CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://doi.org/10.5281/zenodo.7393004

Available online at: http://www.iajps.com *Research Article*

EVALUATION OF ANTI-HYPERGLYCEMIC EFFECTS OF THE HERB CURCUMA CAESIA ROXB.

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Article Received: September 2022 **Accepted:** October 2022 **Published:** November 2022

Abstract:

The aim of this study is to evaluate anti hyperglycemic potential of Curcuma caesia Roxb. The hydroalcoholic extract from roots of Curcuma caesia was tested Streptozotocin induced diabetes rats. The total phenol & flavonoid content was found to be 6.25± 0.001 µg /ml & 15.63 ± 0.005 µg /ml respectively. Rats were divided into different groups, each group consisting of six animals. Experimental diabetes was induced in group II-VI by intraperitoneal injection of STZ. Glibenclamide (500 mcg/kg) was used as a standard drug. Biochemical parameters like total cholesterol, Triglycerides, HDL & oral glucose were used to assess the antidiabetic activity. Result showed that Hydroalcoholic extract of Curcuma caesia (HECC) 200 and 400 mg/kg/p.o. was able to decrease body weight (189.00±5.50 and 186.00 ± 9.70) blood glucose (120.00 ± 5.50 and 116.00 ± 6.00) , cholesterol (139.0 ± 6.00 and 121.0 ± 5.00) & triglycerides (94.00 ± 7.00 and 83.50 ± 5.00) significantly ($p<0.05$) at 21st days. The HECC of 200mg/kg & 400mg/kg increased High- density lipoprotein (HDL) (34.00±1.80 and 45.00±1.90) significantly ($p < 0.05$). It was also noticed that administration of HECC (400 mg/kg b wt.) or Glibenclamide to diabetic rats elicited a significant decrease in blood glucose level at 60 min and beyond. From these finding it can be interpreted that Curcuma caesia Roots possess anti hyperglycemic activity.

Key words: Curcuma caesia, Streptozotocin induced diabetes rats, hydroalcoholic extract

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Please cite this article in press ***Ankita Jatav*** *et al,* ***Evaluation Of Anti-Hyperglycemic Effects Of The Herb Curcuma Caesia Roxb..,*** *Indo Am. J. P. Sci, 2022; 09(11).*

INTRODUCTION:

A worldwide public health issue called diabetes mellitus (DM) is now becoming an epidemic. The term "diabetes mellitus" refers to a number of disorders of improper glucose metabolism that are hyperglycemic in nature. It is linked to variable degrees of peripheral insulin resistance as well as a relative or absolute reduction in insulin secretion (Atkinson & Eisenbarth ,2001).

Diabetes and the associated complications has become a global epidemic disease of 21st century thereby affecting a greater fraction of population worldwide. Based on the current survey of International Diabetes Federation (IDF), there are almost 73.12 million people in India only who are suffering from Diabetes and globally it is expected to rise from 450 million in 2017 to 691 million by 2045. Diabetes is not only a health issue but it is a worldwide disaster which not only causes personal suffering but it drives the families to poverty. It is also creating a lot of burden on the government to provide health schemes or treatment remedies at economical cost (Feldman,2017).

Both conventional and contemporary medicine can benefit from the therapeutic antidiabetic properties of medicinal herbs. Numerous disorders have been treated with herbal remedies, and in recent years, natural health products have gained favour as alternatives or complements to prescription drugs. Numerous ethnopharmacological investigations on medicinal plants that reduce diabetes risk have been published. Numerous traditionally used medicinal herbs have had their hypoglycaemic potential assessed and verified in various animal models (Zimmet *et al*., 2001).

Medicinal plants are the main source of organic compounds such as polyphenols, tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These organic compounds represent a source for the discovery and development of new types of antidiabetic molecules. Many compounds isolated from plant sources have been reported to show antidiabetic activity (Firdous,2014).

One such plant is *Curcuma caesisa*. The perennial herb known as kali haldi, *Curcuma caesia* (family Zingiberaceae), has a bluish-black rhizome and is a member of the Curcuma genus. North-East and Central India are the natural habitats of black turmeric (*C. caesia*). Due to Black Turmeric's rumoured medical capabilities, its rhizomes are of significant commercial value. Fresh rhizomes have a powerful camphoraceous fragrance and are utilised topically to bruises and sprains. Because antibiotics have a short effective life period and are frequently overprescribed and misused, which results in microbial resistance, plant extracts are increasingly being used to treat a variety of bacterial infections. The plant has medical anti-inflammatory, anti-microbial, and antioxidant effects. The main goal of the current study was to assess the anti-hyperglycemia effects of the herb *Curcuma caesia* Roxb.

MATERIALS & METHOD:

Plant materials:

The rhizomes of *Curcuma caesia* were collected from local market of Bhopal in the period of May, 2022.

