

Original Research Article

Pharmacological evaluation of *Typha domingensis* for its potentials against diet-induced hyperlipidemia and associated complications

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Sent for review: 15 December 2021

Revised accepted: 25 February 2022

Abstract

Purpose: To evaluate the hyperlipidemic potential of the methanolic-aqueous extract of *Typha domingensis* Pers.

Method: Hyperlipidemia was induced in Wistar rats with high fat diet (HFD). The animals were divided into five groups; normal control group was administered normal diet and water *ad libitum*; whereas, all other groups were given HFD along with respective treatment; i.e., positive control group (normal saline, 1 ml/kg; p.o.), standard control group (atorvastatin 5 mg/kg; p.o.) and treatment groups received 70 % methanolic-aqueous extract of *Typha domingensis* (TD.Cr) at the 100 and 300 mg/kg p.o. After 28 days, the animals were weighed, blood was collected and sera separated. Aortas were dissected out for histological studies. Sera were analyzed for total cholesterol, triglycerides, HDL and LDL. Hypotensive effects were evaluated through invasive technique followed by diuretic activity.

Results: *Typha domingensis* extract significantly lowered the levels of total cholesterol, triglycerides, and LDL to 132.30 ± 1.145 , 161.50 ± 1.33 and 41.67 ± 1.28 mg/dL, respectively, at 100 mg/kg ($p < 0.001$) and 111.50 ± 1.05 , 157.70 ± 1.74 and 29.17 ± 0.98 mg/dL, at 300 mg/kg, respectively ($p < 0.001$). It produced anti-obesity effects represented as reduction in body weight by 17.00 ± 1.29 to 1.5 ± 7.63 g ($p < 0.001$) from 100 to 300 mg/kg, respectively, compared to control. Histological studies revealed the anti-atherosclerotic effect of the plant extract. A decreasing mean arterial blood pressure revealed hypotensive effects.

Conclusion: *Typha domingensis* has a potential for the treatment of diet-induced hyperlipidemia and associated complications. However, clinical investigations are required to buttress these assertions

Keywords: *Typha domingensis*, High fat diet, Total cholesterol, HDL and LDL

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INTRODUCTION

Hyperlipoproteinemia or hyperlipidemia are metabolic disorders which involve elevations of any of the lipoproteins resulting from

abnormalities in plasma lipid transport, synthesis and degradation of plasma lipoproteins and lipid metabolism [1]. Hyperlipidemia is considered as one of the greatest risk factors for the prevalence of coronary heart diseases. It has been studied

that in majority of population, excess weight, usually due to hyperlipidemia leads to cardiovascular diseases, hypertension, stroke and type II diabetes mellitus etc. Lowering of plasma cholesterol, especially LDL-Ch fractions, may prevent cardiac arrest and reverse coronary atherosclerosis. The goal for management of hyperlipidemia is to reduce the development of ischemic heart disease, cardiovascular and cerebrovascular complications [2].

The scientific and commercial interest in medicinal plants has increased globally, mainly due to their widespread acceptability, immense potential and economy. Due to the increased risk of adverse effects compared to beneficial effects of commercially available synthetic drugs, people are attracted towards natural remedies comprising mostly medicines from plants sources [3].

Typha domingensis Pers. (family Typhaceae) is a tall submerged rhizome with pollen barb tip commonly known as "Cat Tail". It is commonly found in wetland of shallow water, forms dense rhizomes near ponds, canals and river banks. Different species of genus *Typha* are also widely distributed in different forest regions of Pakistan near wetland and *domingensis* species also grows in forest of Lal Sohanra, Bahawalpur [4]. *T. domingensis* has been reported to be used traditionally for wound healing, neuroprotection, anxiety, depression and bleeding disorders like hematuria, hematemesis, dysmenorrheal bleeding, as diuretic, astringent and in food industry [5,6]. The study was designed to evaluate *Typha domingensis* for its potential against diet-induced hyperlipidemia and associated complications.

