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Anti-diabetic activity of *Clematis montana* Buch. -Ham. ex DC. leaves extract in streptozotocin induced diabetic Rats

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Abstract

Clematis montana (Family: *Rananculaceae*) is also known as Himalayan clematis found in the Himalaya, Kashmir, Bhutan, Afghanistan and China at 1500-2000 m height. Plant has been used for the treatment of migraine, nervous disorders, skin infections, liver disorders and hypertension. Current study was carried out to appraise the anti-diabetic potential (subacute) of ethanol extract of *C. montana* leaves. Leaves were extracted with ethanol (CMLEE). The acute oral toxicity study was determined as per the OECD guidelines. The anti-diabetic potential of leaves extract was implemented on streptotozotocin induced diabetic rats for 21 days. In case of acute oral toxicity, mortality was not observed after the administration of leaves extract 2000 mg/kg body weight. Treatment with ethanol extract of leaves at doses 200 mg/kg and 400 mg/kg body weight exhibit significant anti-diabetic activity. The extract also showed significant increase in body weight and HDL in diabetic rats. The existing outcomes indicate that *Clematis montana* leaves have significant anti-diabetic potential in contradiction of diabetic rats.

Key words: Rananculaceae, *Clematis montana*, anti-diabetic, Streptozotocin, HDL, LDL.

Introduction

Diabetes mellitus is a chronic disease, characterized by polydipsia, polyphagia and polyuria due to lack of insulin secretion or tissues become resist for insulin. It is one of the major health problem in all over world. According to WHO survey 285 million people were diabetic worldwide till 2010 and diabetic patient number will be gradually increase up to 439 million till 2030 (Shaw et. al. 2010). As per statistical analysis the number of diabetics in India will be 57 million in the year 2025 and India has a highest number of diabetic patients in all over the world (Satyanarayana 2006). The main reasons for diabetic patient in India are pattern of lifestyle, food habit, and obesity (Eidi 2006). Diabetes mellitus increases the mortality rate due to complication in cardiovascular, nervous and urinary system (Zimmet et al. 1997). Oxidative stress is one of the major causes of diabetes and diabetes related complications (Atalay and Laaksonen 2002). Glucose auto-oxidation, protein or lipid glycation and the sorbitol-aldose reductase pathway *etc.* are general mechanism involved to produce free radicals and reactive oxygen species in diabetic patient (West 2000). It is scientifically proved that the concentration of natural antioxidant like vitamin C decreases when glucose concentration will increase. These free radicals and reactive oxygen species damage the basic cell structure like plasma membrane, DNA, RNA and different enzyme; ultimately produce cardiovascular, alziemer diseases, nephron and nerve damage (Gumieniczek et al. 2002; Aydın et al. 2001). Oral hypoglycemic and insulin are very useful for the treatment of diabetes, but the search for new entities from the plant is always in progress because the present available medicines have many adverse effects (Ratnakar and Murthy 1996). The genus

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Clematis is climbing vines with showy flowers and over 200 species are known. They are famous as flowering plants. *C. montana* found in Garhwal Himalaya region of Uttarakhand and used for the treatment of migraine, diabetes, nervous disorders, skin infections, liver disorders and hypertension (Sastri 1962; Dhar et al. 1968). Triterpenic bisglycosides and saponins have been isolated from this plant (Bahuguna et al. 1990; Thapliyal and Bahuguna 1993a; 1993b). The present study designed to scientifically prove the anti-diabetic activity of ethanol extract of *C. montana* leaves in streptozotocin induced diabetic rats.

Materials and methods

Collection of plant material

In July-Aug 2012, *C. montana* leaves were collected from natural populations in the region of Uttarakhand, India (30.45°N, 78.25°E, altitude: 2286 m). Plant material was authenticated by Dr. J. K. Tiwari, Department of Botany H.N.B Garhwal University, Srinagar Garhwal, Uttarakhand; India, through comparing Garhwal University Herbarium (GUH 1023). The voucher specimen was deposited in Herbarium at the Department of Pharmaceutical Chemistry of the same University. Plant materials were air dried in darkness at room temperature (25-30 °C) for 20 days, powdered and were stored in airtight containers until needed.

Chemicals, drugs and instruments

The chemicals and drugs used in the study were of analytical grade. Streptozotocin was purchased from Himedia, Mumbai, India whereas sodium carboxy methyl cellulose obtained from Merck, Mumbai, India. Rotary evaporator was procured from Heidolph, Schwabach, Germany. Biochemical estimation was done on biochemical autoanalyser model no. EON ONE 00189, vital diagnostic, Italy.