Extraction by maceration method:

Collected plant drugs namely *Curcuma caesia* were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (71.5 gm) and packed in (250 ml) air tight glass Bottle. The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant drugs were subjected to extraction by ethanol and Water (ethanol: water; 70:30) as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated (Khandelwal,2003).

Phytochemical Analysis:

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the extract of *Curcuma caesia*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids (Kokate,2005).

Estimation of total phenolic content:

Estimation of total phenolic content Total phenolic content of all the extracts was evaluated with Folin-Ciocalteu method. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 0.5mL aliquots of 12.5, 25, 50, 100 and 200 µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin– Ciocalteu reagent (diluted ten-fold) and

2.5 mL (7.5 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer 1650 Shimadzu, Japan. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extracts as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per µg /ml of extract (Mohsen & Ammar,2008).

Estimation of total flavonoids content:

The aluminum chloride colorimetric method was modified from the procedure reported. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 0.3, to 6 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of Hydroalcoholic extract solutions (1000 ppm) were reacted with aluminum chloride for determination of Flavonoid content as described (Sultana *et al*., 2009)

Animals:

Wistar albino rats of both sexes (180-220 g) were used for anti-diabetic and anti-obesity study. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were facilitated with standard environmental condition of photo period (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed and water given ad libitum. The use of these animals and the study protocols were approved by (IAEC) Institutional animal ethical committee.

Selection of the doses for animal study:

The animals were randomly selected, marked to permit individual identification and kept in their cages for 7 days prior to dosing to allow for acclimatization to the laboratory condition. In acute toxicity study no toxic symptoms were observed for *Curcuma caesia* up to dose 3000 mg / kg body weight. All animals behaved normally. No neurological or behavioral effect could be noted. No mortality was found up to 14 days study.

Anti-diabetic studies using Streptozotocin induced diabetes Studies:

Rats were divided into different groups, each group consisting of six animals. After overnight fasting (deprived of food for 16 hours had been allowed free access to water) diabetes was induced in group II-VI by intraperitoneal injection of STZ dissolved in 0.1M sodium citrate buffer at pH 4.5, at a dose of 55mg/kg body weight. The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug- induced hypoglycemia1. Diabetes status was confirmed by estimating blood glucose levels after 72 hours of STZ injection. Animals showing fasting blood glucose levels above 250 mg/dl were selected for study (Pushparaj *et al*., 2001)

Animal grouping for anti-diabetic studies:

Table 1: Animal grouping for anti-diabetic studies

Group	Treatment	No. of Animals (n)
Group –I	Normal	6
Group -II	Diabetic control received only STZ (negative control)	6
Group –III	Diabetic rats received Glibenclamide orally at dose of 500 mcg/kg b.wt for 14 days	6
Group –IV	Diabetic rats received Hydroalcoholic extract of *Curcuma caesia* (200 mg/kg/day p.o.)	6
Group -V	Diabetic rats received Hydroalcoholic extract of *Curcuma caesia* (400 mg/kg/day p.o.)	6
Total No. of animal used for the study		30

Biochemical analysis:
Body weight of the experimental rats was taken on pre and post treatment i.e. initial and final day of post treatment by digital balance. The blood glucose level of fasted rats was taken pre and post treatment i.e. 0, 8thand 21thday of post treatment.

At the end of experimental time, all the experimental rats were sacrificed by cervical decapitation. Blood samples were collected and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Biochemical parameters like Estimation of Total Cholesterol (TC), Triglycerides (TG), HDL cholesterol & oral glucose were estimated.

RESULTS:

Extractive values:
The yields were found to be (2.9 % w/w of crude drug) of petroleum ether extract with semisolid mass of brown colour, (5.96% w/w of crude drug) of hydroalcoholic extract with black colour semisolid mass for *Curcuma caesia*. Obtained results were recorded in table 2.

Table 2: Extractive values obtained from *Curcuma caesia*

S.N.	Solvent	% Yield
1.	Petroleum ether	2.9%
2.	Methanol: Water	5.96%

Results of phytochemical analysis:
The positive test results were obtained for carbohydrates, flavonoids, Proteins & Phenol.

