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EFFECT OF *Trichosanthes dioica* ON OXIDATIVE STRESS AND CYP450 GENE EXPRESSION LEVELS IN EXPERIMENTALLY INDUCED DIABETIC RATS

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Abstract

The present study is a systematic and scientific evaluation of antioxidant potential of aqueous extract of *Trichosanthes dioica* fruits on diabetes induced oxidative stress of severely diabetic rats. Its effect on gene expression level of the tissue-specific cytochrome P450 (CYP) was also studied. The dose of 1000mg/kg bw of *T. dioica* extract was administered orally once a day for 28 days to severely diabetic rats. Various oxidative stress parameters were analyzed in selected tissues of control as well as treated diabetic rats. The elevated level of lipid peroxidation was decreased and the decreased activities of antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase were increased significantly ($p<0.05$) in treated rats. Diabetes induced increased expression levels of drug metabolizing gene viz CYP 450 were drastically inhibited after the treatment. Thus the inhibition of gene expression level can be correlated with the exogenous metabolism and hence it effectiveness.

Key words: *Trichosanthes dioica;* antioxidant; diabetes; CYP450.

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Abbreviations: **BGL**: Blood Glucose Level; **bw**: body weight; **CAT**: Catalase; **CDNB**: 1-Choloro-2,4-Di-Nitro Benzene; **cDNA**: Complementary DNA; **dl**: deciliter; **DPPH**: 1,1-Di Phenyl-2-Picrlyl Hydrazyl; **DNA**: De Oxy Ribo Nuclic Acid; **GSH**: Glutathione; **Hb**: Haemoglobin; **LPO**: Lipid Peroxide; **MDA**: Malon Di-Aldehyde ; **PPG**: Post-Prandial Glucose ; **SOD**: Super Oxide Dismutase; **STZ**: Streptozotocin.

INTRODUCTION

The oxidative stress is a crucial factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases like Alzheimer's and also in the ageing process (20). Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the search of novel antioxidants for protection against these diseases is warranted. The antioxidants may mediate their effect by directly reacting with Reactive Oxygen Species (ROS), quenching them or by chelating the catalytic metal ions (25). Several synthetic antioxidants, are commercially available but these are quite unsafe and their toxicity is a serious of health concern (15). Natural

antioxidants, especially polyphenolics, alkaloids, carotenoids, steroids and flavonoids etc, are safe and effective. Moreover, medicinal plants have been a rich source of antioxidant compounds and play an important role in drug discovery and development for the treatment of diabetes mellitus and its complications like oxidative stress etc.

Hence, in the present study antioxidant potential of fresh unripe fruits of *Trichosanthes dioica (T. dioica)* Roxb. (family: Cucurbitaceae), commonly known as 'Sespadula' in English and 'Parwal' in Hindi and widely grown in India, has been explored systematic and scientifically. Fruits of this plant are used as vegetable in Indian traditional food system from times immemorial. In addition to fruits, other parts such as leaves and tender shoots have also been used in the traditional system of medicine since ancient times. Fruits of *Trichosanthes dioica*, exhibit antihyperglycemic effect (23), therefore it could be helpful in managing the diabetes induced oxidative stress, as hyperglycemia effects cellular antioxidant defense system and damage cells and tissues.

Under normal conditions, the ROS generated in our system, are quenched by the antioxidants present in the body, and an equilibrium is maintained between the ROS generated and the antioxidants present. However, this equilibrium is held back, either due to overproduction of ROS or due to inadequate antioxidant defense or both, causing cellular oxidative stress.

