

ORIGINAL CONTRIBUTION

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Protective role of *Pandanus tectorius* Parkinson ex Du Roi in diabetes, hyperlipidemia, liver and kidney dysfunction in alloxan diabetic rats

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Abstract

Background: Diabetes mellitus (DM), an endocrine disease is characterized by increased level of blood glucose. Diabetes may affect other functions of the body and organs including, renal function and lipid metabolism. *Pandanus tectorius* (*P. tectorius*) Parkinson ex Du Roi is a small tree used in folk medicine in treatment of many diseases.

Methods: Diabetes in rats was induced by intraperitoneal injection of alloxan at a dose of 100 mg/kg body weight for 3 days. Water and ethanol extracts of root, stem and leave of *P. tectorius* were given to rats at 200 mg/kg body weight at day 3. Rats were sacrificed on day 4 after 12 h fasting. Serum glucose and other biochemical parameters like liver enzymes; [alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)], kidney parameters (urea & creatinine), lipid profile [total cholesterol, HDL and LDL cholesterol and triglycerides] and coronary artery risk index (CRI) were estimated in blood serum. Antioxidant potential, polyphenol content and phytochemical constituents in different parts of *P. tectorius* were also determined.

Results: Results showed that water extract of stem and ethanol extract of root of *P. tectorius* produced maximum fall in sugar level of alloxan diabetic rats. Extracts of all three plant parts significantly dropped the increased level of urea and creatinine with maximum reduction in creatinine was observed by water extract of root. *P. tectorius* extracts showed positive effect on liver enzymes and lipid profile by bringing them closer to normal range in comparison to alloxan diabetic control rats. Phytochemical screening indicated presence of flavonoids, alkaloids, tannins and saponin in *P. tectorius*. In DPPH free radical scavenging test the highest antioxidant potential was found in water extract of roots, while ethanol extract of stem also showed good activity.

Conclusion: The study demonstrated that different parts of *P. tectorius* have potential to attenuate diabetes and diabetes related complications like, liver, kidney dysfunction and lipid metabolism.

Keywords: *Pandanus tectorius* Parkinson ex Du Roi, Alloxan, Diabetes mellitus, Enzymes, Nephrotoxicity, Lipid profile

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Introduction

Diabetes mellitus (DM) type 2, a metabolic disorder is characterized by the abnormal increase of sugar (glucose) in blood due to insufficient production of insulin by pancreas [1]. Hyperglycemia may affect other body functions and organs, especially kidney, heart, eyes, veins and nerves [2]. Pathogenesis of diabetes mellitus is directly proportional to the decreased antioxidant status of the body [3]. The free radicals (superoxide dismutase, hydrogen peroxide etc.) cause devastation of β cells of pancreas and at last diminished insulin production [4]. The disease may bring about weak digestion of glucose and different biomolecules like lipids, carbohydrates and proteins [5]. According to some estimations type 2 diabetes mellitus has an impact on 415 million people in 2015 and this will increase up to 642 million by 2040 [6]. Different drugs like; sulphonylureas, biguanides, thiazolidinediones and insulin are available to treat diabetes and reduce the risk of complications [7]. Despite remarkable development for treating diabetes by oral hypoglycemic drugs, investigations for new drugs continue, as the existing synthetic drugs have several safety issues [8].

Herbal medicines derived from plants have been utilized for the therapy of type 2 diabetes mellitus [9]. *Pandanus tectorius* Parkinson ex Du Roi is a little tree grown wild in littoral habitats all through the coast of South and Southeast Asia, Australia and Pacific islands of Oceania [10]. In folk medicine, different parts of this plant have been used for the treatment of bronchitis, leprosy, dermatitis, measles, urinary tract ailments and type 2 diabetes mellitus [11]. Its leaves have been used for alleviating hepatitis, asthma, cold and cancer, where its roots were found helpful in managing digestive and respiratory disorders [12]. Ten phenolic compounds isolated from *P. tectorius*, out of which vanillin showed strong antioxidant activity [13]. Various medicinal properties of *P. tectorius* have been reported but, scientific evaluation of these properties received little attention. The present research describes the anti-diabetic, nephron-protective and hypolipidemic activity of water and ethanol extracts of various parts of *P. tectorius* in alloxan diabetic rats.

Materials and methods

Chemicals and reagents

Solvent (ethanol) of analytical grades was purchased from Merck (France). Alloxan, DPPH and Tris (hydroxymethyl) aminomethane were purchased from Sigma Aldrich, U.S.A. All the kits including ALAT, AST, ALP, LDH, urea, creatinine glucose, triglycerides, cholesterol, HDL cholesterol, LDL cholesterol were purchased from Merck (France) and Ecoline (Germany).

Collection of plant material

Pandanus tectorius Parkinson ex Du Roi was collected from Bali Bagh, Malir, Karachi. Fresh plant part of *P. tectorius* (root, stem and leaf) were cleaned with the tap water and stored at -20°C until used. Herbarium sheet was kept in the Department of Botany, University of Karachi, bearing herbarium No. 14/2/2014, G.H: 86550.

Preparation of ethanol extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi

Different parts of *P. tectorius*, leaf, root and stem (500 g each) were cut into small pieces with a knife and then chopped in an electric grinder. The minced material of each part was soaked for 2 weeks in distilled ethanol (96%) and filtered over cotton wool. The filtrate was concentrated to a gummy mass on a rotary vacuum evaporator (Büchi R-200, New Castle, DE) that give the yield of 2.7% from stem, 9.5% from root and 7.5% from leaves respectively. Extracts were stored at a room temperature until used.

Preparation of water extract from different parts of *Pandanus tectorius* Parkinson ex Du Roi

The Leaves, root and stem (100 g each) of *P. tectorius* were cut separately and grinded, soaked in distilled water for 1 h and homogenized using a homogenizer (Polytron (Kinematica) PT-MR 2100), then filtered over through Whatman no. 1 filter paper. The filtrate was lyophilized on a freeze dryer (Eyela, FD-1) that gave the yield of 14.5% from stem, 11.6% from root and 2.6% from leaves and stored at -20°C until used.

Animals

Male (Wistar albino) rats, of body weight 150–200 g were bought from Dow University of Health Science, Karachi. Rats were then kept in cages under standard laboratory conditions ($23 \pm 2^{\circ}\text{C}$ and 12 h light/dark cycle) for 2 weeks prior to experimentation. All the animals were fed with a standard pellet diet and water (ad libitum).

Induction of diabetes mellitus

Diabetes in rats was produced by using method of Zhang et al. [14], where intraperitoneal injection of alloxan monohydrate (Sigma aldrich, St. Louis, MO) at 100 mg/kg BW in saline was given to rats for 3 days continuously. The sugar level was evaluated on the third day by using a glucometer (Gluco trend Roche). The level of blood sugar 250 mg/dl or above was considered as diabetic.

