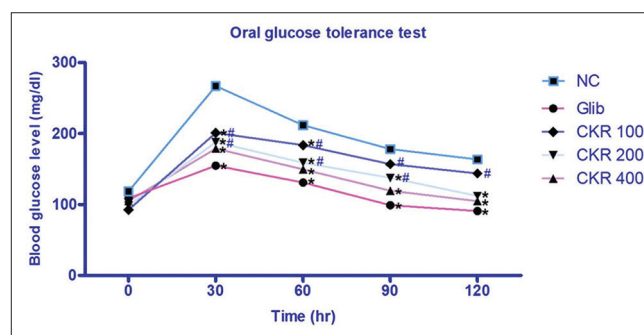


Figure 2: Effect *Chandrakala rasa* on oral glucose tolerance test in normal rats. * $P < 0.05$, compared to normal control; # $P < 0.05$, compared to glibenclamide. (Two-way ANOVA followed by Bonferroni post-test) (NC: Normal control, Glib: Glibenclamide, CKR: *Chandrakala rasa*)

rats with CKR reversed plasma lipid profile near to normal values. This showed that treatment with CKR significantly improved the lipid profile in diabetic animals. The effect of CKR (200 and 400 mg/kg, p.o.) was found comparable with that of GC (10 mg/kg, p.o.).

Effect on body weight

Effect of CKR treatment in rats on body weight and liver glycogen are shown in Table 3. Changes of body weight in DC group were remarkable in comparison to NC groups. The mean body weight of diabetic rats was higher than control group animals on the 21th day when treated with CKR, but it was statistically significant ($P < 0.05$) for only 200 and 400 mg/kg, p.o. of CKR. The increase in body weight by the administration of highest dose CKR was found comparable to that GC treated group. A significant decrease ($P < 0.05$) in liver glycogen content was observed in DC group as compared to NC group. Rats treated with CKR 100 mg/kg, p.o. did not show a significant increase in liver glycogen level; however, rats treated with 200 and 400 mg/kg, p.o. showed pronounced increases in liver glycogen level. GC treatment also significantly increased ($P < 0.05$) liver glycogen level as compared to DC rats.

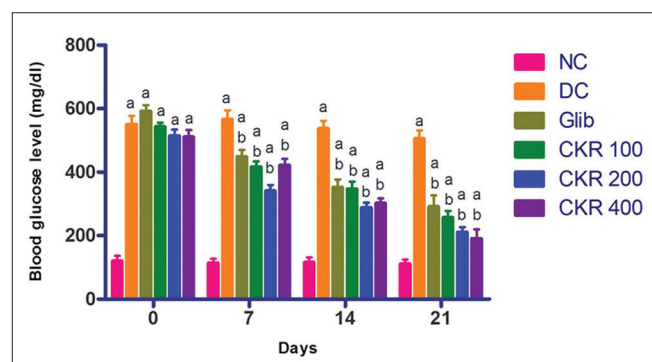


Figure 3: Effect of *Chandrakala rasa* on the blood glucose level of streptozotocin-nicotinamide-induced diabetic rats. ^a $P < 0.05$, compared to normal control; ^b $P < 0.05$, compared to diabetic control. (Two-way ANOVA followed by Bonferroni post-test) (NC: Normal control, DC: Diabetic control, Glib: Glibenclamide, CKR: *Chandrakala rasa*)

Effect on antioxidant enzyme activity

Table 4 represents the concentration of thiobarbituric acid reactive substances (TBARS) in liver samples of normal and experimental rats. There was a significant elevation in tissue TBARS in animals during diabetes as compared to the normal group. Administration of CKR (200 and 400 mg/kg, p.o.) significantly decreased the LPO in diabetic rats. The effect of CKR at the dose level of 400 mg/kg, p.o. was found comparable to GC. Statistical analysis by one-way ANOVA on the effect of CKR on the activity of SOD and CAT showed a significant effect of treatment with CKR. The activity of SOD and CAT were found significantly lower in diabetic rats as compared with their values in NC rats. Treatment with CKR in diabetic rats significantly restored the enzyme levels as compared to untreated diabetes animals.