In severe diabetic conditions ROS are formed disproportionately either by glucose oxidation causing cell damage (9) or by oxidative degeneration of glycated protein causing damage to tissues (2,12). Thus, the oxidative stress as one of the development of diabetic complications is related to the deposition of advanced glycation end-products (AGEs) in tissues (30) and blood vessels (18,2), producing free radicals, causing vessel lipid degradation and therefore accelerating atherogenesis in diabetic patients (18,2). Abnormally high levels of ROS associated with diabetes leads to increased lipid peroxidation, which enhances the formation of AGEs and thus, diminishes the enzymatic activity of antioxidant enzymes such as glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and non-enzymatic scavengers like reduced glutathione (GSH) (12).

Drugs, Synthetic or Natural, are mostly metabolized in liver by CYP enzyme and hence inhibition or induction of these enzymes, due to their gene expression modulation, can alter the plasma concentrations and effects of their substrate drugs. The cytochrome P450, CYP enzymes are heme-containing enzymes of which over 2700 members are known to exist. Oxidation is a common pathway of drugs metabolism by the CYP system (4). CYP enzymes are very important for the pharmaceutical industry as they are responsible for the breakdown of medications mostly by catalyzing oxidative reactions. CYPs most often catalyze the insertion of one of the atoms of molecular oxygen into the substrate being biotransformed, while the second atom of oxygen is reduced to water. Thus, modulation of xenobiotic metabolism is based on CYP gene expression regulation. Hence, the genes responsible for the synthesis of CYP 1A1, CYP 1A2, CYP 2E1, CYP4A3 enzymes were selected for the study of their expression in diabetic control and diabetic treated rats.

MATERIALS AND METHODS

Plant material & preparation of extract

Fresh unripe fruits (6 kg) of *T. dioica* were purchased from the local market of Allahabad (India) and authenticated by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, India. A voucher specimen (AA512) has been submitted. The fruits were cut into small pieces and shade dried. The dried pieces were mechanically crushed and extracted with distilled water using soxhlet at temperature (80 - 100 ^{0}C) up to 36 h. The extract was filtered and concentrated in rotatory evaporator at 35 ± 5 ^{0}C under reduced pressure, to obtain semisolid material, which was then lyophilized to get a powder (yield: 14.9 % w/w).

Experimental animals and Experimental Design

Experiments were performed in 6-8 week old, healthy, male albino Wistar rats, of body weight 150-200 g. Animals obtained from National Institute of Communicable Diseases (NICD) New Delhi, India, were housed under standard environmental conditions (25 ± 2º C temperature, 50 ± 5 % humidity with a 12 h each of dark and light cycle) and maintained with free access of water and a standard laboratory diet (carbohydrates; 30 %, proteins; 22 %, lipids; 12 % and vitamins; 3 %) *ad libitum*. The study was approved by the Institutional Ethical Committee. Diabetes was induced by a single intraperitonial injection of freshly prepared Streptozotocin (purchased from Sigma Aldrich Chem. Co. USA.), 55 mg/kg b.w. (26) in 0.1 m citrate buffer (pH 4.5), to a group of overnight fasted rats. After 2 hour of STZ administration 5 % glucose water was given for next 24 hours to prevent hypoglycemia and then after 3 days rats

with marked hyperglycemia FBG > 250mgdl^{-1} and PPG > 350mgdl^{-1} were used in the study.

The dose of *T. dioica* extract, 1000 mg/kg b.w, was identified as the most effective dose in previous study (24) and therefore, this dose was selected for the present study in order to evaluate the antioxidant potential of *Trichosanthes dioica* fruits extract in the experimentally-induced diabetic rats. The animals were randomly divided into three groups, of six rats each. Group I (normal control) and group II (diabetic control) were treated orally with vehicle (distilled water only). Animals of group III (diabetic treated) were administered *T. dioica* 1000 mg/kg b.w. suspended in distilled water orally once a day. After four weeks of treatment, the rats were deprived of food overnight and sacrificed by cervical dislocation. The vital organs brain, liver, pancreas, kidney and spleen were surgically removed, washed immediately with ice cold saline, dried using filter paper and used fresh as well as stored at –80ºC for further studies.

