ISSN:0975-542X

Effect of 50% Hydroethanolic Leaf Extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on Non-enzymic Antioxidants and other Biochemical Parameters in Liver, Kidney, Serum of Alloxan Induced Diabetic Swiss Albino Rats

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ABSTRACT: The study was undertaken to evaluate the effect of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on non-enzymic antioxidants, liver glycogen, lipid peroxidation, urea, creatinine and LDH levels in the liver, kidney and serum of alloxan induced diabetic wistar rats. Extracts were orally administered for 30 days at a dosage of 250 and 500 mg/kg bodyweight for alloxan induced diabetic rats. A significant ($\rho < 0.05$) decrease was found in urea, LDH and lipid peroxidation (at 500mg/kg bodyweight) levels in the plant extract treated groups. The level of vitamin A and liver glycogen was significantly ($\rho < 0.05$) increased in the treatment and drug treated groups. The results suggest that the plant extracts treated at 500mg/kg body weight treated groups was found to be effective then the 250mg/kg body weight administration. 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) are not only useful in controlling the lipid peroxide level but are also helpful in further strengthening the antioxidant potential.

Keywords: *Ruellia tuberosa* L. *Dipteracanthus patulus* (Jacq.), non-enzymic antioxidants.

INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025. It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025 (1). Though pathophysiology of diabetes remains to be fully understood, experimental evidences suggest that the involvement of free radicals in the pathogenesis of diabetes (2) and more importantly in the development of diabetic complications (3, 4).

Free radicals are capable of damaging cellular molecules, DNA, proteins and lipids leading to altered cellular functions. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models (5) as well as reducing the severity of diabetic complications (6). Medicinal plants are being looked up once again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated (7).

In folk medicine, *Ruellia tuberosa* L. has been used as anti-diabetic, antipyretic, analgesic, anti hypertensive, thirst-quenching, and antidotal (8) and *Dipteracanthus patulus* (Jacq.) leaves are used for treating itches, insect bites, paranychia, venereal diseases, sores, tumours and rheumatic complaints (9). Both the plants belong to *Acanthacea* family.

MATERIALS AND METHODS

Plant leaf collection: Fresh leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) were collected from ABS (Altogether Botanical Species) Medicinal Plants Garden, Karipatti, Salem, Tamilnadu,

India. The plant was identified by the herbarium of Botanical Survey of India (BSI) southern circle, Tamilnadu Agricultural University (TNAU) (No: BSI/SC/5/23/08-09/Tech- 118, 229).

Preparation of 50% hydroethanolic leaf extracts of Ruellia tuberosa L. and Dipteracanthus patulus (Jacq.): The fresh leaves of the plants were collected and shade dried for five days and crushed in to coarse powder. The coarse powder thus obtained was cold macerated with hydro ethanol for 3 days. Then, the water portion of the sample was evaporated to dryness at a low temperature (40^0 C) under reduced pressure in a rotary evaporator. Dark brown colored crystals obtained were used for the studies.

Experimental animals: Male wistar rats of six to eight weeks old weighing about 110-120g were obtained from the animal husbandary of PSG Institute of Medical Science and Research (No: 158/1999/CPCSEA), Coimbatore, India. The rats were grouped and housed in polyacrylic cages and maintained under standard conditions (25 ± 2^0C) with 12±1 h dark/light cycle. The animals were fed with rat pellet feed supplied by Hindustan Lever Ltd., Bangalore, India and water *ad libitum*. All procedures described were reviewed and approved by the Animal Ethical Committee (AEC).

Induction of diabetes mellitus: Alloxan monohydrate was used to induce diabetes mellitus in normoglycemic rats. Animals were allowed to fast for 18 hours and were injected intraperitoneally with freshly prepared alloxan monohydrate in sterile normal saline at a dose of 120 mg/Kg body weight. Blood glucose was measured after 72 hours of alloxan injection and rats showing fasting blood glucose level (approximately 300 mg/dl) were selected for the study. 0.5 to 1 ml of blood was collected from the animal using capillary tube by tail vein route.