EXPERIMENTAL

Chemicals, drugs and assay kits

All the chemicals and kits used in this research were purchased from Merck, Germany, which were of the analytical grade. The drugs were obtained from other sources such as: atorvastatin (Pfizer, USA), diazepam (Roche Pharmaceuticals, Germany), furosemide (Sanofi Aventis Pharmaceuticals, Germany) and ketamine (Abbott Laboratories, USA) and Human assay kits (Human Gesellschaft fur Biochemica und Diagnostica mbH Wiesbaden, Germany).

Animals

Wistar albino rats either sex (180 – 250 g) were kept in poly-carbonated cages (47 × 34 × 18 cm³) and maintained at 23 ± 2 °C with 12 h light

and dark cycles at the animal house of Pharmacology Research Lab, Department of Pharmacology, Faculty of Pharmacy, the Islamia University of Bahawalpur (IUB) according to the guidelines of National Institute of Health, Islamabad. The study design was approved by the Pharmacy Animal Ethical Committee (PAEC), Faculty of Pharmacy, IUB (approval no. PAEC/2020/25).

Plant material

Aerial parts of *Typha domingensis* were collected from Lal Sohanra, an area of district Bahawalpur, Punjab, Pakistan. The material was identified by a taxonomist, Mr. Abdul Hameed, and a voucher specimen {IUB (TD-AP-01-20-158)} was submitted to the herbarium in Department of Pharmacology, Faculty of Pharmacy.

Preparation of crude extract

Aqueous methanolic extract of *T. domingensis* was prepared by macerating 1.7 kg dried material for three days, thrice in 70 % methanol aqueous solvent. After maceration, the macerate was filtered to obtain clear filtrate. The filtrate was concentrated using rotary evaporator (Heidolph, Germany) according to the standard procedure to obtain semisolid mass of the crude extract of *T. domingensis* (TD.Cr) with 11 % yield.

Phytochemical analysis

The TD.Cr was analyzed qualitatively for secondary metabolites like flavonoids, alkaloids, tannins, glycosides, polyphenols, coumarins, terpenes, saponins, proteins and carbohydrates etc. by phytochemical analysis [7].

HPLC analysis

HPLC was performed on Shimadzu LC10-AT VP Liquid Chromatography equipped with SIL-20A auto sampler (Shimadzu, Japan) and SPD-10AV UV VIS Detector. For separation, shim-pack CLC-ODS was used.

Evaluation of potential against diet-induced hyperlipidemia in rats

Normal diet and high fat diet

Normal diet (1 kg) was prepared by mixing 500 g of poultry feed, 350 g of choker and 150 g dry milk. Cholesterol (1 % solution) and cholic acid (0.5 %) were added in the normal diet for preparation of High Fat Diet (HFD).

Animal model

The animals were divided into different groups, each comprising of six animals. All the groups received tap water *ad libitum* throughout the study; i.e., 28 days (4 weeks). Normal control group was given normal feed and positive control group was fed on high fat diet (HFD). Both the groups were administered normal saline (1 ml/kg/day; p.o.), standard control group was given atorvastatin (5 mg/kg/day p.o.) and the treatment group TD.Cr at the doses of 100 and 300 mg/kg/day; p.o. along with HFD. After 28 days of treatment, all the animals were anaesthetized, blood samples were collected by cardiac puncture. The sera were separated by centrifugation at the speed of 5000 rpm for 15 min using a centrifuge machine. The sera from the experimental animals were used for biochemical assays for the determination of TC, TG, LDL and HDL.

Effects of TD.Cr on obesity

The change in body weight of rats during the 28 days was measured as an indicator of obesity as it is related with body fats. The change in body weight was determined by the difference in the body weights at zero day (W_0) and 28th day (W_e) using following formula:

$$\text{Change in Body weight (W)} = W_e - W_0 \text{ (gm)}$$

Effects of TD.Cr on atherosclerosis

To evaluate the anti-atherosclerogenic effects of TD.Cr, aorta of experimental animals were dissected out by sacrificing animals after the treatment for 28 days, fixed in 10 % formalin, paraffin embedded, serially cut with a microtome (5 μ m) and processed for hematoxylin and eosin staining. Histological studies of cross sections of aorta were made at 20 magnification using camera lucida drawings [8].