Biochemical Estimations

The excised rat tissues were rinsed in isotonic ice-cold NaCl (0.9%, w/v) solution, blotted dry and weighed. A 10% (w/v) homogenate of each tissue was prepared in phosphate buffer (100mM, pH 7.4) containing 150mM KCl and centrifuged at 9,000×g for 30min at 4 ^{0}C. The pellet was discarded and the cell-free supernatant was used for estimation of biochemical parameters.

The activity of SOD (E.C.1.15.1.1) was assayed, by spectrophotometric measurement of pyrogallol auto oxidation at 412nm for 3min with and without the enzyme protein (16). One unit of the enzyme activity was expressed as 50% inhibition of auto oxidation of pyrogallol per minute.

The activity of CAT (E.C. 1.11.1.6) was assayed by change in absorbance which was recorded spectrophotometrically at 240 nm for 3min (1). One unit of catalase activity was defined as micromoles of H_2O_2 decomposed per min using molar extinction coefficient of H_2O_2 (43.6 M^{-1} cm^{-1}).

The activity of GPx (E.C. 1.11.1.9) was assayed and expressed as µg of GSH consumed/min/mg protein (26). The extinction coefficient of yellow anion (1.36x10^4 $M^{-1}cm^{-1}$) was used for calculation of activity (5).

The activity of GST (E.C. 2.5.1.18) was assayed and results were expressed as nanomoles of GSH–CDNB (1-Choloro-2,4-di-nitrobenzene) conjugate formed per minute per milligram protein using molar extinction coefficient of conjugate (9.6 ×10^6 M^{-1} cm^{-1}) (29).

Total reduced glutathione GSH (E.C.1.6.4.2) was measured by the use of DTNB assay using Elman's method wherein the incubation mixture at 37°C contained 0.08M sodium phosphate (pH 7.0), 0.08M EDTA, 1.0mM sodium azide, 0.4nM GSH and 0.25mM H_2O_2. GSH was determined at 3-min intervals using DTNB (5).

Lipid peroxidation (LPO) was measured (19) in rat brain and hepatic tissue homogenates by colorimetric estimation of malondialdehyde (MDA) formed. The results were expressed as nmol MDA/mg protein using the extinction coefficient of 1.56x10^5 $M^{-1}cm^{-1}$.

The protein contents in different tissue extracts were determined, using bovine serum albumin (BSA) as a standard (14).

RNA isolation

Total RNA was extracted from liver following manufacturer's protocol using Trizol reagent, a monophasic solution of phenol and guanidium isothiocyanate, (Life Technologies). The protocol used is an improvement to the single-step RNA isolation method (3). Tissue (50 µg) was homogenized in 1.0 ml of Trizol reagent and vortexed briefly after homogenization. Chloroform (200 µl) was added to it and incubated at room temperature for 10 minutes, allowing the separation of aqueous and organic phases. The contents were centrifuged at 8,000×g for 15 minutes, the supernatant was taken carefully and isopropanol (500 µl) was added to the supernatant, vortexed and incubated at room temperature for 10 minutes. It was then again centrifuged at 12,000×g for 10 minutes. The pellet obtained was then washed with 75% ethanol and centrifuged at 7,500×g for 8 minutes. The semi air dried pellet was dissolved finally in diethyl pyrocarbonate (DEPC) treated water (100 µl) for RNA isolation from liver tissue. RNA was quantified in Nanodrop spectrophotometer.