Treatment groups: The animals were divided into seven groups of six animals in each group, after two week acclimatization period. Group I (Normal control + normal saline 5ml/kg body weight), Group II (Diabetic control), Group III (Drug control - Glibenclamide 600 µg/kg body weight), Group IV (Diabetes + 250 mg/kg body weight 50 % HERT), Group V (Diabetes + 500 mg/kg body weight 50 % HERT), Group VI (Diabetes + 250 mg/kg body weight 50 % HEDP), Group VII (Diabetes + 500 mg/kg body weight 50 % HEDP). (50% HEDP/HERT – hydroethanolic leaf extract of *Ruellia tuberosa* L. /*Dipteracanthus patulus* (Jacq.)).

After the end of experimental period (30 days), the rats were fasted overnight and sacrificed by cervical decapitation. The liver and kidney was quickly excised, blotted, dried and stored at -4^0C until the analysis was performed. Serum was separated from the blood collected, by centrifugation and the serum was stored at -4^0C for biochemical analysis.

Preparation of tissue homogenate

The tissues of 1gm were homogenized in 0.1 M cold Tris – HCl buffer (pH 7.4) in a potter – Elvehjam homogenizer fitted with a Teflon plunger at 600 rpm for 30 minutes. The homogenate was centrifuged at 10,000 g for 20 min at 4^0 C and the supernatant was used for enzyme assays.

BIOCHEMICAL ESTIMATIONS

***Estimation of Liver Glycogen* (10):** Liver sample was weighed and grounded finely with 20 ml of 5% TCA. The precipitate of

proteins is filtered off and the clear filtrate was used for the analysis. 2 ml of the liver extract was pipetted in to test tube. To this added 2 ml of 10 N potassium hydroxide solution and kept in a boiling water bath for 1 hour. After cooling the tubes, 1 ml of glacial acetic acid was added to neutralize the excess of alkali. To 2 ml of this solution added 4 ml of anthrone reagent in a cold condition. The tubes were shaken well and kept in a boiling water bath for 10 minutes. The color developed was read at 650 nm in a spectrophotometer.

***Estimation of Urea* (11):** 0.2 ml of sample was taken in the tube labeled 'Test'. The tubes were made up to 2 ml using distilled water and mixed well. The color reagent was prepared fresh at the time of analysis by mixing distilled water, mixed acid reagent and mixed the color reagent in the ratio 1:1:1. Then added 3 ml of color reagent to all the tubes, the tubes were mixed well and kept in a boiling water bath for 15 minutes. After the incubation period the tubes were removed from the water bath and cooled for 5 minutes. The transmittance was measured in a spectrophotometer at 540 nm against reagent blank. A blank was taken in the same manner as described above and instead of sample distilled water was taken.

***Estimation of* Creatinine (12):** Pipetted 0.2-1.0 ml of working standard in a series of test tubes (S1-S5) and 0.2 ml of sample in the tube marked 'Test'. The final volume was made up to 2 ml in all the test tubes using distilled water. Added 3.0 ml of working reagent to all the tubes and mixed well. The tubes were left at room temperature for 30 minutes. After the incubation the transmittance was absorbed at 505 nm. Added 0.2 ml of 30% acetic acid to the 'Test' tubes mixed well and left at the room temperature for 5 minutes. The absorbance was measured at 505 nm.

***Estimation of Lactate Dehydrogenase (LDH)* (13):** In the tube labeled 'Test' taken 0.5 ml of buffered substrate and 100 µl of sample. 100 µl of water was alone added to the 'Blank' marked tube. Then to the 'Test' tubes added 0.1 ml of NAD. Mixed and incubated the tubes at 37ºC for 15 minutes. Exactly after 15 minutes, 0.5 ml of dinitrophenylhydrazine was added to the 'Test'. To the control tube alone, the sample was added after the addition of DNPH. The tubes were kept at room temperature for 15 minutes. Then added 5 ml 0.4 N sodium hydroxide and the color developed was read immediately at 440 nm. Pyruvate was used as standard.