DISCUSSION

The present study was designed to investigate the potential antihyperglycemic, hypolipidemic, and antioxidant activity of CKR in normal, glucose-loaded hyperglycemic, and STZ-NAD-induced diabetic rats. The study revealed that CKR in normoglycemic rats does not exert any significant decline in blood glucose level, signifying that the CKR does not have any hypoglycemic activity. However, the capacity of CKR to lower blood glucose level in the OGTT suggests that animals treated with CKR have better glucose utilization capacity. Oral administration of CKR 100 mg/kg for 21 days caused a significant decrease in blood glucose levels in diabetic rats. Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, basically due to overproduction or decreased utilization of glucose by the tissues.² STZ-induced hyperglycemic condition is a most widely used model for evaluating the antidiabetic drugs. It causes selective pancreatic islet β -cell necrosis mediated through the release of nitric oxide and brings an increase in blood glucose levels.^[26] NAD, a potent antioxidant is added with STZ for induction of Type II diabetes. It prevents the β -cell necrotic action of STZ by free radicals scavenging activity and causes only minor damage to pancreatic β -cell mass producing

Table 2: Effect of CKR on lipid profile of STZ-NAD-induced diabetic rats

Group (n=6)	Treatment (dose in mg/kg)	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
I	NC	68.14±3.47	76.58±6.95	49.95±1.78	23.2±5.86
II	DC	160.91±9.37 ^a	188.77±25.84 ^a	28.36±1.77 ^a	133.52±4.93 ^a
III	Glib (10)	79.71±4.58 ^b	91.7±5.3 ^b	51.16±3.73 ^b	29.89±4.87 ^b
IV	CKR (100)	115.69±19.55 ^a	147.02±12.68 ^a	32.48±1.94 ^a	83.69±11.3 ^b
V	CKR (200)	85.88±10.3 ^b	108.62±12.8 ^b	51.36±4.62 ^b	35.38±8.51 ^b
VI	CKR (400)	78.96±7.6 ^b	89.15±5.06 ^b	54.5±6.55 ^b	24.16±7.8 ^b

Values are mean±SEM of six animals in each group. ^a $P < 0.05$ compared to NC; ^b $P < 0.05$ compared to DC; (One-way ANOVA followed by Tukey's multiple comparison test). NC: Normal control, DC: Diabetic control, Glib: Glibenclamide, CKR: *Chandrakala rasa*, STZ: Streptozotocin, NAD: Nicotinamide, SEM: Standard error of mean

Table 3: Effect of CKR on body weight and liver glycogen in STZ-NAD-induced diabetic rats

Group (n=6)	Treatment (dose in mg/kg)	Body weight (g)		Liver glycogen (mg/g)
		0 th day	21 th day	
I	NC	179.16±6.75	175.26±6.32	28.95±1.36
II	DC	168.50±6.18	120.37±3.65 ^a	13.23±1.38 ^a
III	Glib (10)	174.66±5.72	169.87±5.3 ^b	23.93±1.62 ^b
IV	CKR (100)	162.33±3.71	139.28±4.78 ^a	15.92±0.32 ^a
V	CKR (200)	176.66±9.27	154.38±4.68 ^{ab}	20.08±0.79 ^{ab}
VI	CKR (400)	174.16±5.97	164.36±2.89 ^b	24.72±1.42 ^b

Values are mean±SEM of six animals in each group. ^aP<0.05 compared to NC; ^bP<0.05 compared to DC (one-way ANOVA followed by Tukey's multiple comparison test). NC: Normal control; DC: Diabetic control, Glib: Glibenclamide, CKR: *Chandrakala rasa*, STZ: Streptozotocin, NAD: Nicotinamide, SEM: Standard error of mean

Table 4: Effect of CKR on TBARS, SOD, and CAT in STZ-NAD-induced diabetic rats

Group (n=6)	Treatment (dose in mg/kg)	TBARS	SOD	CAT (μ mol. H ₂ O ₂
		(nmol/mg protein)	(U/mg protein)	consumed/min/mg protein)
I	NC	24.88±1.62	0.81±0.10	241.50±8.78
II	DC	49.91±5.55 ^a	0.42±0.03 ^a	160.85±9.45 ^a
III	Glib (10)	29.79±2.08 ^b	0.78±0.12 ^b	239.86±20.13 ^b
IV	CKR (100)	40.25±2.85 ^a	0.54±0.05	213.90±8.30
V	CKR (200)	33.89±3.96 ^b	0.76±0.05 ^b	235.46±15.95 ^b
VI	CKR (400)	26.42±3.11 ^b	0.86±0.05 ^b	238.69±12.59 ^b

Values are mean±SEM of six animals in each group. ^aP<0.05 compared to normal control; ^bP<0.05 compared to diabetic control. (One-way ANOVA followed by Tukey's multiple comparison test). NC: Normal control, DC: Diabetic control, Glib: Glibenclamide, CKR: *Chandrakala rasa*, TBARS: Thiobarbituric acid reactive substances, SOD: Superoxide dismutase, CAT: Catalase, STZ: Streptozotocin, NAD: Nicotinamide, SEM: Standard error of mean

Type II diabetes.^[27] For evaluating new antihyperglycemic compounds in STZ-NAD-induced diabetes, sulfonylureas such as GC are used as standard drug. Its action is mediated through an increase in intracellular calcium in the β-cell, which in turn stimulates insulin release.^[28]