Effect of ethanol extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi in normal and alloxan diabetic rat's model

The rats were separated into 3 major groups, where the third group was further divided into 6 sub-groups with each consisting of 6 rats.

- *Normal control group*: This group of rats was fed on standard diet and water with no treatment.
- *Alloxan diabetic control group*: This group of rats was intraperitoneally injected with alloxan (100 mg/kg) for 3 days.
- *Ethanol extract of P.tectorius treated group rats*: This group of rats was further separated into 6 sub-groups:
 - *Ethyl alcohol extract of leaves of P.tectorius treated group*: This group of rats was orally dosed with ethanol extract of leaves of *P.tectorius* (200 mg/kg b.w.,) at day 3.
 - *Ethyl alcohol extract of stem of P.tectorius treated group*: This group of rats was orally dosed with ethanol extract of stem of *P.tectorius* (200 mg/kg b.w.,) at day 3.
 - *Ethyl alcohol extract of roots of P.tectorius treated group*: This group of rats was orally dosed with ethanol extract of roots of *P.tectorius* (200 mg/kg b.w.,) at day 3.
 - *Ethyl alcohol extract of leaves of P.tectorius + alloxan dosed group*: This group of rats were intraperitoneally injected with alloxan (100 mg/kg) for 3 days. On day 3 they were fed with ethanol extract of leaves of *P.tectorius* (200 mg/kg b.w.,).
 - *Ethyl alcohol extract of stem of P. tectorius + alloxan dosed group*: This group of rats were intraperitoneally injected with alloxan (100 mg/kg) for 3 days. On day 3 they were fed with ethanol extract from the stem of *P.tectorius* (200 mg/kg b.w.,).
 - *Ethyl alcohol extract of roots of P. tectorius + alloxan dosed group*: This group of rats were intraperitoneally injected with alloxan (100 mg/kg) for 3 days. At day 3 they were fed with ethanol extract of roots of *P. tectorius* (200 mg/kg b.w.).

On the 4th day, rats from each group were decapitated after 12 h fasting [14]. After collection of Blood, serum was obtained by centrifugation to do biochemical analysis.

Effect of water extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi in normal and alloxan diabetic rat's model

Details are same as described in the section of “Effect of ethanol extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi in normal and alloxan diabetic rats’

model”, except extract of water was used as an alternative of ethanol extracts of different parts of *P. tectorius*.

Assessment of anti-diabetic, nephron-protective and cardio-protective effect of *P. tectorius*

Anti-diabetic effect was evaluated by estimating blood glucose level; while nephro-protective effect was determined by using kidney profile (urea and creatinine); whereas cardio-protective effect was examined using hepatic enzyme including i) alanine aminotransferases (ALAT), ii) aspartate aminotransferases (AST), iii) alkaline phosphatase (ALP), iv) lactate dehydrogenase (LDH). Lipid profile i) total cholesterol, ii) triglycerides, iii) HDL cholesterol iv) LDL cholesterol was also determined and coronary artery risk index (CRI) and atherogenic index (AI) were calculated [15]. All estimations were performed on a blood chemistry analyzer {Micro lab 300 (Merck)} using kits (Merck / Ecoline).

Antioxidant activity of ethanol and water extracts of *P. tectorius*

Free radical capturing capacity of extracts of water and ethanol of different parts of *P. tectorius* were estimated using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay [16]. Where 200 μ L of each extract having 0.002, 0.05, and 0.01 mg/ml concentration was mixed with 800 μ l of 100 mM Tris-HCl buffer (pH 7.4). DPPH (30 μ M dissolved in DMSO), 1 mL was added to the mixture and vortex. The absorbance was recorded at 517 nm against blank by using UV-visible spectrophotometer. One ml ethanol in 1 ml DPPH was used as control. Below mentioned formula was used to calculate the activity.

$$(\%) \text{ Antioxidant activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Estimation the phytochemical constituents

For the determination of different phytochemicals in leaves, stem and roots of *P. tectorius* standard methods were used. Polyphenol were extracted from water and ethanol of different parts (root, stem, leaves) of *P. tectorius* by using the method of Jimenez-Escrig et al. [17] and estimated by Chandini et al. [18]. Alkaloids were estimated according to the method of Harborne [19]. Obdoni & Ochuko [20] method was used to estimate saponin. Total tannin was estimated by using the method of Julkunen-Tiitto [21]. Whereas, method of Chang et al. [22] was used for the determination of flavonoid.

Statistical analysis

To analyze the data “Analysis of Variance” (ANOVA) was used and means were compared at [23]. Duncan’s

Multiple Range Test was also performed and means \pm SD was calculated.

Results

Preventive effect of water extract of *P. tectorius* in diabetic and normal rats model

When alloxan was administered, significant increment ($P < 0.05$) of the glucose level in blood as compared to normal control rats was observed. Extracts of water of all three plant parts produced reduction in glucose level towards normal range like root (-61.6%) stem (-65.9%) and leaves (-39.3%) in comparison to diabetic control groups. Water extract of root (-21.68%) and stem (-20.97%) also showed reduction in glucose level in comparison with normal control (Table 1).

The effect of alloxan also resulted in a significant ($P < 0.05$) increase in level of kidney markers (creatinine and urea) when compared to normal control rats. A significant drop in urea and creatinine levels in the extracts of all three parts and remarkable reduction in creatinine was observed by root extract (-36.1%) when compared to diabetic control group. Water extract of leave and root extract did not show any effect on urea while creatinine concentration was reduced in root (-43.4%) and stem extracts (-71.3%) in comparison to normal control rats (Table 1).

Alloxan, besides affecting glucose metabolism it has also disturbed lipid profile and increase in total cholesterol, triglycerides and LDL-cholesterol with lowering in HDL-cholesterol in comparison to normal rat's model. In diabetic animals' administration of water extract of root, stem and leave decreased the alloxan-induced elevated concentration of triglyceride, total cholesterol, and LDL-cholesterol with increased in HDL-cholesterol towards normal range. Alloxan treatment increased the CRI and AI in diabetic control rats; however, treatment with water extract of root, stem and leave reduced the

indexes. Maximum reduction in CRI and AI was observed by water extract of stem (Table 2). Water extract from all three parts of plant positively impact lipid profile and reduced serum cholesterol, triglycerides (TG) and low-density lipoprotein cholesterol (LDL-c) with subsequent increased in high density lipoprotein cholesterol (HDL-c) except water extract of leave (Table 2).