***Estimation of Vitamin A* (14):** 1 ml of the liver homogenate was added with 1 ml of saponification mixture. The mixture was gently refluxed for 20 minutes at 60ºC in the dark. The tubes were cooled at room temperature and added 20 ml of water and mixed well. The solution was transferred to separating funnel and extracted thrice using 25, 15 and 10 ml of petroleum ether. Pooled the extracts and washed thoroughly with 50-100 ml of water, until the wash water was free of alkali. Added Sodium sulphate (anhydrous) to remove the petroleum ether extract and the volume was noted. 3 ml of petroleum ether phase was transferred to a cuvette and read at 420 nm against petroleum ether as blank, without delay to prevent evaporation of the solvent and destruction of carotenoids by light. The reading was marked as A_1. 3 ml of the extract was then evaporated to dryness at 60ºC. The dried tissue was dissolved in 1ml of trichloroacetic acid rapidly and the absorbance taken as 620 nm and it is marked as A_2. Aliquots of the standard (Vitamin A palmitate) were pipetted out into a series of clean dry test tubes. The volumes in all the tubes were made up to 1 ml with chloroform. Added 2 ml of TCA reagent to

the tubes and mixed well. Recorded the absorbance of blue color formed immediately at 620 nm in a spectrophotometer.

***Estimation of Vitamin E* (15):** 2.5 g of sample was weighed in a conical flask. 50 ml of 0.1 N sulphuric acid was added slowly without shaking. Stoppered and allowed to stand overnight. On the next day the contents of the flask were shaken vigorously and filtered through whatmann no.1 filter paper, discarding the initial 10-15 ml of the filtrate. Aliquots of the filtrate were used for the estimation of tocopherol. Into 3 stoppered centrifuge tubes pipetted out 1.5 ml of extract and 1.5 ml of water respectively. To the test and blank 1.5 ml of ethanol was added and to the standard 1.5 ml of water was added and centrifuged. Transferred 1.0 ml of xylene layer in to another stoppered tube, taking care not to include any ethanol or protein. 1.0 ml of 2, 2' dipyridyl reagent was added to each tube, stoppered and mixed. 1.5 ml of the mixture was pipetted out in to colorimeter cuvettes and read the extinction of the test and standard against the blank at 460 nm. To the blank added 0.3 ml of ferric chloride solution, mixed well and exactly after 15 minutes the test and standard read against the blank at 520 nm.

***Estimation of* Ascorbic Acid (16):** Grounded 5 g of sample material with 25-50 ml of 4% oxalic acid solution centrifuged the sample material and collected the fluid. Aliquots of 10ml were transferred to a conical flask and added bromine water drop wise with constant mixing. The enolic hydrogen atoms in ascorbic acid will be removed by bromine. The extract turned orange yellow due to excess bromine and it was expelled by blowing in air. The volumes were made up to a known volume with 4% oxalic acid solution and converted 10ml of stock ascorbic acid solution into dehydro form by bromination. Pipetted standard dehydroascorbic solution (10-100 µg) and different aliquots (0.1 to 2 ml) of brominated sample extract into a series of test tubes. The volumes in each tube were made up to 3 ml by adding distilled water. Added 1 ml of DNPH reagent followed by 1-2 drops of 10% thiourea to each tube. A blank was set with water in the place of ascorbic acid solution. Mixed the contents of the tubes thoroughly and incubated at 37^0C for 3 hours. After the incubation, dissolved the orange-red osazone crystals formed by adding 7 ml of 80 % sulphuric acid and the absorbance was measured at 540 nm.

***Estimation of* Lipid Peroxidation (17):** The tissue homogenate was prepared in Tris – Hydrochloric acid buffer. 1 ml of the homogenate was combined with 2 ml of TCA–TBA–Hydrochloric acid reagent and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. The flocculent precipitate was removed by centrifugation at 1000 rpm for 10 minutes. The absorbance of the sample was read at 535 nm against a blank. Acid was added to the 6ml of the supernatant followed by 2 ml of 0.05 mol 2-thiobarbituricacid and after 40 minutes incubation at 40^0C, the absorbance of the samples was read at 443 nm against the reagent blank.

Statistical Analysis

Data was reported as mean ± SD by using the Statistical Package of Social Sciences (SPSS). The data for all the parameters was analyzed by using Analysis Of Variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). Values are considered statistically significant at $P < 0.05$ (18).