Synthesis of cDNA

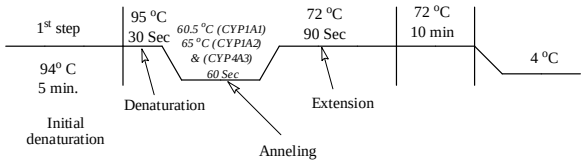
cDNA was synthesized from total RNA, isolated from the liver of diabetic control, as well as treated with *T. dioica* aqueous extract of fruits by the methodology followed by Johri *et al.* (10). RNA of each individual tissue was used for synthesizing complementary DNA (cDNA) by mixing RNA (3.0 µg) with oligo DT primer (1.0 µl) and finally making up its volume upto 12.0 µl by adding DEPC treated water. A quick spin was given to the mixture and was kept in Ependorff thermal cycler at 70°C temperature for 5 minutes (step 1). The mixture was taken out and kept on ice. 4.0 µl of 5x reaction buffer, 2.0 µl dNTP and 1.0 µl RNAse H were added and vortexed briefly, kept in thermal cycler at 37°C for 5 minutes (step 2), cooled on ice after incubation. Finally 1.0 µl reverse transcriptase was added, vortexed briefly and kept in thermal cycler at 42°C for 60 minutes and 70°C for next 10 minutes for final extention. The cDNA was taken out and kept at -20°C.

Amplification of CYP genes & cDNA

Table 1 shows the primer sequences used, already reported for different CYP 450 genes and their expected amplification sizes (8). The PCR reactions for CYP1A1, CYP1A2, CYP2E1 and CYP4A3 genes were carried out. These CYP genes were amplified in 25 µl reaction volume contained 1x PCR buffer, 0.2 m dNTP mix 0.3µM of CYP1A1 or CYP2E1or CYP4A3 primers or 0.4µM of CYP1A2 primer, 0.5 µl cDNA, and 1.5 U *Taq* DNA polymerase from MBI Fermentas. $MgCl_2$ at the final concentration of 3.0 mM was used for CYP1A1 while 1.5mM was used for CYP4A3and CYP2E1 and 1.0mM for CYP1A2. To ensure that PCR reactions were within the linear range, PCR reactions were initially standardized using different concentrations of cDNA and PCR cycles. Optimization of liver and brain of different concentrations of $MgCl_2$, dNTPs, cDNA, primers and Taq polymerase were carried out at a range of 27 to 40 cycles for all the CYPs. 30 cycles of PCR reaction was found most appropriate for all the PCR reactions.

Liver cDNA samples corresponding to 3 ng of total RNA were amplified in 25.0 µl reaction volume as described in an earlier study (10). The final PCR conditions used were initial denaturation at 94°C for 5 min. Then 30 cycles of denaturation at 95°C for 30 sec; annealing at 60.5°C for CYP1A1 and at 65°C for CYP1A2 and for CYP 4A3 for 1 min; and extension at 72°C for 1.5 min. One cycle of final extension at 72°C for 10 min was also used. For CYP2E1, no noticeable amplification was detected so it was

discarded. PCR products were analyzed by agarose gel electrophoresis and photographs were recorded using BIOVIS Gel documentation system Model. The cycles of PCR are given below.



Agarose gel electrophoresis for detection of amplified CYP genes

Agarose solution (1%) in 100ml TAE (tris acetic acid EDTA) (40 mM Tris base, 1 mM EDTA and 2.06% glacial acetic acid) was prepared, boiled, cooled to 55°C, 5.0 µl ethidium bromide stock (10 mg/ml) per 100 ml gel solution for a final concentration of 0.5 ug/ml. After the gel had been prepared, about 2.0 µl of DNA from each sample was mixed with a pinch of gel loading buffer (glycerol: distilled water, 1:1 and ~0.01 mg bromophenol blue), 1.0 µl λ DNA ladder was also added as a reference. Gel was run at 100 V current and when the "front wave" approaches the end of the gel, the current was stopped.

Statistical Analysis

Data were statistically evaluated using one-way ANOVA, followed by a post hoc Newman-Keuls Multiple Comparison Test. The values were expressed as mean ± SD and considered significant at ($p \leq 0.05$).

