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Effect of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on AST, ALT, ACP and ALP levels in serum, liver and kidney of alloxan induced diabetic rats

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ABSTRACT

This study was undertaken to evaluate the relationship and effects of diabetes on liver function. The hepatic effects of diabetes were evaluated *in vivo* using alloxan -induced diabetic rats as an experimental model. The degree of hepatic dysfunction was measured by using biochemical parameters like transaminases (ALT and AST), alkaline phosphatase (ALP) and acid phosphatase (ACP) in serum, liver, kidney. The aim of the study was to investigate the enzyme alterations in alloxan -induced diabetic rats. Diabetes was induced by a single dose of alloxan monohydrate given intraperitoneally in sterile normal saline at a dose of 120 mg/kg body weight. Six albino rats were divided into seven groups, normal control (Group I), diabetic control (Group II), drug treated (Group III) and plant extract treated (IV, V, VI, and VII) which were sacrificed 30 days post treatment, respectively. Increased levels of aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), ALP (alkaline phosphatase) and ACP (acid phosphatase) were observed in the liver. Treatments with 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) significantly reduced the enzyme levels. Our findings suggest that *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) have the ability to moderately repair the kidney and liver damage. The effect of the plant extracts was found to be lower than the standard drug (glibenclamide 600 µg/kg body weight) used.

Key words : *Ruellia tuberosa* L. *Dipteracanthus patulus* (Jacq.), Transaminases, Phosphatases

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with the long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (ADA, 2007). Globally, the estimated incidence of DM and projection for year 2010, as given by International Diabetes Federation (IDF) is 239 million (Karastergiou and Kaski, 2008). A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems (Scartezzini and Sproni, 2000), many of these herbal medicines are used as a single agents or in different oral formulations have been recommended for diabetes mellitus due to the fact that they are less toxic than oral hypoglycemic agents such as sulfonylureas, metformin etc. (Ponnachan *et al.*, 1993;

Chattopadhyay, 1993).

It has been documented that several medicinal plants show their hypoglycemic effects associated with a significant alteration in the activity of the liver kinase enzymes (Bopanna *et al.*, 1997; Kumari *et al.*, 1995). In addition, Bopanna *et al.* (1997) and Eskander *et al.* (1995) demonstrated that the administration of several herb extracts could restore the changes in the activities of serum enzymes, like Alkaline Phosphatase (ALP), Acid Phosphatase (ACP) and transaminases: Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT).

In folk medicine, *Ruellia tuberosa* L. has been used as anti-diabetic, antipyretic, analgesic, anti hypertensive, thirst- quenching, and antidotal (Chiu and Chang, 1995) and *Dipteracanthus patulus* (Jacq.) leaves are used for treating itches, insect bites, venereal diseases, sores, tumours and rheumatic complaints (Murugesu Mudaliar, 1988). Both the plants belong to *Acanthaceae* family.

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Pharmacological and phytochemical studies indicate that it has cardiotoxic activity (Akhtar *et al.*, 1992).

The current review focuses on the restoring capacity of the liver enzymes (AST, ALT, ACP and ALP) by the *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) during the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses.

MATERIALS AND METHODS

Plant material:

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 Agriculture 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° 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 facility 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°C) 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 h 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°C until the analysis was performed. Serum was separated from the blood collected, by centrifugation and the serum was stored at – 4°C for biochemical analysis.

Preparation of tissue homogenate:

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

Estimation of enzymes (AST, ALT, ACP and ALP):

Estimation of aspartate transaminase (AST), alanine transaminase (ALT) was estimated by Reitman and Frank (1957). Acid phosphatase (ACP) and alkaline phosphatase (ALP) was done according to Fiske and Subbarow method (1925).

Statistical analysis:

Data was reported as mean ± SD by using the statistical package of social sciences (SPSS). The data for all the parameters were analyzed by using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant at P < 0.05 (Duncan, 1957).

