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Antidiabetic Activity of *Coccinia indica* in Streptozotocin Induced Diabetic Rats

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The purpose of this study was to investigate the antidiabetic potential of the *n*-hexane extracts of *Coccinia indica* for the treatment of diabetic mellitus in the streptozotocin included diabetic rats at 200 mg/kg body weight for 30 d period. The effect of the compound was found to be 206 ± 23.54 mg/dl of bood, after the extract was given to the diabetic rats.

Key Words: *Coccinia indica*, Antidiabetic activity.

INTRODUCTION

Diabetes mellitus is now recognized as serious global health problem[1]. *Coccinia indica* is grown abundantly in India and have been widely used in the traditional treatment of diabetes mellitus[2]. Contradictory findings on the antidiabetic activity of the different parts of *Coccinia indica* have been reported[3,4].

Nikkhila and Kekki[5] have reported an increase in the cholesterol level in diabetic rats, which was control by fraction treated plant extract. Galletto *et al.*[6] have shown the antidiabetic effect of *Gymnema sylvestre*, similarly Shrivastava *et al.*[7] have reported that 70 % ethanolic extract of *Butea monosperma* where the extract showed increasing insulin in the β-cells of pancreas. Latha and Pari[8] have reported be the effect of aqueous extract of *Scoparia dulcis* on blood glucose level in the rats. Hussain[9] reported the reversal of diabetic retinopathy in STZ induced diabetic rats using traditional Indian antidiabetic plant *Azadirachta indica*. Venkateswaran *et al.*[10] reported the effects of *Coccinia indica* on blood glucose, insulin and hepatic key enzymes in experimental diabetes.

In the present study, it was planned to test a few fractions and delineate the most active fraction of *Coccinia indica*. The *n*-hexane extract of *Coccinia indica* was found to be more active in reducing blood glucose level: this extract was subjected to further fraction and evaluation on antidiabetic activity.

EXPERIMENTAL

Coccinia indica (Wight & Arn.) commonly known as Kanduri belonging to the family Cucurbitaceae. Aerial parts of *Coccinia indica* were collected, dried under shade, powdered and the alcoholic extract was prepared. The dried alcoholic extract was a semi-solid mass and was successively extracted with *n*-hexance, chloroform, ethyl acetate and *n*-butanol, concentrated and dried in a dessicator. The extracts were used for the present study as shown in Table-1.

TABLE-1
PERCENTAGE YIELD OF *Coccinia indica* BY SOXHLET EXTRACTION AT 40-50 ºC

Solvent used	Wt. of powder (g)	Wt. of extract (g)	Yield of crude drug (%)
n-Hexane	150	2.32	1.546
Chloroform	300	2.88	0.960
Ethyl acetate*	200	3.56	1.780
n-Butanol*	300	4.25	1.416

*Both the solutions gave polar compounds.

Chemical analysis of crude drug: The biologically active compound was separated from the crude extract by column chromatography. It was determined by TLC using $CHCl_3$:MeOH:H_2O (2:2:1), $CHCl_3$:CCl_4: acetone (6:6:3) and $CHCl_3$:MeOH:H_2O (2:4:2). This gave three fractions[11,12]. Each fraction was further chromatographed till a single spot was obtained (Table-2).

TABLE-2
TLC OF *n*-HEXANE EXTRACT OF *Coccinia indica*

Solvent system	Obtained fraction	Spot No (s)	Behaviour		R_f value of each spot
			Visible	UV light	
$CHCl_3$:MeOH:H_2O (2:2:1)	C_1	Spot 1	Dark green	Dark green yellow	0.31
$CHCl_3$:CCl_4:H_2O (6:6:3)	C_2	Spot 1	Light green		0.42
		Spot 2	Light yellow	Brown	
		Spot 3	Dark green	Green	
$CHCl_3$:MeOH:H_2O (2:4:2)	C_3	Spot 1	Light brown	Dark brown	0.56
		Spot 2	Yellow brown	Yellow	

Acid hydrolysis of purified fraction: The compound (fraction C_1) was hydrolyzed with 10 % H_2SO_4 in MeOH:H_2O (1:1) at 80 ºC for 4 h. The reaction mixture was neutralized with $BaCO_3$ and filtered. The filtrate was evaporated to dryness in vacuum to give a residue in which glucose was identified.

Methylation: The acid hydrolyzed fractions were methylated after the removal of methanol, the solution was extracted with ethyl acetate thrice.

Glycosidal analysis: The four fractions were tested chemically for glycosides which show the presence of mono and disaccharides in the compound. Iodine and Benedict's tests confirmed the presence of glycosides.

Spectral analysis: The spectral analysis of the compound was carried out for NMR which showed no signals for an aldehylde group at C-23. Instead, signals for two carbonyl carbon (δ 177.6 and 187.9) are present in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed resonance for only five methyl groups on tertiary carbon at δ 0.73, 0.81, 0.98, 1.14 and 1.19 (s, 3H each). A three proton singlets were shown at δ 3.73 with a one bond heteronuclear correlation to the carbon resonance at δ 52.3 and a correlation to the carbonyl signals at δ 178.9, additionally an oxymethylenic group (δ 3.75) was also shown. The compound has the highest molecular mass of the group (δ 3.75) was also shown. The compound has the highest molecular mass of the saponin isolated from 70 % ethanol[13]. The R_f value of the compound was found 0.3-0.5 which indicates the presence of saponin. On the basis of this, a triterpenoid saponin was isolated from *Coccinia indica*.