RESULTS

Effect of aqueous extract of T. dioica fruits on SOD activity

SOD activities (table 2) were found to be significantly high (P <0.001) in treated diabetic rats with aqueous extract of *T. dioica* fruits as compared to diabetic control rats. The SOD levels of diabetic rats treated with extract were increased by 37.3 (brain), 64.1 (liver), 65.5 (kidney), 36.0 (pancreas) and 6.3 % (spleen) compared with diabetic control rats.

Effect of aqueous extract of T. dioica fruits on CAT activity

CAT activities (table 2) were found to be significantly high (P <0.001) in treated diabetic rats with aqueous extract of *T. dioica* fruits as compared to diabetic control rats. The CAT levels of diabetic rats treated with extract were increased by 34.8 (brain), 62.8 (liver), 27.2 (kidney), 34.8 (pancreas) and 10.4 % (spleen) compared with diabetic control rats.

Effect of aqueous extract of T. dioica fruits on GPx activity

GPx activities (table 2) were found to be significantly high (P <0.05) in treated diabetic rats with aqueous extract of *T. dioica* fruits as compared to diabetic control rats. The GPx levels of diabetic rats treated with extract were increased by 66.9 (brain), 63.7 (liver), 29.2 (kidney), 59.5 (pancreas) and 40.4 % (spleen) compared with diabetic control rats.

Effect of aqueous extract of T. dioica fruits on GST activity

GST activities (table 2) were found to be significantly high (P <0.01) in treated diabetic rats with aqueous extract of *T. dioica* fruits as compared to diabetic control rats. The GST levels of diabetic rats treated with extract were increased by 60.0 (brain), 52.1 (liver), 53.6 (kidney), 24.0 (pancreas) and 29.1 % (spleen) compared with diabetic control rats.

Effect of aqueous extract of T. dioica fruits on GSH activity

There was a significant increase in the liver and brain GSH (P <0.001), while no significant increase was observed in the pancreas and spleen GSH

Effect of aqueous extract of T. dioica fruits on LPO levels

A significantly decreased (P <0.01) level of LPO (figure 1) was observed in diabetic treated rats when compared with diabetic control rats. The LPO levels of diabetic rats treated with extract were suppressed by 30.8 (brain), 33.3 (liver), 38.3 (kidney), 20.4 (pancreas) and 27.9 % (spleen) compared with diabetic control rats.

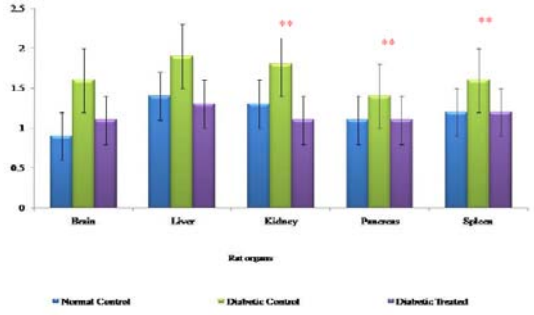


Figure 1. Lipid peroxidation (LPO) has been expressed in terms of nM of malondialdehyde (MDA) produced/ mg protein. Values are expressed as Mean ± SD; n=6, where n=number of determinations. The signs *** indicates, values significantly different from normal control at $p \leq 0.001$, ** denotes values significantly different from diabetic control at $p \leq 0.001$, whereas * shows values significantly different from normal control at $p \leq 0.01$.

Table 1. Primer sequences utilized for reverse transcriptase polymerase chain reaction (RT-PCR) analysis of Cytochrome P450 expression.

CYP450		Primer Sequences	Expected band size
CYP 1A1	F	GATGCTGAGGACCAGAAGACCGC	679 bp
	R	CAGGAGGCTGGACGAGAATGC	
CYP 1A2	F	CTTGGAGAAGCGTGGCCAGG	664 bp
	R	CTACAAAGACAACGGTGGTCT	
CYP 2E1	F	CTGCCCCCAGGACCTTTTCCC	847 bp
	R	ATCAGGAGCCCATATCTCAGAGTTGTGCTGGTGGTCTC	
CYP4A3	F	TCGAGGATCTAAACAACCTGAC	597 bp
	R	GGTTGTGATACCTTTGGGGTATGG	

Table 2. Effects of *T. Dioica* fruit extract treatment on the oxidative stress parameters in different tissues of rats.