As the CKR did not cause the hypoglycemia in normoglycemic rats but reduced blood glucose level in OGTT and STZ-NAD-induced diabetic rats, CKR may act as antihyperglycemic, rather than a hypoglycemic agent. Dyslipidemia is an important factor in the determination of the course and status of the disease. A reduction in insulin secretion causes a variety of derangements in metabolic and regulatory mechanisms leading to accumulation of lipids.^[29] The findings of the present study clearly show that CKR significantly reduced the TG and TC in diabetic rats. Lipid-lowering effect of drugs in diabetes reduces the risk of vascular complications.^[30] Changes in lipid profile (increase the level of TG, TC, LDL, VLDL, and decrease HDL) increases the risk for coronary heart diseases in diabetic patients.^[31] HDL-C reduces the risk of cardiovascular diseases through free radical scavenging and anti-inflammatory actions and promotes the efflux of cholesterol from the peripheral tissues to the liver.^[32]

Altered carbohydrate metabolism promotes increased muscle wasting, structural degradation of proteins or loss of muscle

proteins resulted in a decline in body weight.^[33] An increase in the body weight of diabetic rats treated with CKR (400 mg/kg p.o.) suggesting a protective role of CKR on muscle wasting might be due to the improvement in glycemic control and increase synthesis of structural proteins.^[34] Extracellular glucose concentration and blood insulin level are two key factors for conversion of glucose to glycogen in the liver cells. The observed depletion of liver glycogen level in DC rat was possibly due to the inactivation of the glycogen synthetase systems or increased activity of glycogen phosphorylase, reflecting of insulin deficiency.^[35] The present study showed the significant increase in the liver glycogen in diabetic rats treated with CKR, may be due to the reactivation of glycogen synthetase system which is responsible for the improvement in the liver glycogen synthesis. Hence, CKR interferes with glucose utilization and metabolism by storing excess carbohydrates as glycogen.

Chronic hyperglycemia and impaired insulin secretion may contribute to a reduction in levels of enzymatic (CAT) and non-enzymatic antioxidants (total thiols) along with increased free radicals regeneration, which can lead to increased LPO.^[36] This enhanced oxidative stress on the β-cells was proposed as a key contributor to the development of diabetes mellitus and its complications.^[37] Enhanced levels of TBARS in diabetic rats indicate the excessive formation

of free radicals and activation of LPO system which leads to damage of membrane through LPO of unsaturated fatty acids.^[38] SOD and CAT remove free radicals and play a vital role in maintaining the cell integrity. A decrease in the activity of SOD and CAT can lead to an excessive accumulation of free radicals (superoxide and hydrogen peroxide), which in turn generate ROS, resulting in initiation and propagation of LPO.^[39] Therefore, decreased LPO and improved antioxidant status by the CKR may be one of the mechanisms by which CKR could contribute to the prevention of diabetic complications.^[40]

The result of the present study clearly shows that CKR has free radical scavenging and anti-LPO potential. All the herbal ingredients of CKR have antioxidant activity.^[41-45] The phytochemical screening of CKR reveals the presence of wide range of phytoconstituents such as alkaloids, terpenoid, phenolics, glycosides, steroids, polysaccharides, etc. Triterpenoids of *B. ceiba* were previously reported for antidiabetic activity.^[46] Furthermore, three alkaloids *viz.*, palmatine, jatrorrhizine, and magnoflorine obtained from *T. cordifolia*^[47,48] have been reported for their antidiabetic effect. *Emblica officinalis* also has antidiabetic activity.^[49]

Shilajit, a herbomineral preparation used in the long-term management of diabetes mellitus because of its multifaceted action. It produces a better glycemic control along with improvement in the lipid profile in animals.^[50]

Antidiabetic activity of CKR may be due to the presence of more than one antihyperglycemic principle and their synergistic properties. Certain classes of compounds *viz.* flavonoids, triterpenoids/sterols, alkaloids, and phenolics are known to be bioactive antidiabetic principles. Phenolics are found to be effective antihyperglycemic agents.^[51] The literature reveals that antioxidant activity of plant extract is mainly due to the presence of phenolic compounds, which may exert antioxidant effects as free radical scavengers, as hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators.^[51]

CONCLUSION

In the present investigation, we demonstrated that CKR restored the altered serum glucose level, body weight; lipid profile level near normal in STZ-induced diabetic rats. CKR significantly enhanced the levels of endogenous antioxidant enzymes (CAT, and SOD). On the basis of the findings of the present study, it is clear that CKR has significant hypoglycemic, hypolipidemic, and antioxidant potential.

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Source of Support: Nil. **Conflict of Interest:** None declared.