Evaluation of hypotensive potential of TD.Cr in normotensive rats

TD.Cr was evaluated for its hypotensive effects by using invasive technique. For dissection, animals were placed in supine position. To facilitate respiration, the trachea was cannulated with polyethylene tubing. The right jugular vein was cannulated for IV injection of drugs and TD.Cr solutions. The left carotid artery was also cannulated with cannula filled with heparinized saline (60 IU/mL) and was connected to a pressure transducer coupled with PowerLab 4/30 and LabChart Pro software (AD Instruments, Australia) for blood pressure (BP) and heart rate

(HR) recordings. To prevent blood clotting, Heparinized saline (0.1 mL) was injected to cannulated rat.

The hypotensive and hypertensive response was checked by the administration of acetylcholine (1 μ g/kg) and Nor-adrenaline (1 μ g/kg), separately, of each animal before administration of TD.Cr. After 15 - 20 min of equilibration, 0.1 mL of TD.Cr was injected intravenously at the doses of 1, 3, 10 and 30 mg/kg, followed by same volume of saline flush. MABP was calculated through Lab chart pro software using equation 1. The difference between the steady state values before the administration of dose and the lowest reading after administration of each dose of the test substance was measured as change in blood pressure [9].

$$\text{MABP} = (\text{DBP} + \text{SBP} - \text{DBP})/3 \dots\dots\dots (1)$$

Diuretic assay

Rats were randomly divided into different groups. The control group received normal saline (10 ml/kg, i.p.), furosemide (10 mg/kg, i.p.) was used as standard diuretic for comparison, and the animals of treatment groups were injected TD.Cr at the 100 and 300 mg/kg; i.p. doses. Animals were individually housed in the metabolic cages (Techniplast, Italy) immediately after dosing and the total urine volume was measured for 6 h. The urinary concentrations of sodium and potassium were measured through clinical flame photometer (Sherwood, UK) [10].

Acute toxicity study

Swiss albino mice were used for acute toxicity testing and followed the guideline of Organization for Co-operation and Development (OECD). Animals (18 – 30 g) were divided into different groups, each comprising of five mice and were fasted overnight and received only water *ad libitum*. Normal control group received normal saline (10 ml/kg; p.o.) and other groups received TD.Cr at the doses of 0.5, 1 and 3 g/kg; p.o. Normal diet was given to all groups during the observational period. Behavioral changes and response parameters; i.e. hyperactivity, alertness, grooming, rightening reflex, convulsions, sweating, urination, lacrimation, touch response, pain response, corneal reflex, eyes colour, gripping strength, tremors, change of skin and mortality were observed and recorded at 0.5, 1, 2, 4, 6, 12, 24, 48 and 72 h and then on the 7th and 14th day [11].

Statistical analysis

The results obtained for different parameters were interpreted as mean \pm SEM of each group. All the values obtained were statistically analyzed by using one way ANOVA followed by Tukey's test. The data was computed using GraphPad Prism version 8 and $p < 0.05$ was considered statistically significant.

RESULTS

Phytochemical content

The secondary metabolites in the TD.Cr were flavonoids, alkaloids, saponins, quinolines, coumarins, tannins, phenols, glycosides terpenes and terpenoids.

HPLC findings

The constituents found in TD. Cr include chlorogenic acid, caffeic acid, vanillic acid, p-coumaric acid, quercetin-rhamno-di-hexoside, syringic acid, hyperoside, naringenin, quercetin-3-O-glucopyranoside and trans-ferulic acid (Table 1, Figure 1 A). The chromatogram of TD.Cr was compared with that of standard (Figure 1 B).