Treatment groups	Treatment	Oxidative stress parameters in different tissues of rats				
		Liver	Kidney	Brain	Pancreas	Spleen
		Activity of Superoxide dismutase (SOD), IU/mg protein				
NC	DW	12.3 ± 2.5	11.4 ± 1.8	9.5 ± 2.6	4.3 ± 2.8	3.2 ± 1.9
NT	TE	12.9 ±3.1	10.9 ±2.7	10.4±2.5	4.5±1.1	3.4±0.9
DC	DW	4.2 ± 0.7	4.7 ±0.9	3.5 ± 1.2	1.7 ± 1.0	2.2 ± 0.8
DT	TE	6.8 ± 1.5**	5.9 ± 2.6*	4.7 ± 1.5**	2.5 ± 0.6	2.4 ± 0.5
		Activity of Catalase (CAT), IU/mg protein				
NC	DW	7.32 ± 2.2	6.41 ± 1.7	5.29 ±1.9	3.42 ± 1.1	2.56 ± 0.6
NT	TE	8.2 ± 2.6	7.12 ± 2.3	5.32 ± 1.4	3.67 ± 1.1	2.49 ± 0.7
DC	DW	4.2 ± 1.8	4.7 ± 1.3	3.5 ± 1.0	1.7 ± 0.5	2.2 ± 0.8
DT	TE	6.84 ± 2.5**	5.98± 2.2*	4.72 ± 1.2*	2.51 ± 0.4*	2.43 ± 1.2**
		Activity of Glutathione S transferase (GST), IU/mg protein				
NC	DW	1.52 ± 0.4	1.30 ± 0.8	0.91 ± 0.9	0.72 ± 0.4	0.59 ± 0.2
NT	TE	1.42 ±0.3	1.51 ±0.4	1.25 ±0.2	0.68 ±0.1	0.62 ±0.3
DC	DW	0.92 ± 0.5	0.41 ± 0.2	0.35 ± 0.2	0.50 ± 0.3	0.24 ± 0.1
DT	TE	1.40 ± 0.8***	0.63 ± 0.6**	0.56 ± 0.3*	0.62 ± 0.2	0.31± 0.2
		Activity of Glutathione peroxides (GPx), IU/mg protein				
NC	DW	9.31 ± 2.2	7.96 ± 1.5	6.53 ± 1.7	4.27 ± 1.9	3.52 ± 1.1
NT	TE	9.91 ± 2.4	7.62 ± 2.1	6.84 ± 1.9	4.31 ± 1.3	3.47 ± 1.1
DC	DW	5.40 ± 2.8	5.22 ± 2.2	3.60 ± 2.6	2.32 ± 0.9	2.11 ± 0.6
DT	TE	8.84 ± 2.5***	6.72 ± 1.9**	6.01 ±1.7*	3.67±1.6	2.95± 0.8
		Activity of Glutathione (GSH), nM of DTNB conjugated/mg protein				
NC	DW	12.43 ± 2.2	9.76 ± 1.5	8.43 ± 1.7	5.26 ± 1.9	5.78 ± 1.1
NT	TE	12.98 ± 2.4	9.98 ± 2.1	8.78 ± 1.9	5.89 ± 1.3	6.12 ± 1.1
DC	DW	7.52 ± 2.8	6.12 ± 2.2	4.36 ± 2.6	4.13 ± 0.9	3.14 ± 0.6
DT	TE	11.13 ± 2.5***	8.62 ± 1.9*	5.01 ±1.7*	4.97.67±1.6*	3.45± 0.8

