

Document heading

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine



journal homepage:www.elsevier.com/locate/apjtm

Evaluation of antidiabetic and related actions of some Indian medicinal plants in diabetic rats

Raju N Patil^{1*}, Ravindra Y Patil², Bharati Ahirwar³, Dheeraj Ahirwar¹

¹CEC School of Pharmacy, Lal Khadan, Masturi Road, Bilaspur (C.G.) India ²S U College of Pharmaceutical Sciences and Research, Kharadi, Pune, (M.S.) India ³Department of Pharmaceutics, Guru Ghasidas University, Bilaspur (C.G.) India

doi:

ARTICLE INFO

Article history: Received 2 November 2010 Received in revised form 27 November 2010 Accepted 15 December 2010 Available online 20 January 2011

Keywords: **Diabetes** mellitus Alloxan Serum glucose Hyperlipidemia

ABSTRACT

Objective: To evaluate antidiabetic activity of chloroform extracts of *Acacia arabica* bark, Benincasa hispida fruit, Tinispora cordifolia stem, Ocimum sanctum areal parts and Jatropha curcus leaves. Methods: The chloroform extracts of Acacia arabica bark, Benincasa hispida fruit, Tinospora cordifolia stem, aerial part of Ocimum sanctum and Jatropha curcus leaves were evaluated at different doses (250 and 500 mg/kg body weight.) for antidiabetic potentials in alloxan induced diabetic albino rats. The extracts were administered for two weeks in different groups whereas tolbutamide (80 mg/kg body weight) was used as reference standard throughout study. Results: The result of present study showed test compounds significantly decreases elevated level of serum glucose and also caused to reverse the cholesterol, triglyceride, HDL and LDL values when compared to untreated diabetic rats. Conclusions: Our finding indicates that different test extracts were able to ameliorate the derangements in lipid metabolism caused by diabetes mellitus in alloxan induced diabetic rats towards normal level.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder caused by an absolute or relative lack of insulin and/or reduced insulin activity which results in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism^[1,2]. Management of diabetes without any side effects is still a challenge for medical system. This leads to an increasing search for improved antidiabetic drugs. Few of plant treatments used in traditional medicine for diabetes received scientific scrutiny and WHO has recommended that this area warrants attention^[3]. Acacia arabica (Momosaceae); Benincasa hispida (Cucurbitaceae); Tinisporia cordifolia (Menispermaceae); Jatropa curcas (Euphorbiaceae) and Ocimum sanctum Linn are traditionally used for treatment of diabetes[4]. Acacia arabica possesses antibacterial^[5], antiviral^[6] and antifertility activities^[7]. Tinospora cordifolia was reported to have adaptogenic^[8], antiviral^[9], antihyperglycemic^[10], immunomodulatory^[11], antineoplastic^[12], cardioprotective^[13] and antioxidants^[14,15],

Tel: +91 2112 254447, +91 9730080522

activities. Ocimum sanctum showed antioxidant, antifungal^[16], antimicrobial^[17] and wound healing^[18] activities. Jatropha curcus possesses anti-diabetic^[19], wound-healing^[20], antitumor^[21], anti-inflammatory^[22] and antifungal^[23] activities. Benincasa hispida was reported to have antinociceptive, anti-pyretic^[24] and anti-stress^[25] activities. The objective of present study was to evaluate antidiabetic activity of chloroform extracts of Acacia arabica bark, Benincasa hispida fruit, Tinispora cordifolia stem, Ocimum sanctum areal parts and Jatropha curcus leaves.

2. Material and methods

2.1. Plant material

The bark of Acacia arabica, fruit of Benincasa hispida, stem of Tinospora cordifolia, aerial part of Ocimum sanctum and leaves of Jatropha curcus were collected from Pune District, Maharashtra, India in August 2008. The plants were identified and authenticated by Dr. R. B. Deshmukh, Department of Botany, Sharadabai Pawar College, Malegaon(bk) Tal- Baramati Dist- Pune.(India). The voucher specimens (AA-08, BH-08, TC-08, OS-08 and

^{*}Corresponding author: Mr. Raju N. Patil, Department of Pharmacy, CEC School of Pharmacy, Lal Khadan, Masturi Road, Bilaspur (C.G.) India. E-mail: patilrn31@rediffmail.com

JC-08 respectively) are deposited in herbarium. The samples were cleaned, dried in shade and powdered. The powdered samples were kept in airtight container for further studies.