Administration of alloxan also disturbed cardiac and liver enzymes and increased ALAT, ALP, ASAT, LDH as compared to normal control rats. Treatment with water extracts from root, stem and leave dropped the elevated level of all enzymes markedly. Highest lowering effect was produced by the water extract of stem, ALAT (-50.6%), ASAT (-28.8%), ALP (-53.4) and LDH (-20.7%) close to normal control rats (Table 3).

Preventive effect of ethanol extract of *P. tectorius* in diabetic and normal rats model

Treatment with ethanol extracts of root, stem and leaf showed marked reduction in glucose concentration respectively (-48.8%), (-31.33%) and (-16.4%) in alloxan diabetic rats as compared to diabetic alone groups. Whereas, ethanol extracts of root and stem also significantly reduced glucose in normal rats in comparison to normal control rats. Urea and creatinine concentrations were also significantly decreased by the all three ethanol extracts ranging from creatinine (-71.42% to -89.86%) and urea (-24.37% to 50.0%) in comparison to diabetic control groups. Significant decrease in creatinine and urea levels were also found by the ethanol extract of root when compared with normal control rats. Root extract was ranked top with respect to overall effect on glucose and kidney markers (Table 4).

Treatment with alloxan again resulted in significant hypercholesterolemia and hypertriglyceridemia with lowering of HDL-cholesterol in comparison to normal control animals. Treatment with ethanol extract from

Table 1 Effect of water extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi on blood glucose level and kidney markers in normal and diabetic rat's model

Treatments	Normal Rats Model			Diabetic Rats Model		
	Glucose mg/dl	Creatinine mg/dl	Urea mg/dl	Glucose mg/dl	Creatinine mg/dl	Urea mg/dl
Control	95.33 ^b \pm 2.51	0.23 ^a \pm 0.05	48 ^a \pm 2	391 ^a \pm 8.5	1.66 ^a \pm 0.11	139 ^a \pm 1
Water extract of root	74.66 ^c \pm 1.15 (-21.68%)	0.13 ^{ab} \pm 0.05 (-43.47%)	47.33 ^a \pm 1.52 (-1.45%)	150 ^c \pm 5 (-61.6%)	1.06 ^b \pm 0.05 (-36.14%)	38.33 ^d \pm 1.52 (-72.42%)
Water extract of stem	75.33 ^c \pm 0.57 (-20.97%)	0.16a ^b \pm 0.05 (-30.43%)	33 ^b \pm 1.73 (-31.25%)	133 ^d \pm 2 (-65.98%)	1.16 ^b \pm 0.15 (-30.12%)	41.66 ^{cd} \pm 4.72 (-70.02%)
Water extract of leave	101 ^a \pm 2.51 ($+5.9\%$)	0.23 ^a \pm 0.05 (0%)	47.66 ^a \pm 3.7 (-0.7%)	237 ^b \pm 5 (-39.38%)	1.23 ^b \pm 0.37 (-25.90%)	93.33 ^b \pm 1.52 (-32.85%)
LSD_{0.05}	6.12	0.11	4.57	9.2	0.36	5.03

Mean values in column bearing same superscript letters are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Mean values in columns showing differences greater than LSD values are significantly different at $p < 0.05$

Values are mean \pm Standard deviation with $n = 6$

Values in parenthesis are showing percent increased or decreased as compared to their respective control

Table 2 Effect of water extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi on lipid profile in normal and diabetic rat's model

Treatments	Lipid Profile									
	Normal Rats Model					Diabetic Rats Model				
	Total cholesterol mg/dl	Triglycerides mg/dl	HDL-cholesterol mg/dl	LDL-cholesterol mg/dl	AI	Total cholesterol mg/dl	Triglycerides mg/dl	HDL-cholesterol mg/dl	LDL-cholesterol mg/dl	AI
Control	95 ^a ± 2	137 ^a ± 2.6	36.6 ^c ± 0.57	30.9 ^a ± 1.1	2.53 ± 0.05 ^a	115 ^a ± 2.6	209 ^a ± 4	23.3 ^e ± 1.52	49.8 ^a ± 2.13	4.9 ± 0.2 ^a
Water extract of root	86.6 ^b ± 3.05 (- 8.7%)	110.6 ^b ± 1.52 (- 19.2%)	44 ^b ± 2 (+ 20%)	20.5 ^b ± 2.9 (- 33.5%)	1.93 ± 0.11 ^c	81.3 ^{bc} ± 4.04 (- 29.2%)	88 ^d ± 3 (- 57.8%)	49 ^b ± 1 (+ 110%)	14.7 ^d ± 3.91 (- 70.4%)	1. ± 0.1 ^d
Water extract of stem	75 ^c ± 2 (- 21%)	70.33 ^c ± 4.04 (- 48.6%)	50 ^a ± 5 (+ 36%)	10.9 ^c ± 2.8 (- 64.7%)	1.5 ± 0.1 ^d	78.3 ^c ± 4.72 (- 31.8%)	76 ^e ± 2.64 (- 63.6%)	54 ^d ± 2.64 (+ 131.4%)	10.4 ^d ± 3.0 (- 79%)	1.4 ± 0.1 ^d
Water extract of leave	97 ^a ± 3 (+ 2.1%)	115.3 ^b ± 2.30 (- 15.8%)	42.3 ^b ± 2.51 (+ 15.4%)	31.6 ^a ± 1.03 (2.2%)	2.2 ± 0.05 ^b	87 ^b ± 2 (- 24.3%)	121 ^b ± 4 (- 42.1%)	42 ^b ± 2 (+ 80%)	20 ^c ± 2.44 (- 59.6%)	1.9 ± 0.05 ^c
LSD_{0.05}	4.83	5.24	5.62	4.06	0.16	7.03	6.14	3.87	5.19	0.2

Mean values in column bearing same superscript letters are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test Mean values in column showing differences greater than LSD values are significantly different at $p < 0.05$ Values are mean ± Standard deviation with $n = 6$

Values in parenthesis are showing percent increased or decreased as compared to their respective control

Table 3 Effect of water extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi on liver and cardiac enzymes in normal and diabetic rat's model