The treatment groups were normal control (NC), normal treated (NT), Diabetic control (DC) and diabetic treated (DT). The treatment regimen and doses have been discussed in materials and method section. DW and TE denote distilled water and *T. Dioica* fruit extract respectively. The oxidative stress parameters were estimated in terms of activities of SOD, CAT, GST, GPX and lipid peroxidation were determined. One international unit (IU) of enzyme activity has been defined as fifty percent inhibition of pyrogallol auto oxidation per min. One international unit (IU) of catalase activity was defined as micromoles of H_2O_2 decomposed per minute**.** One international unit (IU) of GST activity was defined as expressed as nanomoles of GSH–CDNB conjugate formed per minute whereas, glutathione peroxidase activity was expressed as µg of GSH consumed/min/mg protein. Specific activity of enzymes is expressed as activity (IU)/mg protein. ***Indicates values significantly different from DC at $p \leq 0.001$, **means values are significantly different from DC at $p \leq 0.01$ and * indicates value significantly different from DC at $p \leq 0.05$.

Table 3. Effect of most effective dose of *Trichosanthes dioica* fruit aqueous extract on BGL of severely diabetic rats (mean ± S.D.).

Experimental animals	Treatment (Aq. extract)	Pre-treatment levels	Post-treatment levels			
			7 days	14 days	21 days	28 days
FBG (mg/dl)						
Normal (control)	D W	82.2 ± 3.5	85.6 ± 2.8	84.6 ± 4.6	83.9 ± 4.8	85.2 ± 3.9
SD (control)	D W	287.7 ± 5.7	290.9 ± 3.4	302.4 ± 4.6	299.9 ± 4.9	298.7 ± 4.9
SD (treated)	1000 mg/kg	298.2 ± 7.5	274.3 ± 4.6*	256.5 ± 4.5**	224.41± 4.9	212.3 ± 5.2
PPG (mg/dl)						
Normal (control)	D W	160.8 ± 5.2	162.8 ± 4.7	163.2 ± 4.9	162.1 ± 5.1	161.8 ± 4.1
SD (control)	D W	447.7 ± 4.8	456.1 ± 5.1	458.9± 3.6	452.4 ± 4.5	462.8 ± 3.8
SD (treated)	1000 mg/kg	438.2 ± 3.5	386.3 ± 5.2**	354.6 ± 4.1*	322.2 ± 4.3*	303.3 ± 4.5**

Effect on FBG and PPG of severely diabetic rats

Table 3 describes the antidiabetic effect of long term treatment of 4 weeks with the dose of 1000mg/kg of extract on blood glucose levels of severely diabetic rats. Rats were treated with the dose of 1000 mg/kg of aqueous extract once a day in noon for one month. At the end of the treatment, the animals were compared with their own initial values and showed a significant reduction of 28.7 % ($P<0.001$) in FBG and 30.7 % ($P<0.001$) in PPG levels.

Effect of aqueous extract of T. dioica fruits on CYP gene expression levels

Expression levels of drug metabolizing gene viz. CYP1A1, CYP1A2, CYP2E1 and CYP4A3 were studied in liver tissue of rats of both the groups, diabetic control and diabetic treated. It is worth pointing out that the genes CYP1A1 and CYP2E1 were found to have detectable levels of expression in liver whereas, other genes viz CYP1A2 and CYP4A3 were not having detectable change in their levels of expressions, suggesting thereby improvement in methodology adopted so that detectable levels of their transcripts could be amplified in order to come to a meaningful conclusion, However, gene expression levels of CYP1A1 and CYP2E1 were drastically inhibited (down regulated) in liver of rats treated with aqueous extract of *Trichosanthes dioica* for four weeks (figure 2).