2.2. Chemicals and drugs

Alloxan monohydrate, tolbutamide and chloroform were purchased from Sigma Chemicals, St. Louis. Chemical kits for estimation of blood glucose, cholesterol, triglyceride, LDL and HDL were purchased from Erba Diagnostics, Mannheim.

2.3. Animals

The male albino rats (Wistar strain weighing 150–180 g) and albino mice (weighing 20–30 g) were procured from SVPM's College of Pharmacy, Malegaon (bk), Tal– Baramati Dist–Pune, India and housed in animal house. They were kept at 27 ± 3 °C (Relative humidity: $65\%\pm10\%$ and light / dark cycle for 12 h. All the animals were fed with rodent pellet diet (Gold Mohr, Lipton India Ltd.) and water was allowed ad–libitum under strict hygienic conditions. Institutional Animal Ethics Committee (IAEC) approved all the protocol of study (Reg. No. 1214/ac/08/CPCSEA).

2.4. Preparation of extract

Dried powders each of 100 g of *Acacia arabica* bark (CEAA), *Benincasa hispida* fruit (CEBH), *Tinospora cordifolia* stem (CEOS), *Ocimum sanctum* aerial part and leaves of *Jatropha curcus* (CETC) were extracted with chloroform by Soxhlet extraction and solvents were evaporated by rotary evaporator. Concentrated extracts were dried on water bath at controlled temperature to yield CEAA 12.30%w/w, CEBH 14.08%w/w, CETC 9.69%w/w, CEOS 15.07%w/w and CEJC 11.31%w/w respectively.

2.5. Toxicity studies

An acute toxicity of CEAA, CEBH, CETC, CEOS and CEJC were carried out in female albino mice (25–30 g). The mice were divided in to six groups (n=6). The animals were fasted for overnight prior to the experimentation. The fixed dose method of CPCSEA (Mrs. Prema Veeraraghavan. Expert consultant, CPCSEA, OECD guide line No. 420; Oct 2000) was adopted for toxicity studies.

2.6. Preparation of diabetic rats

Alloxan monohydrate dissolved in saline was injected in rats intraperitoneally at dose of 150 mg/ kg body weight. After a fortnight, rats with marked hyperglycemia were selected and used for the study.

2.7. Experimental design

The rats were divided into thirteen groups (n=5). Group I (controlled group) administered 0.5 mL saline, Group II (untreated diabetic rats), group III (diabetic rats receiving

tolbutamide orally at 80 mg/kg body weight in saline), Groups IV to XIII (diabetic rat treated with CEAA, CEBH, CETC, CEOS and CEJC at dose of 250 and 500/mg kg body weight). Tolbutamide was used as the standard antidiabetic throughout the experimentation. The animals were carefully monitored at every day. Animals described as fasted were deprived of food for at least 12 h but allowed to free access for drinking water. Fasting blood glucose measurement was done on day 0, 7th and 14th of the study. Blood samples were collected by retro orbital method at weekly intervals till the end of study and processed for estimation of serum glucose and serum lipid[26, 27, 28].

2.8. Biochemical estimation

Serum samples from all the experimental rats were collected for estimation of biochemical parameters, serum glucose (GOD-POD method), cholesterol (CHOD-PAP method), triglycerides (GPO-Triender method), HDL and LDL^[29].

2.9. Statistics

All values are expressed as Mean \pm SEM. The differences were compared using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. *P* values < 0.05 were considered as significant.

3. Result

In untreated diabetic rats, glucose, cholesterol, triglycerides, LDL values were elevated to high level during the study where as HDL value was decreased. Chronic treatment with CEAA, CEBH, CETC, CEOS and CEJC at 250 and 500 mg/kg significantly (*P*<0.0001) causes decrease in serum glucose on 7th and 14th day as shown in Figure 1, 2 and 3. Serum cholesterol triglycerides HDL and LDL levels also reversed with treatment of test extracts and standard as shown in table 1.

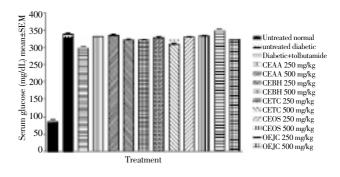


Figure 1. Effect of CEAA, CEBH, CETC, CEOS and CEJC on serum glucose level in diabetic rats on 0 day.