Treatments	Enzymes							
	Normal Rats Model				Diabetic Rats Model			
	ALAT U/L	ASAT U/L	ALP U/L	LDH U/L	ALAT U/L	ASAT U/L	ALP U/L	LDH U/L
Control	35 ^a ± 2	144.3 ^a ± 4.72	22 ^a ± 2	48 ^a ± 2	45 ^a ± 5	130.66 ^a ± 6.65	138.3 ^a ± 4.16	282.66 ^a ± 1.52
Water extract of root	20 ^b ± 1 (-42.8%)	109.6 ^c ± 3.51 (-23.8%)	18.6 ^a ± 4.50 (-15%)	198.3 ^b ± 1.52 (-313.1%)	25.6 ^c ± 2.08 (+42.9%)	101 ^c ± 3.46 (-22.7%)	114.6 ^b ± 4.6 (-17.1%)	198 ^e ± 8 (-29.9%)
Water extract of stem	17 ^b ± 2 (-51.4%)	125 ^b ± 2.6 (-13.3%)	19.3 ^a ± 0.5 (-12.1%)	245 ^a ± 4 (+410.4%)	22.3 ^c ± 2.51 (-50.6%)	93 ^d ± 3 (-28.8%)	64.3 ^c ± 4.3 (-53.5%)	224 ^c ± 5.5 (-20.7%)
Water extract of leave	18.3 ^b ± 1.5 (-47.6%)	110 ^c ± 2 (-23.7%)	17.6 ^a ± 2.08 (-19.7%)	198.3 ^b ± 1.52 (+313.1%)	35 ^b ± 4.5 (-22.2%)	137.3 ^a ± 3.51 (-5.10%)	135.3 ^a ± 4.5 (-2.16%)	273.6 ^b ± 3 (-3.4%)
LSD_{0.05}	3.1	6.3	5.0	4.6	6.7	7.2	7.7	9.1

Mean values in columns bearing the same superscript letters are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Mean values in columns showing differences greater than LSD values are significantly different at $p < 0.05$

Values are mean ± Standard deviation with $n = 6$

Values in parenthesis are showing percent increased or decreased as compared to their respective control

different parts of the plant significantly decreased these values. Highest increase in HDL-cholesterol (+ 112.2%) and decreased in LDL-cholesterol (- 67.37%) was demonstrated by leaves extract. CRI and AI showed increases in both indexes when treated with alloxan, whereas the level was decreased by three ethanol extracts as compared to diabetic control rats. Whereas, normal control rats in all the three extracts, showed deduction in triglyceride, total cholesterol, and LDL-cholesterol with an increase in HDL-cholesterol (Table 5).

Treatment with ethanol extract of root, stem, and leave, also decreased elevated liver enzymes in alloxan diabetic rats. All the three extracts showed remarkable reduction in enzymes levels, ranging from ALP (- 55.8% to - 66.7%), ALAT (- 51.4 to 55.1%), ASAT (- 21.05% to - 52.3%) and LDH (- 19.2 to 51.4%) in comparison with diabetic control rats (Table 6). Ethanol extract of root

was also found to reduce ALAT (- 27.2%), ASAT (- 73.3%), ALP (- 21.0%) and LDH (- 41.8%) as compared to control rats (Table 6).

Determination of antioxidant activity in water and ethanol extracts of *P. tectorius*

In order to determine activity of antioxidants in water extract different concentrations 0.002, 0.01, and 0.05 mg/ml were used (Fig. 1). The effectivity was concentration dependent. The highest activity was found in root (34.2%) at 0.05 concentrations, which was followed by stem (27.4%) and leave (19.7%) at the same concentration level. Root extract at 0.05 mg/ml concentration showed activity (34.2%) slightly higher than the standard ascorbic acid (31.06%). Lowest activity was recorded by leaf extract.

Table 4 Effect of ethanol extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi on blood glucose level and kidney markers in normal and diabetic rat's model

Treatments	Normal Rats Model			Diabetic Rats Model		
	Glucose mg/dl	Creatinine mg/dl	Urea mg/dl	Glucose mg/dl	Creatinine mg/dl	Urea mg/dl
Control	97.3 ^a ± 1.5	1 ^a ± 0	47.6 ^a ± 4.7	250 ^a ± 6	1.26 ^a ± 0.2	105.3 ^a ± 3.7
Ethanol extract of root	66.6 ^b ± 2.5 (-31.5%)	0.83 ^b ± 0.1 (-17%)	25.6 ^c ± 3.5 (-46.1%)	128 ^d ± 2.8 (-48.8%)	0.13 ^c ± 0.05 (-89.8%)	52.6 ^c ± 2.5 (-50.0%)
Ethanol extract of stem	76 ^b ± 3 (-21.9%)	1.03 ^a ± 0.1 (+3%)	36.6 ^b ± 4.1 (-23.1%)	171.6 ^c ± 4.0 (-31.3%)	0.36 ^b ± 0.11 (-71.4%)	79.6 ^b ± 4.50 (-24.3%)
Ethanol extract of leave	95 ^c ± 6.2 (-2.3%)	1 ^a ± 0 (0%)	39.3 ^b ± 2.8 (-17.47%)	209 ^b ± 4.3 (-16.4%)	0.26 ^{bc} ± 0.05 (-79.36%)	58 ^c ± 4 (-44.9%)
LSD_{0.05}	7.0	0.1	7.3	7.4	0.2	6.4

Mean values in columns bearing the same superscript letters are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Mean values in column showing differences greater than LSD values are significantly different at $p < 0.05$

Values are mean ± Standard deviation with $n = 6$

Values in parenthesis are showing percent increased or decreased as compared to their respective control

Table 5 Effect of ethanol extract of different parts *Pandanus tectorius* Parkinson ex Du Roi of lipid profile in normal and diabetic rat's model

Treatments	Lipid Profile					
	Normal Rats Model			Diabetic Rats Model		
	Total cholesterol mg/dl	HDL-cholesterol mg/dl	LDL-cholesterol mg/dl	CRI	AI	
Control	78 ^a ± 2	13 ^c ± 1	35.9 ^a ± 2.5	6 ± 0.5 ^a	2.7 ± 0.2 ^a	116 ^a ± 2
Water extract of root	47.6 ^c ± 2.5 (-38.8%)	20 ^b ± 2 (+53.8%)	7 ^c ± 2.02 (-80.5%)	2.3 ± 0.2 ^b	0.3 ± 0.1 ^b	81 ^c ± 1 (-30.1%)
Water extract of stem	62.6 ^b ± 2.1 (-19.6%)	26 ^a ± 3 (+100%)	13.1 ^b ± 3.2 (-63.6%)	2.3 ± 0.2 ^b	0.4 ± 0.2 ^b	86.3 ^b ± 1.52 (-25.5%)
Water extract of leave	60.6 ^b ± 2.8 (-22.2%)	21.3 ^b ± 3.05 (+64%)	12.2 ^b ± 3.1 (-66%)	2.8 ± 0.4 ^b	0.5 ± 0.2 ^b	81.3 ^c ± 1.52 (-29.8%)
LSD_{0.05}	4.5	4.5	5.2	0.7	0.3	2.8
						8.1
						4.0
						5.5
						1.0
						0.7

Mean values in columns bearing the same superscript letters are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Mean values in column showing differences greater than LSD values are significantly different at $p < 0.05$

Values are mean ± Standard deviation with $n = 6$

Values in parenthesis are showing percent increased or decreased as compared to their respective control