Preparation of extract

Powdered sample (100 g) was extracted with ethanol in soxhlet extractor for 72 hrs. The solvent from total extract was distilled off and concentrated on to syrupy consistency and then evaporated to dryness. The yield was found to be 8.98%.

Preliminary phytochemical screening

The qualitative chemical tests of ethanol extract of *C. montana* were done by using standard procedure to determine the presence of various phytochemicals (Kokate 2015).

Experimental animals

Wistar albino rats weighing 150-200 g were used for the study. All the animals were housed in clean polypropylene cages and were maintained at standard conditions of temperature (22 ± 1 °C), 12:12 h light/dark cycles and 60-70% relative humidity. The animals were maintained with standard pellet diet (Hindustan Lever Ltd, India) and had free access to water *ad libitum*. Ethical clearance was obtained from Institutional Animal Ethical Committee (Ref. No. NCFT/14/561).

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Acute toxicity testing

Acute toxicity study was performed for ethanol extract according to the OECD guidelines (O.E.C.D. 2004). Five healthy albino rats were selected for the study and then the animals were kept away for overnight fasting providing only water, after that ethanol extract was administered orally at the dose of 300 mg/kg body weight and animals were observed for 24 h. If the mortality was observed in three out of five animals, then this dose was assigned as toxic dose. If the mortality was observed in two animals, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, then the ethanol extract administered orally at the dose of 2000 mg/kg body weight and the toxicity measure as mentioned above.

Experimental induction of diabetes

To induce the diabetes, streptozotocin 55 mg/kg body weight were injected intraperitonially (Aslan et al. 2000; 2003). After 72 h treatment blood glucose levels of all the rats were determined and rats having blood glucose level more than 250 mg/dl were selected for the sub-acute study. After the induction of diabetes, animals were randomly divided into five groups (II to XI). Group I contained animals don't have diabetes and this group act as normal control. Group II known as diabetic control. Groups I and II were treated with vehicle 1% CMC during the whole experiment. The standard drug glibenclamide (10 mg/kg) were given to Group III and groups from IV to VI received the extract at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively. The vehicle, standard drug and extract were administered for 21 days continuously. The blood samples were collected at 1^{st}, 7^{th}, 14^{th} and 21^{st} days after drug administration. The body weight variation also measured at 21^{st} day after the treatment.

Biochemical estimations

On day 21, blood was collected and serum was separate out, then the cholesterol, triglycerides, HDL, VLDL, LDL, SGOT, SGPT, urea, uric acid, creatinine, total protein, albumin and alkaline phoshphatase were estimated in serum by diagnostic kit method (Natelson et al. 1951; Brod and Sirota 1948; Caraway 1963).

Statistical analysis

Data was represented as mean ± S.E.M. for anti-diabetic activity. Statistical analysis was carried out by one-way ANOVA followed by Dunnett test by using Graph-Pad prism software version 5.03. P value was regarded as significant, when $P < 0.05$.

Results and discussion

Preliminary phytochemical screening

Preliminary phytochemical screening of ethanol extract of *C. montana* leaves extract revealed the presence of carbohydrates, tannins, steroids, triterpenoids, saponins and flavonoids (Table 1).

Table 1 Results of preliminary phytochemical investigation of ethanol extracts of *C. montana* leaves

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S. No.	Test	Leaves
1	Alkaloids	-
2	Carbohydrates	+
3	Steroids and sterols	+
4	Flavonoids	+++
5	Tannins	+++
6	Triterpenoids	+
7	Saponines	+

Acute toxicity

In case of acute toxicity study, animals did not show any behavioral changes or mortality after the administration of extract doses ranging from 300 to 2,000 mg/kg body weight. According to acute toxicity results, the one tenth of maximum dose of the extract tested for acute toxicity was selected as middle dose (200 mg/kg), double and half of middle dose was selected for higher and lower dose (400, 100 mg/kg) for the anti-diabetic activity.

Anti-diabetic activity

Before to treatment beginning, all the groups showed similar values of blood glucose levels (diabetic control = 298.42 ± 0.21; diabetic standard = 315.22 ± 0.59; diabetic extract 100 = 326.36 ± 0.29; diabetic extract 200 = 330.39 ± 0.79; diabetic extract 400 = 315.41 ± 0.84). On the last day of the experiment, treated diabetic rat shows significant reduction of 15.93% (100 mg/kg CMLEE), 19.57% (200 mg/kg CMLEE), 32.62% (400 mg/kg CMLEE) and 44.06% (10 mg/kg standard) glucose levels when compared to non-treated diabetic rats (Table 2).