RESULT

Vitamin A Level in the Kidney

The results show that (Table I and II) the diabetes developed rats showed a decrease in the vitamin A level. A significant ($\rho < 0.05$) increase in the vitamin A level was seen in the plant extracts treated groups. Treatment with 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w considerably increased the vitamin A levels to 50.1% and 87.5%. Administration of 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) increased the vitamin A levels to 62.4% and 82.4% in 250 and 500 mg/kg b.w dosage levels respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) raised the vitamin A level to 96.0% as compared to the diabetic control group.

Vitamin C Level in the Kidney

Vitamin C level was found to be decreased in alloxan induced animals and it was considerably increased in the plant extract treated groups. There is no significant increase in the vitamin C level except in the administration of 500 mg/kg b.w of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) (Table I and II). Administration of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w increased the vitamin C levels to 18.5% and 32.4%. Treatments with 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) at dosages of 250 and 500 mg/kg b.w showed 9.2% and 29.6% increase in the vitamin C level respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) raised the vitamin C level to 37.0% as compared to the diabetic control group.

Vitamin E Level in the Kidney

Vitamin E level was found to be reduced in the alloxan induced groups. There is no significant increase in the vitamin E level except in 500 mg/kg b.w of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. extract treated groups (Table I and II). Treatment with 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w increased the vitamin E levels to 4.4% and 62.8%. Administration of 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) increased the vitamin E levels to 1.4% and 60.2% in 250 and 500 mg/kg b.w dosage levels respectively. Glibenclamide (600 µg/kg b.w) treated group showed 64.5% increase in the vitamin E level as compared to the diabetic control group.

Table I: Effect of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on vitamin A, C and E levels in alloxan induced diabetic rat kidney.

S.No	Groups	Vitamin A (µg/ g tissue)	Vitamin C(mg/ g tissue)	Vitamin E (µg/ g tissue)
1	Group I	22.85 ± 2.63	1.62 ± 0.33	5.26 ± 2.31
2	Group II	7.93 ± 2.61	1.08 ± 0.26	1.36 ± 1.03
3	Group III	15.55 ± 2.80	1.48 ± 0.24	3.83 ± 2.08
4	Group IV	11.91 ± 2.61^{a}	1.28 ± 0.26*	1.30 ± 0.50*
5	Group V	14.87 ± 2.62^{b}	1.43 ± 0.23^{a}	3.66 ± 2.25^{a}
6	Group VI	12.88 ± 2.62^{d}	1.18 ± 0.27*	1.34 ± 0.58*
7	Group VII	14.47 ± 2.90^{c}	1.40 ± 0.25^{b}	3.42 ± 2.29*

Values are expressed as mean ± SD of six rats from each group, $\rho < 0.05$ as compared to diabetic control. Group II vs. IV, V, VI and VII. Mean with different subscripts (a, b, c, d) differ from diabetic control significantly, * denotes no significant difference.

Table II: Vitamin A, C, E levels (kidney) in percentage

S.No	Groups	Vitamin A	Vitamin C	Vitamin E
1	Group I	---	---	---
2	Group II	---	---	---
3	Group III	96.0	37.0	64.5
4	Group IV	50.1	18.5	4.4
5	Group V	87.5	32.4	62.8
6	Group VI	62.4	9.2	1.4
7	Group VII	82.4	29.6	60.2

Vitamin A Level in the Liver

The results show that (Table III and IV) the diabetes developed rats showed a decrease in the vitamin A level. A significant ($\rho < 0.05$) increase in the vitamin A level was seen in the 500 mg/kg b.w administration of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) plant leaf extracts treated groups. Treatment with 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w considerably increased the vitamin A levels to 31.5% and 63.1%. Administration of 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) increased the vitamin A levels to 25.6% and 50.3% in 250 and 500 mg/kg b.w dosage levels respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) raised the liver vitamin A level to 82.0% as compared to the diabetic control group.

Vitamin C Level in the Liver

Vitamin C level was found to be decreased in alloxan induced animals and it was considerably increased in the plant extract treated groups. There is no significant increase in the vitamin C level except in the administration of 500 mg/kg b.w of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. (Table III and IV). Administration of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w increased the vitamin C levels to 25.4% and 31.9%. Treatments with 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) at dosages of 250 and 500 mg/kg b.w showed 22.1% and 24.5% increase in the vitamin C level respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) raised the liver vitamin C level to 27.8% as compared to the diabetic control group.