All values are expressed as Mean \pm SEM (*n*=5). Statistical comparisons between each treatment and untreated diabetic were carried out by one way ANOVA followed by Tukey's multiple comparison tests. *** P < 0.0001 when compare to untreated diabetic group. Table 1

Comparative effect of CEAA	CEBH. CETC	. CEOS and CEIC on se	rum cholesterol, triglyceride	s. HDL and LDL (mg/d	l) in diabetic :
domparative encer or diffin	, order, ordere	, de ob una dej a on be	full enoiceteroi, ingrecentee	, IID L und LD L (mg a	i) in anabono.

C		Average serum lipid profile (mg/dL)				
Groups	Treatment -	Total cholesterol	Triglycerides	HDL cholesterol	LDL cholesterol	
Ι	Normal	64.28±3.51	79.64±3.12	48.60 ± 3.72	48.53 ± 4.36	
II	Diabetic	95.28±4.01	143.88±3.52	26.14 ± 5.61	108.23±7.59	
III	Diabetic + Tolbutamide 80 mg/ kg	58.78±3.61***	$81.10 \pm 4.00^{***}$	$41.86 \pm 5.94^{**}$	55.34±5.46**	
IV	Diabetic+CEAA 250 mg/kg	72.46±2.69****	109.60±3.06***	30.14 ± 4.18	81.34±2.30****	
V	Diabetic+CEAA 500 mg/kg	61.39±2.06 ***	97.59±2.33****	35.24±4.84	65.71±0.60****	
VI	Diabetic+CEBH 250 mg/kg	72.01±2.21 ***	109.56±1.23****	31.16±5.14	86.73±1.58**	
VII	Diabetic+CEBH 500 mg/kg	64.69±1.08 ***	87.41±2.95****	39.30±4.19	$63.24 \pm 1.60^{***}$	
VIII	Diabetic+CETC 250mg/kg	79.43±3.07*	$100.79 \pm 2.82^{***}$	30.18±3.51	$89.24 \pm 2.60^{**}$	
IX	Diabetic+CETC 500mg/kg	66.82±1.62 ***	82.91±2.70****	36.13±3.24	71.10±0.60***	
Х	Diabetic+CEOS 250mg/kg	80.23±2.65 *	92.80±1.18****	31.24±4.14	$91.46 \pm 1.69^*$	
XI	Diabetic+CEOS 500mg/kg	65.26±2.71 ***	83.47±2.91****	37.62±3.31	64.18±0.50***	
XII	Diabetic+CEJC 250mg/kg	81.52±2.69 *	87.06±2.02***	29.92±4.12	93.24±1.65	
XIII	Diabetic+CEJC 500mg/kg	72.18±3.01 ****	95.48±3.74***	38.81±5.21	72.09±1.90***	

Values are expressed as Mean \pm SEM; (n = 5), a P < 0.05, b p < 0.01, c P<0.001 compared with untreated diabetic rats.

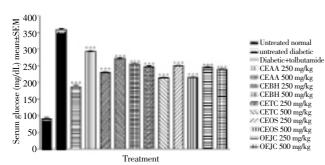


Figure 2. Effect of CEAA, CEBH, CETC, CEOS and CEJC on serum glucose level in diabetic rats on 7th day.

All values are expressed as Mean \pm SEM (*n*=5). Statistical comparisons between each treatment and untreated diabetic were carried out by one way ANOVA followed by Tukey's multiple comparison tests. *** P < 0.0001 when compare to untreated diabetic group.

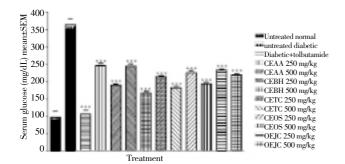


Figure 3. Effect of CEAA, CEBH, CETC, CEOS and CEJC on serum glucose level in diabetic rats on 14th day.

All values are mean \pm SEM (*n*=5). Statistical comparisons between each treatment and untreated diabetic were carried out by one way ANOVA followed by Tukey's multiple comparison tests. *** *P* < 0.0001 when compare to untreated diabetic group.

4. Discussion

Alloxan induces diabetes by damaging the insulin secreting cells of pancreas leading to hyperglycemia^[30].