Potential of TD.Cr against diet induced hyperlipidemia in rats

The values of serum total cholesterol (TC), triglycerides (TGs), low density lipoproteins (LDL), high density lipoproteins (HDL) and change in body weight after 28 days of experiment are presented in Table 2. The HFD profoundly increased the serum level of total cholesterol, triglycerides, LDL and also increased the weight of the animals. Atorvastatin reduced the levels of TC, TGs, LDL and body weight while HDL increased.

TD.Cr reduced the serum concentration of TC, TGs, LDL and inhibited increase in body weight in diet-induced hyperlipidemic rats at the tested doses of 100 and 300 mg/kg. The extract also exhibited highly significant decrease in total cholesterol ($p < 0.0001$) when compared to that of positive control group. It was also observed that reduction in the TC was dose dependent from 100 to 300 mg/kg dose with maximum effects at 300 mg/kg dose. The reduction in the serum triglycerides was also highly significant ($p < 0.0001$) when compared to the positive control. Both doses (100 and 300 mg/kg) produced similar decrease in the levels of TGs in rats.

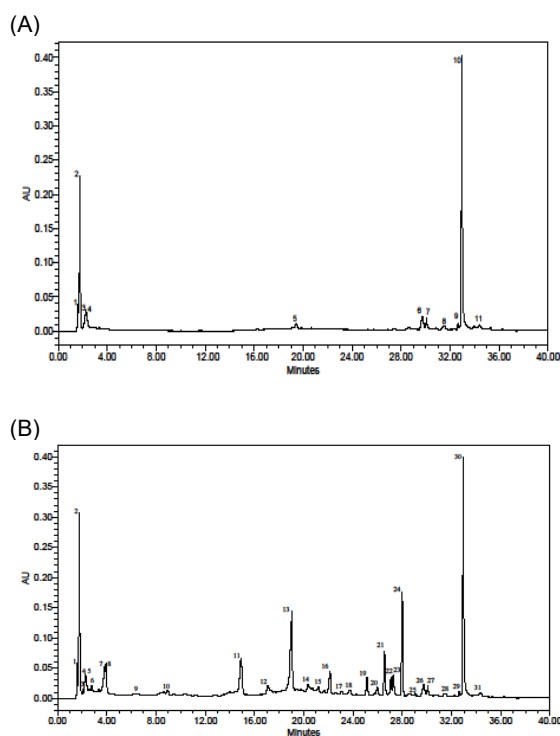


Figure 1: HPLC chromatograms of (A) TD.Cr (B) standard

Table 1: Phytochemical profile of 70 % methanolic-aqueous extract of *Typha domingensis*

Retention time (min)	Compound identified	Similarity of retention time with standard (%)
1.60	Chlorogenic acid	98
1.81	Caffeic acid	98
2.22	Vanillic acid	97
2.46	p-Coumaric acid	98
19.38	Quercetin	98
29.76	Quercetin-rhamno-di-hexoside	100
30.14	Syringic acid	100
31.44	Hyperoside	99
32.71	Naringenin	99
33.04	Quercetin-3-O-glucopyranoside	100
34.39	Trans-ferulic acid	100

Table 2: The serum levels of Total Cholesterol, Triglycerides, HDL, LDL and change in body weight of animal after 28 days of treatment

Group	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Change in body weight (g)
Normal Control (NS 1 ml/kg; p.o.) + ND	198.80±1.57	212.30±2.65	59.50±0.43	96.83±1.25	43.00±2.51
Positive Control (NS 1 ml/kg; p.o.) + HFD	279.80±1.57	258.50±1.17	52.67±1.28	175.20±3.71	68.50±1.19
Standard (atorvastatin 5 mg/kg) + HFD	188.80±2.56***	188.20±2.92***	59.33±0.42	91.83±1.70***	23.50±0.96***
TD.Cr (100 mg/kg TD.Cr) + HFD	132.30±1.145***	161.50±1.33***	59.67±0.42**	41.67±1.28***	17.00±1.29***
TD.Cr (300 mg/kg TD.Cr) + HFD	111.50±1.05***	157.70±1.74***	50.67±0.55	29.17±0.98***	1.5±7.63***