Vitamin E Level in the Liver

Vitamin E level was found to be reduced in the alloxan induced groups. There is no significant increase in the vitamin E level of the plant extract treated groups (Table III and IV). Treatment with 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w increased the vitamin E levels to 16.9% and 46.7%.

Table III: Effect of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on vitamin A, C, E levels in alloxan induced diabetic rat liver

S.No	Groups	Vitamin A	Vitamin C	Vitamin E
1	Group I	31.80 ± 2.36	1.62 ± 0.34	7.58 ± 2.66
2	Group II	15.85 ± 2.75	1.22 ± 0.17	3.08 ± 2.42
3	Group III	28.86 ± 2.60	1.56 ± 0.30	6.88 ± 2.62
4	Group IV	20.85 ± 2.74^{a}	1.53 ± 0.26*	3.71 ± 2.64*
5	Group V	25.86 ± 2.73^{b}	1.61 ± 0.30^{a}	5.78 ± 2.60*
6	Group VI	19.91 ± 2.73^{c}	1.49± 0.26*	3.90 ± 2.72*
7	Group VII	23.83 ± 2.50^{d}	1.52 ± 0.29*	4.60 ± 2.45*

Values are expressed as mean ± SD of six rats from each group, $\rho < 0.05$ as compared to diabetic control. Group II vs. IV, V, VI and VII. Mean with different subscripts (a, b, c, d) differ from diabetic control significantly, * denotes no significant difference.

Administration of 50% hydroethanolic leaf extract of

Dipteracanthus patulus (Jacq.) increased the vitamin E levels to 21.0% and 33.0% in 250 and 500 mg/kg b.w dosage levels respectively. Glibenclamide (600 µg/kg b.w) treated group showed 55.2% increase in the vitamin E level as compared to the diabetic control group.

Table IV: Vitamin A, C, E levels (liver) in percentage

S.No	Groups	Vitamin A	Vitamin C	Vitamin E
1	Group I	---	---	---
2	Group II	---	---	---
3	Group III	82.0	27.8	55.2
4	Group IV	31.5	25.4	16.9
5	Group V	63.1	31.9	46.7
6	Group VI	25.6	22.1	21.0
7	Group VII	50.3	24.5	33.0

Urea Level

The results show that (Table V and VI) the diabetes induced rats showed an increase in the urea level in the serum. A significant ($\rho < 0.05$) decrease in urea level was found in the plant extract treated groups. Administration of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w decreased the urea levels to 30.7% and 32.7%. Treatments with 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) at dosages of 250 and 500 mg/kg b.w showed 24.5% and 27.6% decrease in the urea level respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) reduced the serum urea level to 31.6% as compared to the diabetic control group.

Creatinine Level

Creatinine level was found to be elevated in alloxan induced animals and it was considerably reduced in the treatment groups. There is no significant ($\rho < 0.05$) decrease in the creatinine level of the plant extract treated groups with respect to diabetic control group (Table V and VI). Administration of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w decreased the creatinine levels to 1.29% and 14.9%. Treatments with 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) at dosages of 250 and 500 mg/kg b.w showed 2.5% and

9.7% decrease in the creatinine level respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) reduced the serum creatinine level to 9.0% as compared to the diabetic control group.

Lactate Dehydrogenase Level

The results show that (Table V and VI) the diabetes developed rats increased the lactate dehydrogenase level in the serum. A significant ($\rho < 0.05$) decrease in the lactate dehydrogenase level was seen in the 250 and 500 mg/kg b.w administration of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) plant leaf extracts treated groups. Treatment with 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w decreased the lactate dehydrogenase levels to 17.6% and 25.2%. Administration of 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) decreased the lactate dehydrogenase levels to 10.1% and 18.8% in 250 and 500 mg/kg b.w dosage levels respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) reduced the serum lactate dehydrogenase level to 30.4% as compared to the diabetic control group.

Liver Glycogen Level

The results show that (Table V and VI) the diabetes developed rats showed a decrease in the liver glycogen level.

Table V: Effect of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on urea, creatinine, lactate dehydrogenase (LDH), liver glycogen and lipid peroxidation (Lipid Peroxidation) levels in serum and liver of alloxan induced diabetic rat.