Table 6 Effect of ethanol extract of different parts of *Pandanus tectorius* Parkinson ex Du Roi on liver and cardiac enzymes in normal and diabetic rat's model

Treatments	Enzymes							
	Normal Rats Model				Diabetic Rats Model			
	ALAT U/L	ASAT U/L	ALP U/L	LDH U/L	ALAT U/L	ASAT U/L	ALP U/L	LDH U/L
Control	29.3 ^a ± 4	95 ^a ± 4.5	31.6 ^a ± 2.88	232 ^a ± 4.58	45.3 ^a ± 5.03	133 ^a ± 1.52	94.3 ^a ± 5.8	345.3 ^a ± 3.5
Ethanol extract of root	21.3 ^b ± 1.52 (-27.2%)	25.3 ^d ± 1.52 (-73.3%)	25 ^{bc} ± 1 (-21%)	135 ^c ± 3 (-41.8%)	20.3 ^c ± 4.61 (-55.1%)	105 ^b ± 4.5 (-21%)	31.3 ^c ± 1.2 (-66.7%)	272 ^b ± 3 (-21.2%)
Ethanol extract of stem	14.6 ^c ± 0.57 (-50%)	58.3 ^c ± 8.02 (-38.6%)	21.6 ^c ± 1.52 (-31.5%)	220 ^b ± 8.3 (-5.17%)	21 ^c ± 2.6 (+53.6%)	87 ^c ± 4.5 (-34.5%)	41.6 ^b ± 4.50 (+55.8%)	167.6 ^d ± 8.5 (-51.4%)
Ethanol extract of leaf	29 ^a ± 4.5 (-1.12%)	82 ^b ± 5.29 (-13.6%)	29.3 ^{ab} ± 4.61 (-7.35%)	230 ^{ab} ± 4.2 (-0.86%)	22 ^c ± 3.6 (-51.4%)	63.3 ^d ± 1.52 (-52.3%)	32.6 ^c ± 2.51 (-65.37%)	279 ^b ± 6.8 (-19.2%)
LSD _{0.05}	5.	10.1	5.4	10.2	6.8	6.5	6.8	9.9

Mean values in column bearing same superscript letters are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Mean values in column showing differences greater than LSD values are significantly different at $p < 0.05$

Values are mean ± Standard deviation with $n = 6$

Values in parenthesis are showing percent increased or decreased as compared to their respective control

The antioxidant potential of root, stem and leaf in ethanol extract are given in Fig. 2. The activity was determined at the concentration of 0.002, 0.01 and 0.05 mg/ml. The scavenging activity was concentration dependent in all parts. Highest antioxidant potential was observed in stem (35.9%) at the concentration level of 0.05 followed by root (29.6%) and leaf (19.4%). The activity in the stem (35.9%) was higher than standard ascorbic acid (28.2%) at the concentration of 0.05 mg/ml.

Estimation of polyphenols

The polyphenol content in water and ethanol extract ranged from 2.2 mg% GA to 9.54 mg% GA. The highest polyphenol content was found in water extract of stem (9.54 ± .2 mg% GA). In ethanol extract, highest polyphenol

content was found in root (4.28 ± .1 mg% GA), while stem and leaf extracts showed low content (Fig. 3).

Estimation of flavonoids and tannin

To estimate Flavonoids ethanol and water extracts were used and results are expressed as rutin equivalents (RE)/ mg% of extract. The concentration of flavonoids in ethanol and water extracts is given in Fig. 4. The values in water extract ranged from 0.30 RE/ mg% of extract to 2.5 RE/mg % of extract. Ethanol extract showed many fold high value of flavonoids. The values ranged from 0.79 ± .01 RE/mg % of extract to 20.0 RE/ mg % of extract. Highest flavonoids were found in ethanol extract of leaves (20.0 ± 0.9 RE/ mg % of extract followed by root (10.6 ± 0.2 RE/ mg % of extract).

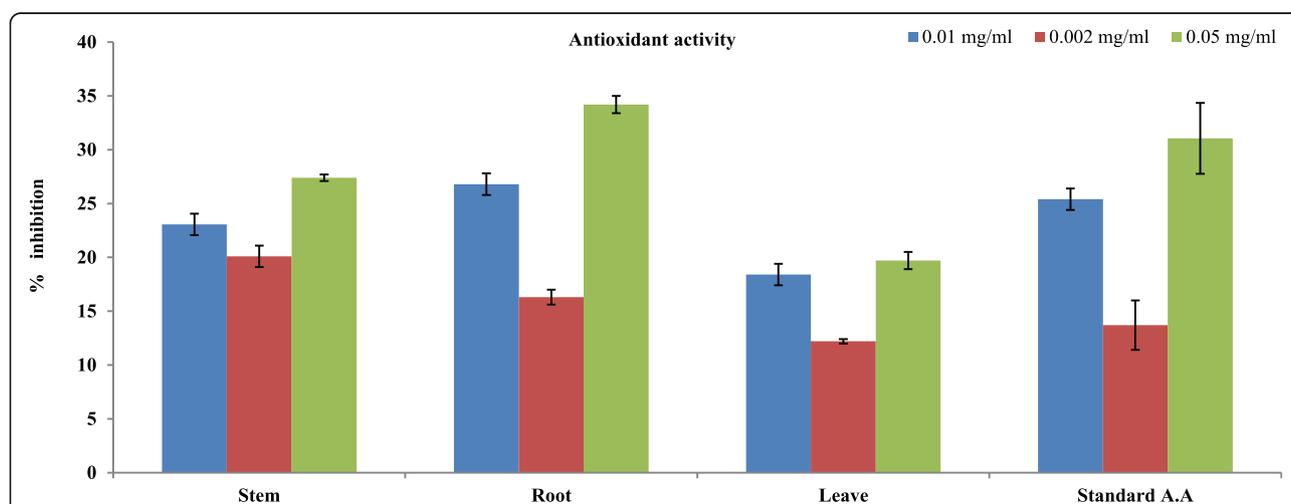
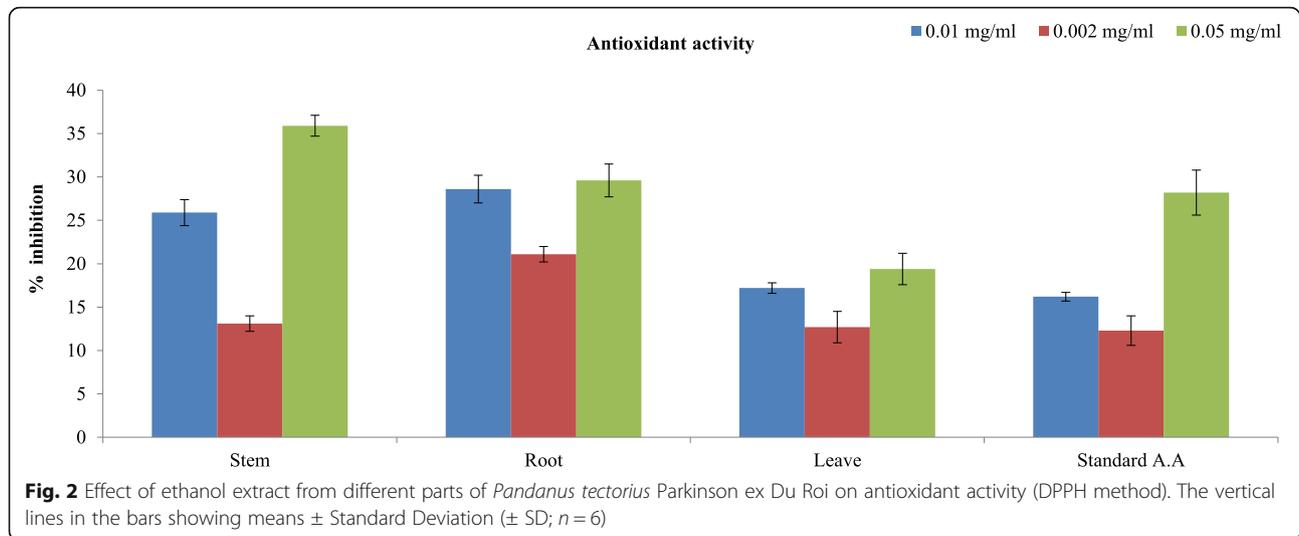