ND = Normal diet, HFD = High fat diet, NS = Normal saline, (***) if $p < 0.001$

The serum HDL levels increased at 100 mg/kg of TD.Cr and showed further significant increase ($p < 0.001$) as compared to positive control while TD.Cr did not produce significant ($p > 0.05$) effect at the dose of 300 mg/kg when compared to the positive control. The extract of *Typha domingensis* exhibited significant reduction in the serum LDL levels in the experimental rats at 100 and 300 mg/Kg. The results showed dose dependent decrease in the serum LDL levels. TD.Cr produced highly significant ($p < 0.0001$) reduction of serum LDL level at both doses when compared with positive control (Table 2).

Potential against atherosclerosis

Histological study of cross section of aorta (Figure 2) showed normal endothelial anatomy in normal control group (A); whereas, foam cells were seen in the positive control group (B) with erosion in endothelial membrane which was a sign of first stage atherosclerosis, the experimental groups receiving different doses of TD.Cr (D & E) showed normal endothelial structure. Therefore, the images showed the anti-atherosclerotic effects of TD.Cr at the given doses of 100 and 300 mg/kg.

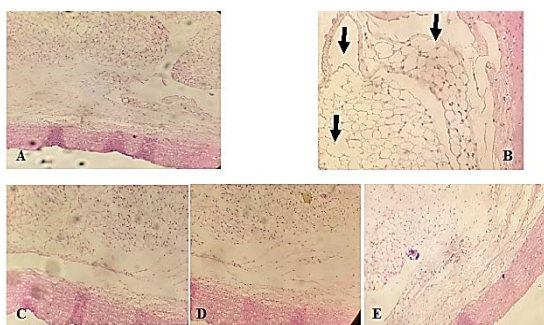


Figure 2: Histological images of aorta; (A) Normal control, (B) Positive control, (C) Standard control, (D) TD.Cr 100 mg/kg, (E) TD.Cr 300 mg/kg. Arrows indicate foam cells and erosion

Hypotensive potential in normotensive rats

The results showed that TD.Cr produced hypotensive effects. It reduced both systolic and

diastolic blood pressure. Mean arterial blood pressure was also decreased in experimental rats at all the given doses; 1, 3, 10 and 30 mg/kg. The heart rate was also decreased in rats. It was further evaluated for diuretic potential (Table 3, Figure 3).

Table 3: Hypotensive effects of 70 % methanolic-aqueous extract of *Typha domingensis*

Observation (Dose)	MABP (mmHg)	% Fall in MABP
Normal	129	-
Acetyl choline (1µg/kg)	65	50
TD.Cr (1mg/kg)	96	25
TD.Cr (3mg/kg)	80	38
TD.Cr (10mg/kg)	71	45
TD.Cr (30mg/kg)	66	49

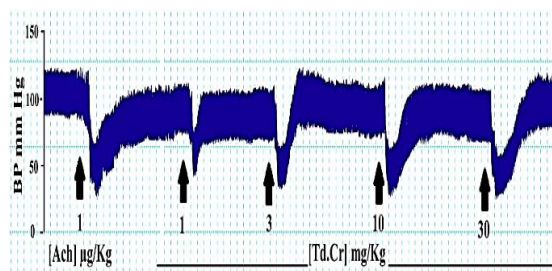


Figure 3: A tracing representing the effects of Acetylcholine (1µg/Kg) and different doses of TD.Cr on blood pressure (BP) of anesthetized rat. Arrows indicate the time of administration

Diuretic potential of TD.Cr

The urine volume of normal control per 100 g of rat in 6 h was compared with that of different doses of TD.Cr and furosemide (10 mg/kg). The standard control (furosemide 10 mg/kg) showed significant increase ($p < 0.001$) in urine volume and output of electrolytes. TD.Cr significantly increased urine volume and Na^+ and K^+ excretion in urine. It was also observed that the effects of TD.Cr at 100 and 300 mg/kg were less than that of furosemide. Na^+ and K^+ concentration showed natriuretic effects (Table 4).