Diabetes mellitus is a group of syndromes characterized by hypoglycemia, altered metabolism of lipids, carbohydrates and proteins. It is an increased risk of complications from vascular diseases^[31].

rats

An observation in this study correlates with the previous research finding, in that the blood glucose levels significantly increased in alloxan induced untreated diabetic rats. In the present study, the continuous treatment of different test compounds for a period of two weeks caused a significant decrease in blood glucose of treated diabetic rats. These results have confirmed the earlier results of our preliminary studies[32]. We have also observed an increase in concentration of cholesterol, triglyceride, LDL and decrease in HDL in alloxan untreated diabetic rats. Hyperlipidaemia is recognized consequence of diabetes mellitus^[33–35]. Chronic administration of different tests extracts normalized serum lipid profile i.e. secondary to diabetic state. Diabetes induced hyper lipidaemia is attributable to excess mobilization of fat from adipose due to the under utilization of glucose^[36]. The regression of the diabetic state due to chronic administration of different test extract at different doses increased the utilization of glucose, thereby depressing the mobilization of fat.

In conclusion, the present investigation supports the hypoglycemic effects of extracts of ethnic folk medicine which mitigates pathological states like diabetes mellitus.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are gratefully acknowledged the Principal, Management of CEC School of Pharmacy, Bilaspur (CG) and CSVTU, Bhilai (CG) for facilities provided during the study.

References

- Bhatena SJ, Velasquez MT. Beneficial role of dietary phytoesrogens in obesity and diabetes. *Am J Clin Natr* 2002; 76:1191-201.
- [2] Bayens JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40: 405-12.
- [3] The WHO Expert Committee on diabetes mellitus. *Technical report series 646*. Geneva: World Health Organization; 1980, p.61.
- [4] Kirtikar KR, Basu BD. *The Indian medicinal plants*. 2nd ed. Bombay: International book distributor; 1987.
- [5] Tambekar DH, Khante BS, Chandak BR. Screening of antibacterial potentials of some medicinal plants from Melghat forest in India. *Afr J Tradit Complement Altrn Med* 2009; 6(3): 228–322.
- [6] Bhanuprakash V, Hosamani M, Balamurugan V. In vitro antiviral activity of plant extracts on goatpox virus replication. Indian J Exp Biol 2008; 46(2):120–7.
- [7] Azad Chowdhary AK, Khaleque RA, Chakder SK. Antifertility activity of a traditional contraceptive pill comprising Acacia catechu, Acacia arabica & Tragia involucerta. Indian J Med Res Sep 1984; 80: 372-4.
- [8] Savrikar SS, Dole V, Ravishankar B, Shukla VJ. A comparative pharmacological investigation of three samples of 'Guduchi ghrita' for adaptogenic activity against forced swimming induced gastric ulceration and hematological changes in albino rats. *Int J Ayurveda Res* 2010; 1(2): 67–72.
- [9] Akhtar S. Use of *Tinospora cordifolia* in HIV infection. *Indian J Pharmacol* 2010; 42(1): 57.
- [10]Patel SS, Shah RS, Goyal RK. Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats. *Indian J Exp Biol* 2009; 47(7): 564–70.
- [11]Kalikar MV, Thawani VR, Varadpande UK. Immunomodulatory effect of *Tinospora cordifolia* extract in human immuno–deficiency virus positive patients. *Indian J Pharmacol* 2008; 40(3): 107–10.
- [12]Jagetia GC, Rao SK. Evaluation of the antineoplastic activity of guduchi (*Tinospora cordifolia*) in Ehrlich ascites carcinoma bearing mice. *Biol Pharm Bull* 2006; 29(3): 460–6.
- [13]Rao PR, Kumar VK, Viswanath RK, Subbaraju GV. Cardioprotective activity of alcoholic extract of *Tinospora* cordifolia in ischemia-reperfusion induced myocardial infarction in rats. *Biol Pharm Bull* 2005; 28(12): 2319–22.
- [14]Prince PS, Kamalakkannan N, Menon VP. Restoration of antioxidants by ethanolic *Tinospora cordifolia* in alloxan–induced diabetic wistar rats. *Acta Pol Pharm* 2004; 61(4): 283–7.
- [15]Subramanian M, Chitalwar GJ, Chattopadhyay S. Antioxidant properties of a *Tinospora cordifolia* polysaccharide against ironmediated lipid damage and gamma-ray induced protein damage. *Redox Rep* 2002; 7(3): 137–43.
- [16]Khan A, Ahmad A, Manzoor N, Khan LA. Antifungal activities of Ocimum sanctum essential oil and its lead molecules. *Nat Prod Commun* 2010; 5(2): 345–9.
- [17]Agarwal P, Nagesh L Murlikrishnan. Evaluation of the antimicrobial activity of various concentrations of Tulsi (Ocimum sanctum) extract against Streptococcus mutansan in vitro study. Indian J Dent Res 2010; 21(3): 357–9.
- [18]Goel A, Kumar S, Singh DK, Bhatia AK. Wound healing potential