DISCUSSION

The diabetes-inducing agents produces reactive free radicals, which have been shown to be cytotoxic to the β cells of the pancreas (7). During diabetes, elevated blood glucose level also enhances the production of oxygen-free radicals, due to autoxidation of glucose, causing cell damage (30). However, there is an evidence to suggest that the incidence of diabetes involves the formation of superoxide anion and hydroxyl radicals causing tissue injury as well (30). The deleterious effects of superoxide anion and hydroxyl radicals can be counteracted by antioxidant enzymes, such as SOD, CAT and GPx. In addition to these enzymes, glutathione reductase (GSH-R) provides glutathione in its reduced form (GSH) and glutathione *S*-transferase (GST) helps to neutralize toxic electrophiles, by conjugating them with GSH. LPO is also one of the features of chronic diabetes and lipid peroxide-mediated damage has been observed in both type I and type II diabetes mellitus. Depletion of tissue glutathione and increase in LPO have been observed in diabetes (17). NADPH dependent LPO also increases in diabetic patients. Administration of *T. dioica* aqueous extract significantly increased the activities of SOD, CAT, GPx, GSH and GST in diabetic models. The treatment tends to bring the LPO level to near-normal level.

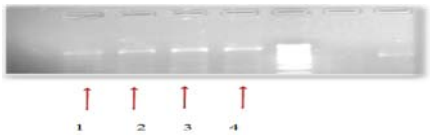


Figure 2. Gene expression of drug metabolizing CYP enzyme Where, 1= CYP1A1 inhibited expression of diabetic treated rats; 2= CYP2E1 inhibited expression of diabetic treated rats; 3 = CYP1A1 increased expression of diabetic control rats; 4= CYP2E1 increased expression of diabetic control rats.

The capacity of *Trichosanthes dioica* aqueous extract to significantly decrease the elevated blood glucose close to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. These findings coincide with those of the earlier studies which report the antidiabetic activity of the plant (21-22). One of the consequences of hyperglycemia is increased metabolism of glucose by sorbitol pathway. Besides this, other pathways, such as fatty acid and cholesterol biosynthesis also compete for NADPH with GSH. The decrease in GSH level in liver during diabetes is probably due to its increased utilization by the hepatic cells which could be the result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes (13). We have also observed the decrease in GSH in liver and kidney in diabetic control group. A marked improvement (48 %) in GST activity was observed in liver of treated diabetic rats.

In conclusion, the treatment of STZ induced diabetic rats with aqueous extract of *Trichosanthes dioica* for four weeks appears to mitigate the oxidative stress generated in different organs efficiently. The results from our study establish that brain and pancreas are the most responsive organs to treatment with *Trichosanthes dioica* aqueous extract followed by liver and kidney. The presence of flavonoids and vit C in the extract may be contributing either directly by scavenging the free radicals or through protection of activities of the antioxidant enzymes.

Moreover, the effect of antidiabetic *Trichosanthes dioica* fruit extract feeding, on the modulation of xenobiotic metabolism and oxidative stress in rats with diabetes was also studied. The long term effects of STZ induced diabetes in diabetic control group and on xenobiotic metabolism in extract treated group were studied on tissue specific cytochrome P450 (CYP) in liver tissue of rats. During diabetes an increased expression of CP1A1, CYP2E1 and CYP4A1 isozymes has already been seen by Western blot analysis (24). *Trichosanthes dioica* fruits extract feeding modulates the enzyme expression and catalytic activation in a tissue-specific and isozyme- specific manner. A marked improvement (52.1 %) in GST activity was observed in liver of treated diabetic rats. These results have suggested that the modulation of xenobiotic metabolism and oxidative stress in various tissues may be related to altered metabolism of endogenous substrates and hormonal status during diabetes. The findings may have significant implications in elucidating the therapeutic use of antidiabetic drugs and management of diabetes in severely diabetic patients. Thus, the effectiveness of the extract can be correlated with the inhibited (down regulated) gene expression levels of CYP isozymes, which also suggests that the drug (exogenous) metabolism will be slowed down and hence it will be retained for a longer period in blood and thus more effective.

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Other articles in this theme issue include references (31-46).

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