Table 2 Effect of ethanol extract of the leaves of *C. montana* on blood glucose level in streptozotocin induced diabetic rats (mg/dl) subacute study

Group	Treatment	Blood glucose level (±SEM)			
		1st day	7th day	14th day	21st day
1	Normal	95.5 ±0.24	114.12±0.42	112.26± 0.55	115.72 ±0.31
2	D.C.	298.42 ±.21	285.56±0.66	301.27 ±1.45	299.33± 2.35
3	D.G. (10 mg/kg)	315.22 ±0.59	251.66*± 0.86	200.77**±1.30	167.42***±0.98

4	D.E. (100 mg/kg)	326.36 ±0.29	300.12 ± 1.28	282.11 ± 0.95	251.63* ±0.88
5	D.E.(200 mg/kg)	330.39±0.79	305.62 ±0 .95	281.46 *±2.34	240.75** ±0.55
6	D.E. (400 mg/kg)	315.41 ±0.84	289.36 ± 1.06	241.22* ±0.87	201.67** ±2.33

D.C.: Diabetic control, D.G.: Diabetic + glibenclamide, D.E.: Diabetic + Extract, *p<0.05, **p<0.01, ***p<0.01significant compared to diabetic control.

At the 1st day of experiment, all the animals from different group showed approximately similar body weight. Treatment with standard drug and extract at a dose of 100 mg/kg and 200 mg/kg body weight, significantly increase the body weight of diabetic rats (Table 3).

Table 3 Effect of ethanol extract of the leaves of *C. montana* on the body weight in normal and streptozotocin induced diabetic rats

Group	**Treatment**	**Change in body weight**	
		Initial day	**21st day**
1	Normal	175.21±0.61	174.92±1.19
2	Diabetic control	190.89±1.55	165.11±0.79
3	Diabetic + glibenclamide (10 mg/kg)	178.22±0.94	195.77*±1.11
4	Diabetic + extract (100 mg/kg)	169.32±0.69	172.66*±0.95
5	Diabetic + extract (200 mg/kg)	172.66±2.76	170.78±1.01
6	Diabetic + extract (400 mg/kg)	179.56±2.23	181.53* ±1.15

*p<0.05significant compared to diabetic control.

The results of blood profile of all groups are represented in (Table 4 and 5). The extract treated animals exhibited dose dependent increase in blood HDL, albumin and protein level when compared with the non-treated animals. The other blood parameters like cholesterol, LDL, VLDL, urea, uric acid, triglyceride, creatinine, liver enzyme etc. significantly decreased in extract treated animals in comparison to non-treated animals.

Table 4 Effect of ethanol extract of the leaves of *C. montana* on serum biochemical parameters in streptozotocin induced diabetic rats

Groups	**Cholestrol (mg/dl)**	**Triglyceride (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**	**HDL (mg/dl)**	**SGPT (U/L)**	**SGOT (U/L)**
Normal	155.29±4.09	106.32±0.55	67.57±0.72	29.61±0.65	45.21±1.78	29.98±0.88	28.11±0.44

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Diabetic control	291.76±1.98	281.85±2.34	184.23±1.56	74.11±0.96	27.18±0.75	52.64±3.44	61.45±2.21
Glibenclamide (10 mg/kg)	147.63±0.39***	125.75±0.82***	69.22±0.95**	34.36±0.19**	52.92±0.76**	30.12±0.48**	30.69±0.27**
Extract (100 mg/kg)	215.55±0.58*	235.48±1.88*	178.26±1.64	70.39±0.73	31.17±0.79	49.19±2.24	41.76±0.99
Extract (200 mg/kg)	200.11±0.79*	188.29±0.71*	151.63±0.88*	61.22±1.22	40.86±0.84*	44.66±0.90	38.38±0.83*
Extract (400 mg/kg)	191.67±1.34**	142.66±2.54**	120.29±1.66*	56.94±0.75*	47.62±0.89**	42.69±0.97*	36.74±0.89*

Table 5 Effect of ethanol extract of the leaves of *C. montana* on serum biochemical parameters in streptozotocin induced diabetic rats

Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Total proteins (g/dl)	Albumin (g/dl)	Alkaline phoshphatase (U/L)
Normal	26.66±0.85	6.25±0.77	0.67±0.33	8.5±1.29	4.99±0.66	119.24±1.59
Diabetic Control	75.14±0.42	14.33±0.41	2.01±0.56	2.92±0.87	3.26±1.12	320.26±0.93
Glibenclamide (10 mg/kg)	35.17±0.59***	7.73±2.54***	0.89±0.97***	8.29±3.78***	5.96±0.79***	136.25±1.09***
Extract (100 mg/kg)	45.59±0.79	13.92±1.22	1.58±o.99	4.51±0.61	3.34±2.77	276.26±0.96
Extract (200 mg/kg)	42.52±2.45*	11.21±0.87	1.40±0.81	5.33±0.39*	3.99±3.11	254.22±0.55*
Extract (400 mg/kg)	41.77±1.12*	11.39±0.76*	1.06±0.11*	5.99±0.55**	4.76±0.98*	220.66±0.67**

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Phytochemical screening test is important for identification of active phytochemical in plant extract. Identified secondary metabolites (phytochemical) are responsible for bioactivity of plant. The anti-diabetic potential of *C. montana* leaves extract may be due to the presence of detected phytochemical such as flavonoids, carbohydrates, steroids, tri-terpenoids and saponins. Throughout world, to investigate the anti-diabetic activity of medicinal plants, different *in vivo* and *in vitro* experiment models has been established. But, knowledge about the safety aspect of plant-derived medicines is important, many medicinal plants used to treat diabetes by large number of population in all over the world and many side effects may be observed after the use of medicinal plants. So, it is necessary to perform experimental investigation in animals like mice and rats regarding the any acute toxic effects of medicinal plant extracts (Jesus et al. 2012; Mu et al. 2011). The outcome from our study shows that *C. montana* leaves ethanol extract is safe at the highest dose of 2000 mg/kg as per OECD guidelines and no mortality has been occurred under the observation period of 24 hr. In diabetic patient insulin secretion either decrease or body become resist, the result is high glucose level mainly due to the gluconeogenesis, glycogenolysis and decrease the consumption of glucose by the tissues. So it is an urge to be develop new compounds that can regulate the normal glucose metabolism for diabetes treatment (Leney and Tavare 2009; Wu et al. 2005). Streptozotocin or streptozocin (STZ) is a naturally occurring glucosamine-nitrosourea compound obtained from strain of the soil microbe *Streptomyces achromogenus*. STZ is particularly toxic to the β cells of Islet of Langerhans of the pancreas and used to induce hyperglycemia an animal model. The above study confirms that the ethanol extract of leaves of *C. montana* has antidiabetic activity against STZ induced diabetic rats. The insulin secretion may be increased by pancreatic β-cell after the administration of extract same like glibenclamide antidiabetic effect. Glibenclamide is a known oral hypoglycemic drug (sulfonyl urea), which is effective in moderate diabetic state but ineffective when pancreatic β-cells completely destroyed in severe diabetic condition (Tatiya et al. 2010). In case of STZ induced diabetic rats, triglycerides, cholesterol, low density lipoprotein (LDL) are increased and high density lipoprotein (HDL) decreased, these parameters were reversed in *C. montana* treated rats (Okoli et al. 2010). The elevation of AST, ALT and ALP enzymes in diabetic rats indicates the hepatic damage (Rathod et al. 2009). These elevated enzymes were significantly decrease after the administration of *C. montana* extract, which showed its ability to restore the normal functional status of the damaged liver. After the induction of diabetes by STZ renal function parameter such as serum creatinine, urea and uric acid increased, which indicates the dysfunction of kidney, these parameters were inhibited by the treatment with *C. montana* extract (Kakadiya et al. 2010a; 2010b). Our study also shows significant increase in body weight in comparison of nontreated group. The above results suggest that *C. montana* not only control the blood glucose level but also improve the parameters like body weight and lipid profile, so may be of useful for the treatment of diabetes. Further studies are required to isolate the chemical constituents who are responsible for anti-diabetic activity and their mechanism of action.

Conclusion

In conclusion, ethanol extract of *C. montana* leaves was assessed for anti-diabetic potential (subacute) and acute toxicity test. The current results indicate that *Clematis montana* leaves have significant anti-diabetic potential in contradiction of diabetic rats. Further feature studies are essential to determine clinical usefulness of *C. montana*.

Conflict of interest statement

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We declare that we have no conflict of interest.

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