of *Ocimum sanctum* linn. with induction of tumor necrosis factoralpha. *Indian J Exp Biol* 2010; **48**(4): 402–6.

- [19]Obatomi DK, Bikomo EO, Temple VJ. Anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. J Ethnopharmacol 1994; 43(1): 13–7.
- [20]Villegas LF, Fernandez ID, Maldonado H. Evaluation of the wound-healing activity of selected traditional medicinal plants from Perú. J Ethnopharmacol 1997; 55(3): 93–200.
- [21]Lin J, Yan F, Tang L, Chen F. Antitumor effects of curcin from seeds of Jatropha curcas. Acta Pharmacol Sin 2003; 24(3): 241–6.
- [22]Mujundar AM, Misar AV. Anti-inflammatory activity of Jatropha curcas roots in mice and rats. J Ethnophramacol 2004; 90(1): 111-5.
- [23]Saetae D, Suntornsuk W. Antifungal activities of ethanolic extract from Jatropha curcas seed cake. J Microbiol Biotechnol 2010; 20(2): 319–24.
- [24]Qadrie ZL, Hawisa NT, Khan MW. Antinociceptive and antipyretic activity of *Benincasa hispida* (thunb.) cogn. in wistar albino rats. *Pak J Pharm Sci* 2009; 22(3): 287–90.
- [25]Shetty BV, Arjuman A, Jorapur A, Samanth R. Effect of extract of *Benincasa hispida* on oxidative stress in rats with indomethacin induced gastric ulcers. *Indian J Physiol Pharmacol* 2008; 52(2):178-82.
- [26]Grover JK, Vats V, Rathi SS. Antihyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J Ethnopharmacol 2000; 73: 461–70.
- [27]Stanley P, Menon VP. Hypoglycemic and other related actions of Tinospora cordifolia roots in alloxan induced diabetic rats. J Ethnopharmacol 2000; 70(1): 9–15.
- [28]Claudia EN, Momo Julius E. Antidiabetic and hypolipidemic effects of *Laportea ovalifolia* (Urticaceae) in alloxan induced diabetic rats. *Afr J Trad CAM* 2006; **3**(1): 36–43.
- [29]Ajit Kar, Choudhary BK, Bandopadhay NG. Comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats. *J Ethnopharmacol* 2003; 84: 105–8.
- [30]Szuldelski T. The mechanism of alloxan and streptozotocin action in beta cells of the rat pancreas. *Physiol Res* 2001; 50: 536–46.
- [31]Jing-Tian Xian, Anbao Wang, Sangeeta Mehendale. Antidiabetic effects of *Gymnema yunnananense* extract. *Pharmacol Res* 2003; 47: 323–9.
- [32]Patil RN, Patil RY, Dheeraj Ahirwar. Study of some medicinal plants for antidiabetic activity in alloxan induced diabetes. *Pharmacologyonline* 2010; 1: 53–60.
- [33]Pushparaj P, Tan CH, Tan BKH. Effect of Averrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin diabetic rats. *J Ethnophrmacol* 2000; 72: 69–76.
- [34]Pepato MT, Baviera AM, Vendramini RC. Cissus sicyoides (Princess wine) in the long term treatment of streptozotocindiabetic rats. Biotechnol App Biochem 2003; 37: 15-20.
- [35]Sharma SB, Hasir A, Prabhu KM, Murthy PS, Dez G. Hypoglycemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolona* in alloxan–induced diabetic rabbits. J Ethnopharmacol 2003; 85: 201–6.
- [36]Krishnakumar K, Augustti KT, Vijayammal PL. Hypolipidaemic effect of *Salacia oblonga* wall root bark in streptozotocin diabetic rats. *Med Science* 2000; 28: 65–7.