RESULTS AND DISCUSSION

Table 1, 2, 3 show the activity of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT),

Table 1 : Effect of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on aspartate transaminase, alanine transaminase, alkaline phosphatase and acid phosphatase level in alloxan induced diabetic rat liver

Sr. No.	Group	Aspartate transaminase (μ moles of pyruvate/ min/ mg protein)	Alanine transaminase (μ moles of pyruvate/min/ mg protein)	Alkaline phosphatase (μ moles of phenol /min/mg protein)	Acid phosphatase (μ moles of phenol /min/ mg protein)
1.	Group I	45.71 \pm 2.57	63.68 \pm 2.52	29.97 \pm 2.54	23.54 \pm 2.79
2.	Group II	72.66 \pm 2.88	161.81 \pm 2.45	87.07 \pm 2.83	91.15 \pm 2.85
3.	Group III	40.60 \pm 2.94	75.75 \pm 2.61	39.01 \pm 2.86	44.78 \pm 2.88
4.	Group IV	63.70 \pm 2.51 ^a	144.43 \pm 2.91 ^a	56.95 \pm 2.55 ^a	71.82 \pm 7.21 ^a
5.	Group V	51.51 \pm 2.65 ^b	126.53 \pm 2.68 ^b	43.06 \pm 2.86 ^b	62.36 \pm 1.99 ^b
6.	Group VI	65.81 \pm 2.73 ^c	153.75 \pm 2.68 ^c	69.06 \pm 2.71 ^c	71.50 \pm 2.77 ^c
7.	Group VII	57.73 \pm 2.78 ^d	141.60 \pm 2.62 ^d	53.0 \pm 2.52 ^d	69.25 \pm 2.78 ^d

Values are expressed as mean \pm SD of six rats from each group, $p < 0.05$ 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 2 : Effect of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on aspartate transaminase, alanine transaminase, alkaline phosphatase and acid phosphatase level in the serum of alloxan induced diabetic rat

Sr. No.	Group	Aspartate transaminase (μ moles of pyruvate/ min/ mg protein)	Alanine transaminase (μ moles of pyruvate/min/ mg protein)	Alkaline phosphatase (μ moles of phenol /min/mg protein)	Acid phosphatase (μ moles of phenol /min/ mg protein)
1.	Group I	48.88 \pm 2.62	39.96 \pm 2.68	40.95 \pm 2.53	36.98 \pm 2.63
2.	Group II	100.95 \pm 2.61	80.10 \pm 2.67	63.0 \pm 2.62	60.06 \pm 2.73
3.	Group III	68.90 \pm 2.61	57.08 \pm 2.58	43.91 \pm 2.71	41.03 \pm 2.73
4.	Group IV	74.0 \pm 2.73 ^a	67.06 \pm 2.63 ^a	57.95 \pm 2.75 ^a	51.10 \pm 2.83 ^a
5.	Group V	67.10 \pm 2.41 ^b	61.0 \pm 2.69 ^b	49.06 \pm 2.83 ^b	43.91 \pm 2.69 ^b
6.	Group VI	83.95 \pm 2.60 ^c	74.03 \pm 2.49 ^c	58.50 \pm 3.32 ^c	57.11 \pm 2.67 ^c
7.	Group VII	80.91 \pm 2.67 ^d	68.08 \pm 2.83 ^d	51.03 \pm 2.64 ^d	49.06 \pm 2.52 ^d

Values are expressed as mean \pm SD of six rats from each group, $p < 0.05$ 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 3 : Effect of 50% hydroethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) on acid phosphatase, alkaline phosphatase, aspartate transaminase and alanine transaminase levels in alloxan induced diabetic rat kidney

Sr. No.	Group	Aspartate transaminase (μ moles of pyruvate/ min/ mg protein)	Alanine transaminase (μ moles of pyruvate/min/ mg protein)	Alkaline phosphatase (μ moles of phenol /min/mg protein)	Acid phosphatase (μ moles of phenol /min/ mg protein)
1.	Group I	26.51 \pm 2.55	22.05 \pm 1.70	26.94 \pm 2.60	51.59 \pm 2.65
2.	Group II	67.41 \pm 2.14	52.61 \pm 2.69	52.58 \pm 2.87	150.73 \pm 2.39
3.	Group III	41.56 \pm 2.39	25.66 \pm 2.90	31.68 \pm 2.40	100.60 \pm 2.66
4.	Group IV	60.70 \pm 2.37 ^a	33.65 \pm 2.81 ^a	40.49 \pm 3.28 ^a	115.72 \pm 3.03 ^a
5.	Group V	56.60 \pm 2.70 ^b	27.60 \pm 2.53 ^b	36.12 \pm 2.19 ^b	110.68 \pm 2.54 ^b
6.	Group VI	62.39 \pm 3.39 ^c	36.70 \pm 2.97 ^c	42.83 \pm 2.74 ^c	133.58 \pm 2.78 ^c
7.	Group VII	58.48 \pm 2.47 ^d	30.80 \pm 2.39 ^d	39.72 \pm 2.4 ^d	128.70 \pm 2.32 ^d

Values are expressed as mean \pm SD of six rats from each group, $p < 0.05$ 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.