S.No	Group	Urea (mg/ dl)	Creatinine (mg/ dl)	LDH(mg/dl)	Liver Glycogen (mg/gm tissue)	Lipid Peroxidation (U/mg Protein)
1	Group I	45.92 ± 2.72	1.28 ± 0.26	40.68 ± 2.62	63.83 ± 2.63	0.46 ± 0.29
2	Group II	98.05 ± 2.57	1.54 ± 0.27	78.98 ± 2.72	24.75 ± 2.73	1.53 ± 0.26
3	Group III	66.98 ± 2.53	1.40 ± 0.24	54.97 ± 2.78	52.93 ± 2.61	0.53 ± 0.33
4	Group IV	67.94 ± 2.67[a]	1.52 ± 0.29[*]	65.05 ± 2.67[a]	38.85 ± 2.63[a]	1.28 ± 0.26[*]
5	Group V	65.97 ± 2.59[b]	1.31 ± 0.25[*]	59.02 ± 2.80[b]	46.73 ± 2.60[b]	0.59 ± 0.36[a]
6	Group VI	73.94 ± 2.52[c]	1.50 ± 0.25[*]	70.95 ± 2.76[c]	30.90 ± 2.61[c]	1.29 ± 0.26[*]
7	Group VII	70.94 ± 2.60[d]	1.39 ± 0.26[*]	64.11 ± 2.56[d]	39.70 ± 2.73[d]	0.60 ± 0.38[b]

Lipid peroxidation (LIPID PEROXIDATION) - n moles of monaldehyde/mg of protein, LDH – lactate dehydrogenase

Values are expressed as mean ± SD of six rats from each group, $\rho < 0.05$ as compared to diabetic control. Group II vs. IV, V, VI and VII. Mean with different subscripts (a, b, c, d) differ from diabetic control significantly, * denotes no significant difference.

Table VI: Urea, Creatinine, LDH, Liver Glycogen, Lipid Peroxidation levels in percentage

S.No	Group	Urea	Creatinine	LDH	Liver Glycogen	Lipid Peroxidation
1	Group I	---	---	---	---	---
2	Group II	---	---	---	---	---
3	Group III	31.6	9.0	30.4	53.2	16.3
4	Group IV	30.7	1.29	17.6	36.2	61.4
5	Group V	32.7	14.9	25.2	47.0	15.6
6	Group VI	24.5	2.5	10.1	19.9	60.7
7	Group VII	27.6	9.7	18.8	37.6	65.3

A significant ($\rho < 0.05$) increase in the liver glycogen level was seen in the plant extracts treated groups. Treatment with 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w considerably increased the liver glycogen levels to 36.2% and 47.0%. Administration of 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) increased the liver glycogen levels to 19.9% and 37.6% in 250 and 500 mg/kg b.w dosage levels respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) raised the liver glycogen level to 53.2% as compared to the diabetic control group.

Lipid Peroxidation Level

Lipid peroxidation level was found to be increased in alloxan induced animals and it was considerably reduced in the treatment groups. There is no significant decrease in the lipid peroxidation level of the plant extracts treated groups except in the treatment of 500 mg/kg b.w of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. was observed (Table V and VI). Administration of 50% hydroethanolic leaf extract of *Ruellia tuberosa* L. at dosages of 250 and 500 mg/kg b.w decreased the lipid peroxidation levels to 16.3% and 61.4%. Treatments with 50% hydroethanolic leaf extract of *Dipteracanthus patulus* (Jacq.) at dosages of 250 and 500 mg/kg b.w showed 15.6% and 60.7% decrease in the lipid peroxidation level respectively. The drug treated group (glibenclamide at 600 µg/kg b.w) reduced the lipid peroxidation level to 65.3% as compared to the diabetic control group.

DISCUSSION

In diabetes mellitus there will be a severe renal damage due to the abnormal glucose regulation, including elevated glucose and glycosylated protein tissue levels, haemodynamic changes within the kidney tissue and increased oxidative stress (19). Plasma urea and creatinine levels were found to be higher in the non-treated diabetic rats than in diabetic control group. Damage or loss of glomeruli in diabetes could lead to such an increase. The level of these substances had reduced after one month of treatment by 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.), which may indicate the ability of this plant extracts to enhance the renal function.