Fig. 1 Effect of water extract from different parts of *Pandanus tectorius* Parkinson ex Du Roi on antioxidant activity (DPPH method). The vertical lines in the bars showing means ± Standard Deviation (± SD; $n = 6$)



Tannin was determined in ethyl alcohol and water extracts of root, stem and leaf. Water extracts revealed high levels of tannins in terms of mg% (+)-catechin in all three parts. The level of tannin in water extract of root (46.2 ± 2.4 mg% (+)-catechin), stem (66.4 ± 0.2 mg% (+)-catechin), and leaf (41.7 ± 1.3 mg% (+)-catechin) was many fold higher than ethanol extract except leaf extract of ethanol (49.5 ± 2.1 mg% (+)-catechin) (Fig. 4).

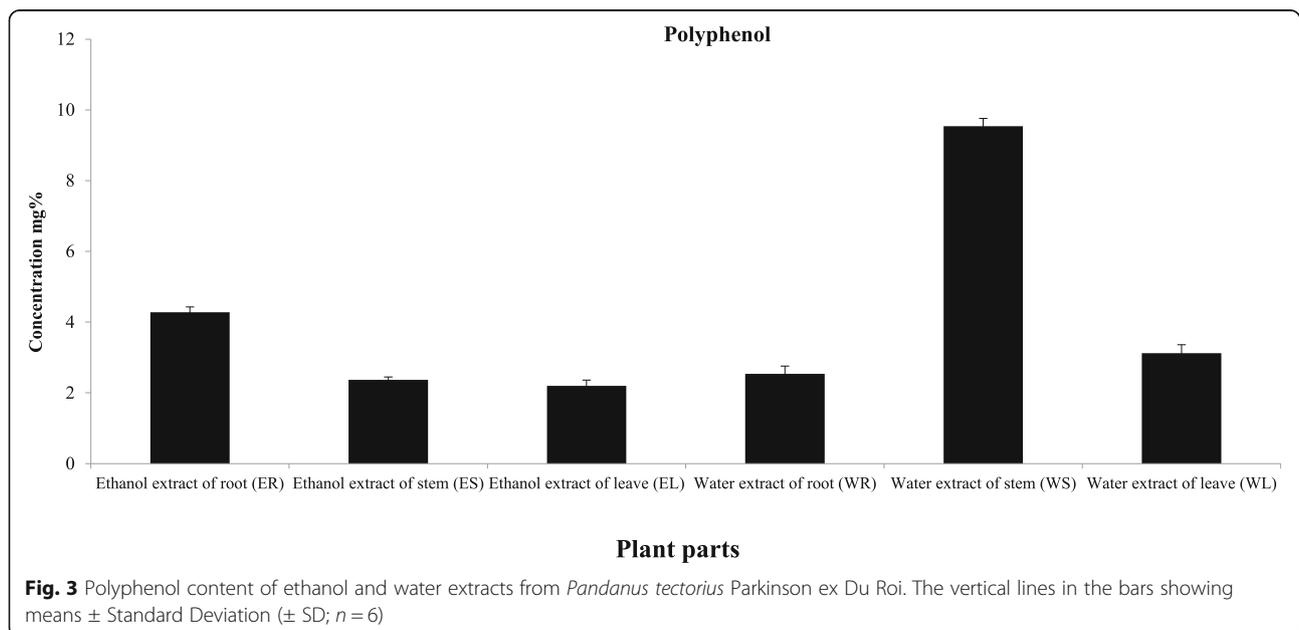
Estimation of alkaloid and saponin

The concentration of alkaloid and saponin in different parts of the plant are shown in Fig. 5. Highest alkaloid concentration was observed in leaf ($0.948 \pm .015\%$ w/w)

followed by stem ($0.766 \pm .01\%$ w/w) and root ($0.14 \pm 0.01\%$ w/w). Whereas, highest saponin level was observed in stem ($1.31 \pm 0.1\%$ w/w) followed by root ($0.99 \pm 0.06\%$ w/w) and leaves ($0.94 \pm 0.08\%$ w/w).

Discussion

In the present study root, stem and leaf of plant *Pandanus tectorius* Parkinson ex Du Roi were tested for their hypoglycemic potential in healthy and alloxan induced diabetic rats. The results showed that both water and ethanol extracts exhibited anti-hyperglycemia in normal rats, as well as in diabetic rat models. Medicinal plants have been reported to possess hypoglycemic