Table 4: Diuretic effects of 70 % methanolic aqueous extract of *Typha domingensis*

Group	Urine volume ml/100g/6h	Diuretic index	Na ⁺ conc (mmol/L)	K ⁺ Conc (mmol/L)
Control (NS 10 ml/kg; i.p.)	1.10±0.138	-	50.10±3.47	12.0±0.74
Standard (Furosemide 10 mg/kg; i.p.)	4.31±0.056	3.9	108.20±4.41	26.27±2.61
TD.Cr 100 mg/kg; i.p.	2.51±0.090	2.3	51.43±4.83	15.97±1.52
TD.Cr 300mg/kg; i.p.	2.80±0.21	2.5	70.32±2.76	15.28±1.32

Acute toxicity

TD.Cr was safe at doses up to 3 g/kg after oral administration and did not show any sign of toxicity.

DISCUSSION

The results show that *Typha domingensis* possess antihyperlipidemic effects and also reduces the incidence of associated complications like obesity, atherosclerosis and hypertension. The results show that the plant extract reduced the levels of serum total cholesterol, triglyceride and LDL in a dose dependent manner. The reduction in the rate of increased plasma lipids is clinically beneficial for controlling cardiovascular events and mortality. The prevalence of coronary heart diseases increases in patients with raised levels of triglycerides.

Even among patients with normal levels of total cholesterol, cardiovascular incidences are reported to be increased with raised levels of LDL-cholesterol and triglycerides [12]. In diabetic patients, high levels of LDL-cholesterol is linked with the precipitation of cardiovascular complications and the reduction in LDL-cholesterol markedly reported to decrease cardiovascular complication [13]. The histological studies show that *T. domingensis* produced anti-atherosclerotic effects which were evident from the decrease in the levels of LDL and triglycerides, which are the main causes of atherosclerosis. *Typha domingensis* also caused hypotensive effects. One of the explored mechanisms being diuretic potential, however other mechanisms may also be involved thereby contributing to its hypotensive effects.

The pharmacological effects of *Typha domingensis* could be due to the presence of identified polyphenols, flavonoids and glycosides such as chlorogenic acid, ferulic acid, hyperoside, naringenin, quercetin-rhamno-di-hexoside and quercetin-3-O-glucopyranoside. All these substances have scientifically been reported to possess potential therapeutic effects [14,15]. For example, green coffee rich in chlorogenic acid has been reported to reduce the risk of cardiovascular diseases. Chlorogenic acid

also increased the activity of hepatic lipases in the liver which play a key role in lipid metabolism through the activity of peroxisome proliferator activated receptors PPARs *in vivo* in rats [14,15]. Ferulic acid has been reported to produce free radical scavenging (antioxidant) properties and hypotensive effects in spontaneously hypertensive rats which was associated with nitric oxide mediated vasodilation [16]. Naringenin has been reported to exert modulation of signaling pathways related to fatty acids, lowering their accumulation in the liver thereby preventing the fatty liver [17]. Quercetin-rhamno-di-hexoside and quercetin-3-O-glucopyranoside have also been effective antioxidant agents [18].

CONCLUSION

The results show that *Typha domingensis* is effective in the treatment of diet-induced hyperlipidemia and associated complications like obesity, atherosclerosis and hypertension in rat models. Further studies will pave way for the development of new therapeutic agent(s) from *Typha domingensis* with lesser side effects.

DECLARATIONS

Acknowledgement

Authors acknowledge the contributions of Dr. Naveed Aslam, Dr Imran, Hafiz Muhammad Farhan Rasheed and Mr Moqheet Sumroo who provided research support.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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