The cytotoxicity of xenobiotics can be evaluated using serum marker enzymes. One such enzyme is lactate dehydrogenase, which though distributed throughout the body, it possess isoenzymes recognized as marker for liver muscle lesion. It is important to exclude the possibility that the diabetes itself damage the tissue in such a way that activities of lactate dehydrogenase are attached (20). Elevated serum lactate dehydrogenase levels in diabetic rats indicate cardiac muscular damage (21). The quantity of enzyme released from damaged tissue is a measure of the number of necrotic cells. Alloxan induced diabetic animals showed increased release of lactate dehydrogenase in serum, the levels were reduced in the 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) treated groups.

Excessive hepatic glycogenolysis and gluconeogenesis, associated with decreased utilization of glucose by tissue is the fundamental mechanism underlying hyperglycemia in diabetic state (22). Aberration of liver glycogen synthesis by glycogenolysis in diabetes may be due to the lack of or resistance of insulin, which is essential to activate glycogen synthase system. The significant increase of liver

glycogen level in 50% hydro-ethanolic extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) treated diabetic groups may be due to reactivation of the glycogen synthase system by improving insulin secretion.

The involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes has been reported by several workers (23). It has been reported that in diabetes mellitus, oxygen free radicals are generated by stimulating hydrogen peroxide *in vitro*, as well as *in vivo* and in the pancreatic beta cells (24). The increase in lipid peroxidation indicates an increased oxidative stress as a result of excessive generation of free radicals. Hypoinsulinnaemia increases the activity of the enzyme, fatty acyl coenzyme, coenzyme A oxidase, which initiates β-oxidation of fatty acids resulting in the lipid peroxidation (25). Administartion of 50% hydro-ethanolic extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) decreased the lipid peroxidation index. The reduction in lipid peroxidation can be attributed to the antioxidant activity of various phytochemicals present in 50% HERT and HEDP.

Vitamin A is a free radical scavenger (singlet O_2) and chain breaking antioxidant (26). It protects the cells from the oxidative damage (27). It assists vitamin E for the inhibition of lipidperoxidation by recycling the vitamin E. It is one of the most important free radical scavenging chain-breaking antioxidant in the membrane (28). Vitamin E reduces the lipidperoxides generated during the process of peroxidation and protects cell structure from the damage (29). The decreased level of vitamin E found in liver and kidney of the diabetic rats as compared with control rats could be due to the increased oxidative stress (30, 31). Vitamin C, a major extracellular non-enzymatic antioxidant, has a crucial role in scavenging several reactive oxygen species. Vitamin C contributes at 20% of total peroxyl radical trapping capacity. The decrease may be due to increasing utilization in the trapping of free radicals (32).

Vitamin C or ascorbic acid is an excellent hydrophilic antioxidant in plasma and disappear faster than other antioxidants on exposure to reactive oxygen species (33). The decreased level of ascorbic acid in the diabetic rats may be due to either increased utilization as an antioxidant defense against increased reactive oxygen species or to a decreased glutathione level since glutathione is required for the recycling of ascorbic acid (34, 35). In our study there was considerable reduction in vitamin A, C, E in liver and kidney of diabetic induced animals. The levels were increased in the 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) treated groups.

The presence of phytochemicals such as flavonoids, saponins, glycosides and phenolic compounds were found in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) (36). The flavonoids such as apigenin and luteolin were shown to possess anti-hyperglycemic (37, 38) and antioxidant activity (39). The leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) possess flavonoids such as luteolin and apigenin, lupeol which have potent antioxidant and anticancer activity (40, 41, 42, 43).

We suggest here that the mode of action of 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) may be due to their presence of phytochemicals (Flavonoids, glycosides,

phenols and saponins) and their potent antioxidant and free radical scavenging activity (Manikandan and Victor Arokia Doss, 2009), which might have increased the vitamin levels and decreased the lipid peroxidation levels in alloxan induced diabetic animals. Thus our findings suggest that the 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) at 500 mg/kg body weight showed better results when compared to 250 mg/kg body weight. Treatment with standard drug was found to be effective then the plant extracts. However, further pharmacological investigations are needed to find out the mechanism of action of the active components involved.

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