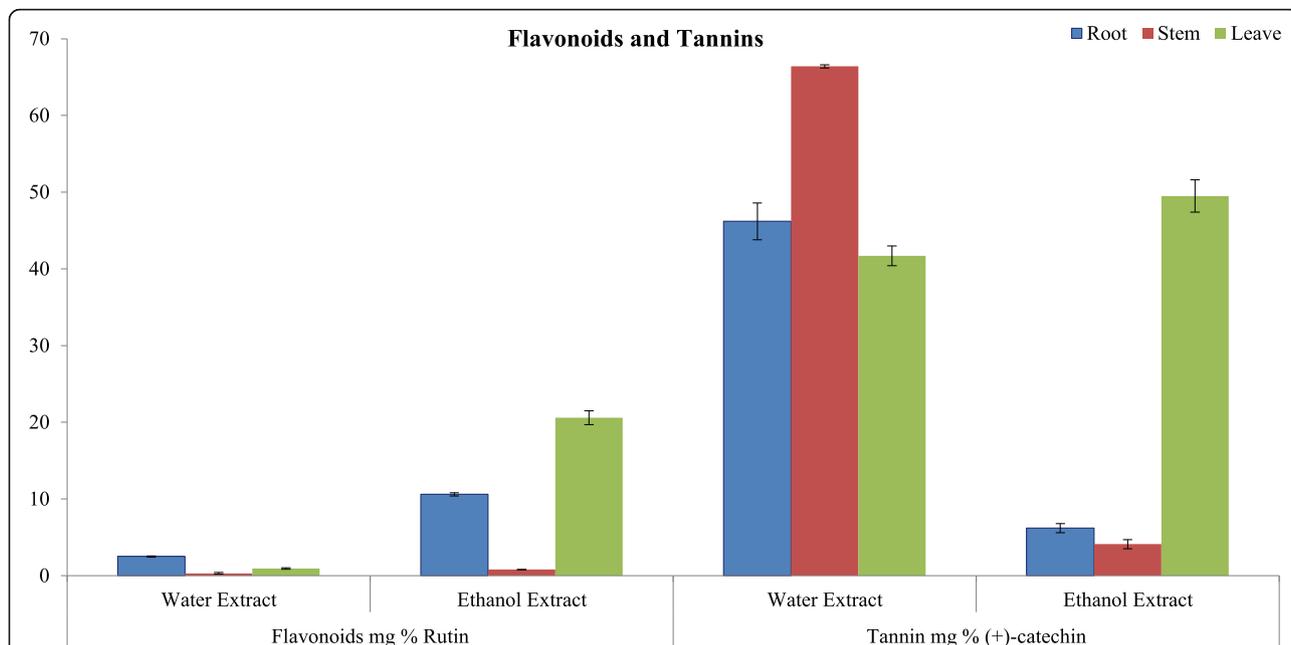


Fig. 4 Phytochemicals; flavonoids and tannin in different parts of *Pandanus tectorius* Parkinson ex Du Ro. The vertical lines in the bars showing means \pm Standard Deviation (\pm SD; $n = 3$)

potential which can be used to treat diabetes mellitus [24]. Many mechanisms of action have been proposed by which extracts of medicinal plants produced hypoglycemia. The proposed mechanisms include the effect of plant material on β -cells of pancreas, which may diminish synthesis of insulin, or release, or cell regeneration. The plants also produce anti-diabetic effects by improving peripheral glucose utilization or enhancing

the synthesis of liver glycogen or may inhibit absorption of glucose from intestine [25]. For anti-diabetic activity, plants are responsible via their capability to reestablish the function of pancreas either by increasing the secretion of hormone or by decreasing the absorption of glucose from intestine [26, 27]. In this study water extract of root, stem (65.98%) and leaf displayed a marked reduction in serum glucose concentration in alloxan

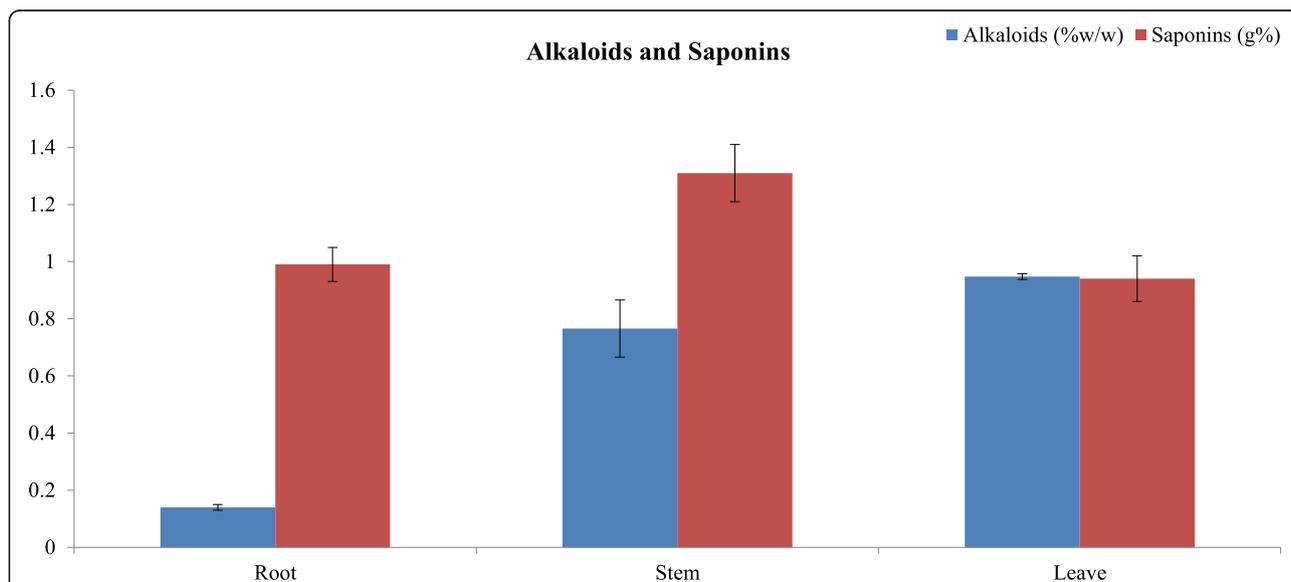


Fig. 5 Phytochemical (alkaloid and saponin) in different parts of *Pandanus tectorius* Parkinson ex Du Roi The vertical lines in the bars showing means \pm Standard Deviation (\pm SD; $n = 3$)

diabetic rats. Similarly, ethanol extract of root, stem and leaf also significantly decreased the level of glucose in comparison with their respective diabetic control (untreated) rats, this indicates stimulation of insulin release from the pancreas [28]. Alloxan causes heavy destruction of pancreatic β -cells, which results in reduced synthesis and release of hormones from the pancreas. The normal functioning of insulin is impaired which causes hyperglycemia [29]. In our study alloxan induced hyperglycemia, but treatment with ethanol and water extract significantly reduced glucose level. The water extract of *Euonymus alatus* decreased the streptozotocin induced elevated level of serum glucose level [30]. Alloxan causes permanent damage to β -cells [31]. In our study alloxan treated rats after treatment with ethanol and water extract of root stem and leaf demonstrated variable but significant reduction in glucose level, so we concluded that these extracts produced anti-diabetic effects by a mechanism other than the stimulation of insulin release from β - cells of pancreas.