Alkaline Phosphatase (ALP) and Acid phosphatase (ACP) in serum, liver and kidney of alloxan induced diabetic rats. In comparison to the normal control rats, the activities of these enzymes were found to be increased in the serum, liver and kidney of non-treated diabetic rats. Oral

administration of 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) for 30 days resulted in a significant ($r < 0.05$) decrease in the levels of AST, ALT, ALP and ACP in the serum, liver and kidney in the diabetic rats.

Conclusion:

In transaminase reaction, one amino acid is converted to the corresponding keto acids with simultaneous conversion of another keto acid to an amino acid. The aminotransferases (enzymes) involved in this reaction are: Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) (Ganong, 2001). These enzymes are present in hepatocytes and leak into blood with liver cell damage. In our study, the activity of these enzymes in plasma and liver were higher in non-treated diabetic rats than in control group. This increase may reflect hepatocellular damage associated with diabetes (Begum and Shanmugasundaram, 1978).

The activity of these enzymes have reduced after one month of treatment by 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.), indicating that this plant pulp extract could, as well, have the ability to repair liver tissue damage. The enzyme Alkaline Phosphatase (ALP) is present in the canalicular and sinusoidal membrane of the liver and is also present in many other tissues. In the present study, activity of this enzyme was raised in plasma, liver and kidney of non-treated diabetic rats compared to control group (Kumar and Clark, 2005).

One month of treatment with 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) has led to a reduction in the activity of this enzyme. Acid phosphatase and alkaline phosphatase enzymes are having clinical and toxicological importance as change in their activities is indicative of tissue damage by toxicants. Increased activities of phosphatases in diabetes may affect the transport of metabolites across the membrane due to alteration in dephosphorylation reactions. Enhanced level of phosphatases causes increased intracellular inorganic phosphate, which further affects the efficiency of ionic pumps, which further reflected in decreased activities of Na⁺, K⁺, ATPases in diabetes (Sailaja, 2000). The most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzyme such as serum alkaline phosphatase in the circulation after cellular damage (Sallie *et al.*, 1991).

In addition the soluble enzyme acid phosphatase is released when injury involves in the organelles such as, liver and kidney (Senthil, *et al.*, 2003). In our study the acid phosphatase and alkaline phosphatase enzyme levels were found to be elevated in alloxan induced diabetic animals, which was reduced in 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) treated groups. Andallu and Varadhacharyulu (2001), have also reported that administration of *Aloe vera* extract and *Mullberry* leaves

extract significantly lowered the activities of phosphatases in alloxan induced diabetic rats.

These results are in accordance with those of Rawi *et al.* (1998), who found that the decrease of transaminase activities with treatments may be attributed to the improved liver function to its normal rate. In agreement with the present results, Defronzo, (1999) and Roy *et al.* (1998) showed a slight increase of ALP activity after induction of diabetes by alloxan. Moreover, Roy *et al.* (1998) and Vuksan *et al.* (2000) reported that alloxan diabetes exerted 2 fold increases in serum ALP activity and the treatment of the diabetic animal with ginseng caused a marked decrease in the elevated enzyme. Flavonoids are one of the most numerous and widespread groups of phenolic compounds in higher plants (Carini *et al.*, 2001). The flavonoids such as apigenin and luteolin were shown to possess anti-hyperglycemic (Matsuda *et al.*, 1995; Asgary *et al.*, 2002) and antioxidant activity (Ramonova *et al.*, 2001).

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 (Harbone, 1967; Yoon *et al.*, 2006; Hirano *et al.*, 2006; Akhtar *et al.*, 1992). The preliminary phytochemical analysis revealed the presence of secondary metabolites such as flavonoids, phenol, saponin, glycosides etc. (Manikandan and Victor Arokia Doss, 2009). We suggest here that the mode of action of 50% hydro-ethanolic leaf extracts of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.) may be caused by their contents of phytochemicals (Flavonoids: Luteolin, Apigenin and Lupeol) which reduce the increased AST, ALT, ACP and ALP enzyme levels during diabetes.

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