Antidiabetic activities: The fresh fruits of *Coccinia indica* were washed with water and air-dried at room temperature. The air-dried fruits were grinded to powder about 40-60 mesh size. About 150 g known amount of powdered substance was then extracted successively with solvent ethanol to increase the polarity. The crude extract obtained was filtered using Whatmann filter paper No. 1 and the solvent was evaporated to dryness under reduced pressure in vacuum evaporator at 40-50 ºC. The freshly prepared extract was chemically tested for the presence of different constituents using standard method[14].

Induction of diabetes: The animals were starved overnight then diabetes was induced by a single interaperitoneal injection of a freshly prepared streptozotocin (STZ) solution (50 mg/kg body weight). Streptozotocin was dissolved in 0.1 M freshly prepared citrate buffer solution (pH 4.5). The animals were allowed to drink 5 % glucose solution overnight to overcome. After 5 d streptozotocin administration, rats showing diabetes. Control rats were injected with citrate buffer alone. The extract in aqueous solution was administered orally through a garage at a concentration of 200 mg/kg body weight/rat/day for 30 d.

Experimental design: The animals were divided into four groups for the analysis of biochemical parameters. Each group has six animals.
Group I: Normal control rats; Group II: Diabetic control rats; Group III: Diabetic rats treated with *Coccinia indica* extract 200 mg/kg body weight orally and Group IV: Diabetic treated with standard drug.

RESULTS AND DISCUSSION

Data are expressed as mean ± SEM. The biochemical parameters were analyzed statistically using one-way Anova followed by Dunnett's multiple comparison test. The glucose levels before and after the administration of

different fractions were compared using paired students t-test. The minimum level of significance was fixed at $p < 0.05$. The result of present study indicates that the *n*-hexane fraction was found to be quite active, decreasing the blood glucose level -27.2 %. This fraction was found to possess triterpenoid saponin (Table-3).

TABLE-3
EFFECT OF VARIOUS EXTRACTS OF *Coccinia indica* ON THE BLOOD GLUCOSE LEVEL IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Group	Doses (mg/kg)	Glucose level (mg/dl) after streptozotocin		% After drug treatment
		Before drug treatment	After drug treatment	
Solvent control	–	269.15 ± 17.57	305.66 ± 31.32	+13.56
Positive control (Phenformin)	30	284.67 ± 30.16	140.60 ± 33.14	-50.60
n-Hexane	200	283.00 ± 21.28	206.00 ± 23.54*	-27.2
Chloroform	200	257.00 ± 15.31	223.12 ± 12.36	-13.18
Ethyl acetate	200	288.12 ± 17.05	238.13 ± 12.34	-17.35
n-Butanol	200	261.67 ± 26.00	281.67 ± 21.15	+07.64

The values are mean ± SEM (n = 6 in each group. "+" Denotes increase in hyperglycemic activity. *$p < 0.05$ in comparison to corresponding value before treatment.

REFERENCES

1. American Diabetes Association, 1701 North Beauregard Street, Alexandria, VA, USA (2000).
2. R.N. Chopra, I.L. Chopra, K.L. Handa and L.D. Kapur, Indigenous Drugs of India, UN Dhar and Sons Pvt. Ltd., Calcutta, India edn. 2 (1958).
3. N. Singh, S.P. Singh, S. Vvat, N. Mishra, K.S. Dixit and R.P. Kohli, *Indian J. Med. Sci.*, **39**, 27 (1985).
4. H.D. Brahmachari and K.T. Augusti, *J. Pharm. Pharmacol.*, **16**, 411 (1963).
5. E.A. Nikkhila and M. Kekki, *Metabolism*, **22**, 1 (1973).
6. R. Galletto, V.L.D. Siqueira, E.B. Ferreira, A.J.B. Obliveira and R.B. Bazotte, *Brazil. Arch. Biol. Technol.*, **47**, 545 (2004).
7. V. Shrivastava, N. Jain, S.M. Jain and R.C. Saxena, Antidiabetic Effect of Flowers of *Butea monosperma* in Albino Rats, 36th Annual Conference of the Indian Pharmacological Society, p. 109 (2003).
8. M. Latha and L. Pari, *Brazil. J. Med. Biol. Res.*, **37**, 577 (2004).
9. H.E.M.A. Hussain, *Indian J. Clin. Biochem.*, **17**, 115 (2002).
10. S. Venkateswaran and L. Pari, *Pharmacol. Biol.*, **40**, 165 (2002).
11. M.C. Koli, R. Choudhry, S. Kumar, S. Thakur, D. Shukla and R.C. Saxena, *Asian J. Chem.*, **14**, 1673 (2002).
12. S. Sharma, P.N. Shrivastava and R.C. Saxena, *Asian J. Chem.*, **18**, 2766 (2006).
13. R.C. Saxena, Muscles Relaxant Activates on Amaranthus Aspara, WOCAMP, 3rd World Conference on Medicinal and Aromatic plants, Chiang May Yniversity, Thailand, OP01-13: p. 41 (2003).
14. G.E. Treas and W.C. Evans, Pharmacognosy Bailliere Tindall, Vol. 386, edn. 13, pp. 167-197 (1994).

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