Results of total phenolic content:

Table 3: Total phenolic content of hydroalcoholic extract of *Curcuma caesia*

Sample	Total phenolic content GAE µg /ml
Hydroalcoholic extract 1000µg/ml	6.25± 0.001

n=3, values are given in SEM

Results of total flavonoid content

Table 4: Total flavonoid content of hydroalcoholic extract *Curcuma caesia*

S. N.	Extract	Flavonoid content Quercetin equivalent µg /ml
1	Hydroalcoholic extract (1000µg/ml)	15.63 ± 0.005

n=3, values are given in SEM

Toxicity studies:

Table 5: General appearance and behavioral observations in control and treated groups

Observation	Control Group	Treated Group
Behaviours	Normal	Normal
Skin and fur	Normal	Normal
Sleep	Normal	Normal
Eyes	Normal	Normal
Salivation	Normal	Normal
Diarrhea	Normal	Normal
Lethargy	Normal	Normal

Table 6: Mean Body Weight Change

Group	Drug	Dose	Body weight (g)	
			Onset of study	End of study
I	Normal Control	Normal saline	190.15±6.83	210.18±6.83
II	Diabetic Control	Normal saline	200.20± 10.00	180.00±10.00
III	Glibenclamide	500 mg/kg p.o.	210.22± 8.26	180.40±8.26*
IV	HECC	200 mg/kg p.o.	220.10± 5.50	189.00±5.50
V	HECC	400 mg/kg p.o.	217.10± 5.00	186.00 ± 9.70*

Table 7: Antidiabetic activity of (HECC) on blood glucose level in STZ-induced diabetic rats

Groups	Treatment	Dose	Blood glucose (mg/dl)		
			Days 0	Days 8	Days 21
I	Normal Control	Normal saline	83.00 ± 5.00	86.00 ± 4.00	93.00 ± 4.00
II	Diabetic Control	Normal saline	298.00 ± 6.00	384.00 ± 7.00#	391.00± 7.00#
III	Glibenclamide	500 mg/kg p.o.	264.00± 5.20	139.00 ± 6.50**	110.00 ± 6.50**
IV	HECC	200 mg/kg p.o.	264.00 ± 5.50	159.00 ± 5.50 *	120.00 ± 5.50*
V	HECC	400 mg/kg p.o.	262.00± 6.00	154.10 ± 6.00*	116.00 ± 6.00**

Table 8: Effect of (HECC) on total cholesterol level in STZ-induced diabetic rats

Group	Drug	Dose	Total Cholesterol(mg/dl)
I	Normal Control	Normal saline	81.00 ± 6.00
II	Diabetic Control	Normal saline	198.0 ± 5.00
III	Glibenclamide	500 mg/kg p.o.	105.0 ± 5.00***
IV	HECC	200 mg/kg p.o.	139.0 ± 6.00
V	HECC	400 mg/kg p.o.	121.0 ± 5.00**

Table 9: Effect of (HECC) on triglyceride level in STZ -induced diabetic rats

Group	Drug	Dose	Triglyceride (mg/dl)
I	Normal Control	Normal saline	70.00 ± 5.00
II	Diabetic Control	Normal saline	145.5 ± 6.00
III	Glibenclamide	500 mg/kg p.o.	81.00 ± 5.00**
IV	HECC	200 mg/kg p.o.	94.00 ± 7.00*
V	HECC	400 mg/kg p.o.	83.50 ± 5.00*

Table 10: Effect of (HECC) on HDL in STZ-induced diabetic rats

Group	Drug	Dose	HDL (mg/dl)
I	Normal Control	Normal saline	52.80±1.10
II	Diabetic Control	Normal saline	27.00±2.70
III	Glibenclamide	500 mg/kg p.o.	49.50±2.00***
IV	HECC	200 mg/kg p.o.	34.00±1.80*
V	HECC	400 mg/kg p.o.	45.00±1.90**

Table 11: Effect of (HECC) on glucose tolerance in control and experimental diabetic rats

Group	Drug	Dose	0 min	30 min	60 min	90 min	120 min
I	Normal Control	Normal saline	100	190	140	130	120
II	Diabetic Control	Normal saline	150	240	200	190	180
III	Glibenclamide	500 mg/kg p.o.	140	170	160	155	120
IV	HECC	200 mg/kg p.o.	150	200	170	155	130
V	HECC	400 mg/kg p.o.	150	200	170	165	122

CONCLUSION:

The anti-hyperglycemic effect of hydroalcoholic extract of *Curcuma caesia* (HECC) on the STZ-induced diabetic rats suggest that its main mechanism may not be due to stimulating insulin release from pancreatic cells, but may exert a direct action by promoting glucose utilization by peripheral tissues.

In the present study a significant decrease in total cholesterol, triglyceride and LDL cholesterol levels was observed after treating the STZ induced diabetic rats with hydroalcoholic extract of *Curcuma caesia* (HECC) leaves at 200 and 400 mg/kg body weight leading to a significant increase in the HDL cholesterol levels.

In levels of SGOT and SGPT were significantly altered. The decreased glucose levels than negative control in diabetic + hydroalcoholic extract of *Curcuma caesia* (HECC) group and overall condition of animals were indicative of classical anti-diabetic activity of hydroalcoholic extract of *Curcuma caesia* (HECC).

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