Hyperlipidemia is characterized by enhanced levels of triglycerides, cholesterol, low density lipoprotein cholesterol (LDL-c). The results of the in vivo study revealed that both ethyl alcohol and water extracts of root, leaf and stem significantly reduced the level of cholesterol, triglycerides, and LDL-c with subsequent increase in HDL-c in normal and diabetic rats in comparison with their respective control. All three extracts corrected hyperlipidemia. Hyperlipidemia is the leading endangerment for the premature development of heart disease like coronary artery disease, atherosclerosis and hypertension. Many studies on hyperlipidemia suggested that decrease in lipid profile: cholesterol, triglycerides and LDL-c and increase in HDL-c reduces risk of developing ischemic heart diseases [32]. Prolonged hyperglycemia produced more oxidative stress. Oxidation is one of the destructive activities in which various molecules are broken down. Oxidative stress is the main factor which relates hyperlipidemia with pathogenesis of atherosclerosis [33]. These reactive oxygen species react with lipids and cause lipid peroxidation, which results in membrane necrosis. Lipid peroxidation is declined which leads to curtailment in arterial wall cholesterol and deduction in atherosclerosis caused by hyperlipidemia [34]. In the study the coronary risk index (CRI) and atherogenic index (AI) increased in alloxan induced diabetic rats, whereas indexes reduced or came towards normal range after treatment with extract, this indicates reduction in risk for development of coronary disease. Several indices have been derived from lipid profile to establish an index for the prediction of cardiac risk, among these AI is considered as a strong index for predicting risk of ischemic diseases [35].

Free radicals also play a vital role in the production of secondary entanglement in diabetes mellitus and affect or impair the function of kidney, blood vessels, eye and nerves [36]. The elevated level of oxidative stress increases the lipid profile [37]. In this study different parts of *P.tectorius* showed significant antioxidant activity. There is a report that destruction of β cells of pancreas is prevented by antioxidants [38]. The anti-diabetic activity of ethyl acetate fraction of *E. alatus* has been reported in alloxan treated mice evidenced by reduced plasma glucose, triglyceride and total cholesterol level with elevated SOD. This study also revealed that the ethyl acetate fraction contains flavonoids and polyphenols, including quercetin and kaempferol as main components with strong antioxidant activities [39].

Plasma urea and creatinine is widely accepted as a measurement of renal function [40]. In the study it was observed that urea and creatinine increased significantly in the alloxan diabetic control group. Administration of ethanol and water extract significantly decreased the serum level of both. The reduction in the level of these metabolites by the extract indicates the protective role of extracts against renal disorder due to diabetes [41, 42]. Anti-diabetic, anti-hyperlipidemia and nephron-protective (via reducing urea and creatinine level in serum of diabetic rats) has been reported [43].

Determination of cardiac and hepatic enzymes activity in serum or plasma is significantly important in order to diagnose the progression of disease. In this study the anti-diabetic activity and decrease in the level of ALP, ALAT, ASAT and LDH in alloxan induced diabetic rats might be due to the presence of antioxidant molecules in different parts of the plant. Certain enzymes are confined to certain tissues, and they enter in the blood only when these tissues are damaged. The presence of a significant increased amount of these specific enzymes in the blood reveals cell damage or destruction [44]. In present study enzyme activity of alanine aminotransferase (ALAT)/ serum glutamic pyruvic transaminase (SGPT), aspartate aminotransferase (ASAT)/ serum glutamic oxaloacetic acid (SGOT) alkaline phosphatase, and lactate dehydrogenase (LDH) in serum were reduced by the treatment of water and ethanol extracts of root, stem and leaf as compared to alloxan induced diabetic rats. Attenuation of increased activities of all tested enzymes as compared to control rats indicate the protective and nontoxic nature of plant extract [45]. ALP is a liver marker enzyme and high level of enzyme in diabetic control indicates damage to structural integrity of liver. However diabetic rats treated with plant extract showed decreased in ALP level indicating healing effect of the extract. The transaminases ALAT and ASAT are also liver markers and used to detect toxicity to the liver [46]. Increased in activity of both transaminases in

diabetic rats suggest damage of liver cells [47]. In this study LDH increased significantly in diabetic rats, and treatment with water and ethanol extract reduced the level towards normal. The increase in LDH activity after in diabetic rats may result from loss of membrane integrity [48].

Phytochemical estimation revealed the highest phenol content in water extract of stem. Extracts from different solvents showed a variable amount of phenol. Phenols are responsible for producing multiple biological effects including antioxidant and anti-diabetic [49, 50]. Our study also demonstrated the presence of a variable number of flavonoids, tannin, alkaloid and saponin in different parts of the plant. Many reports are available suggesting antioxidant and anti-diabetic and hypolipidemic potential of plants due to the presence of these constituents [29, 30, 49].

Conclusion

Diabetes is a serious metabolic disorder affecting peoples worldwide. Long term hyperglycemia results in many complications like irregular functions of liver, heart and kidney. It also damages nerves, blood vessels and eyes. Treatment with oral hypoglycemic agents and insulin are not only expensive but also produce many adverse effects. We studied the anti-diabetic potential of water and ethanol extract of leaves, stem and root of *P. tectorius* in alloxan diabetic rats. This study demonstrated that all parts of plants have tremendous effect in lowering the blood glucose level and as well as attenuating the diabetes related complications like heart related problems and kidney dysfunction. In this study, different parts of *P. tectorius* showed good antioxidant activities and also showed presence of polyphenols. Dietary polyphenols have been reported to possess antidiabetic activity and inhibit α -amylase and α -glucosidase activities which may inhibit glucose absorption in the intestine and stimulate insulin secretion [51]. Polyphenols from *P. tectorius* could be a dietary therapy for the prevention and management of Type 2 diabetes. However, to confirm these effects and to make dietary recommendations for patients with type 2 diabetes, further studies are necessary.

Abbreviations

ALAT: Alanine aminotransferases; AST: Aspartate aminotransferases; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; HDL: High density lipoprotein; LDL: Low density lipoprotein; CRI: Coronary artery risk index; AI: Atherogenic index; DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; *P. tectorius*: *Pandanus tectorius* Parkinson ex Du Roi; DM: Diabetes mellitus

Authors' contributions

SS & JA conceived and designed the study. SS and KH performed experimental work. SS & NS analyzed and interpreted the results. AT and VS did chemical work. SS, H and NS wrote the manuscript. SE & JA improved the manuscript. All authors read and approved the final manuscript.

Funding

Authors are thankful to the Dean, Faculty of Science, University of Karachi for financial assistance.

Availability of data and materials

Research data (Lab notebook) and materials can be provided on request.

Declarations

Ethics approval and consent to participate

The experiment was conducted according to the rules of Board of Advanced Studies & Research/ Institutional Animal Ethics Committee (BASR No.01402/ SC dated March 05, 2013), University of Karachi.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 3 April 2020 Accepted: 26 April 2021

Published online: 28 May 2021

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