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Anti-diabetic Activity of Hydroethanolic Leaf Extract of *Delonix regia* on Streptozotocin Induced Diabetic Rats

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Abstract: The present investigation was carried out to study the anti-diabetic effect of the hydroethanolic extract of Delonix regia in streptozotocin induced diabetic rats. The oral administration of hydroethanolic leaf extract of *Delonix regia* at high doze level (400mg/kg body weight) and low doze level (200mg/kg body weight) was done on streptozotocin induced diabetic rats for 21 days. The effect was compared with oral dose of 120mg/kg body weight Glibenclamide. The determination of blood glucose level by GOD-POD kit method. Decreased blood glucose level of the test animals shows that the extract exhibit significant anti-diabetic activity when compared to diabetic control group.

Keywords: *Delonix regia, Anti-diabetic activity, Streptozotocin, GOD-POD kit.*

1. INTRODUCTION

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrates, protein, and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes that are the major causes of morbidity and death (Kameswararao, 2003). Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or resistance to the action of the hormone at the cellular level (Wadkar et al., 2008).

The presence of DM confers increased risk of many devastating complications such as cardiovascular diseases (CVD), Peripheral vascular disease (PVD) and complication such as coronary artery disease (CAD), stroke, neuropathy, renal failure, retinopathy amputations, and blindness (Bajaj et al., 1995). World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for oral therapy (Patel et al., 1997). Recently, many reviews on medicinal plants possessing experimental and clinical anti-diabetic activity that have been used in traditional systems of medicine. The present study undertaken to evaluate the anti-diabetic activity of ethanolic extract of *Delonix regia* in streptozotocin induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant material: The leaf of *Delonix regia* were collected from local market of Coimbatore, Tamilnadu. In this plant authentified by Dr. M. Palanisamy, Scientist 'c'-In-charge, Botanical Survey of India, Southern Region Centre, Coimbatore where a voucher specimen (No: BSI/SRC/5/23/ 2013-14/Tech/1418) has been deposited for further reference.

2.2 Extraction: About 1 kg of coarse powder of *Delonix regia* leaves (Fig.1) were soaked in 50 % alcohol and cold macerated for three days. During the maceration period occasional stirring was done. After three

days, the suspension was filtered through a fine muslin cloth. The residue was removed. The filtrate was taken in a round-bottomed glass flask and the sample was evaporated to dryness at a low temperature in a rotary evaporator. Finally dark brown colored crystals were obtained. It was stored in an air tight desiccator. When needed the residual extracts were dissolved in distilled water and used in the anti-diabetic study.

Fig.1*: Delonix regia* plant

2.3 Animals: Adult male Wister rats 120-140gm were procured from PSG Institute of medical science, and research Coimbatore, Tamilnadu. The study protocol was approved by Institutional Animal ethics committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India (CPCSEA No.221/2014/IAEC). The animals were housed in large spacious hygienic cages inside a well-ventilated room. The animals were fed with rat pellet feed and adequate supply of water.

2.4 Induction of diabetes: Streptozotocin induced diabetic rats were used for the study of anti-diabetic activity. Diabetes mellitus was induced in overnight fasted rats by a single intra-peritoneal injection of streptozotocin (60mg/kg body weight). After 3 days, fasting glucose levels were measured and the animals showing blood glucose level 225mg/dl and above were used (Prasanna shama et al., 2012). Diabetes animals were grouped, five days after induction of diabetes effect of hydroethanolic extract of *Delonix regia* leaves in streptozotocin induced diabetes in rats.

2.5 Experimental design: The experimental rats were divided into 5 groups of 6 animals in each group.

Group I: Normal control rats

Group II: Streptozotocin induced diabetic rats (60mg/kg body weight)

Group III: Diabetic rats treated with Glibenclamide (120mg/kg body weight)

Group IV: Diabetic rats treated with 50% ethanolic extract of *Delonix regia* (200mg/kg)

Group V: Diabetic rats treated with 50% ethanolic extract of *Delonix regia* (400mg/kg)

After the end of experimental treatment period, the animals were fasted overnight and sacrificed by cerivical dislocation under mild chloroform anesthesia. Blood was collected by cardiac puncture and the serum was separated by centrifugation at 2500 rpm for 15 minutes.

2.6 Biochemical estimations

2.6.1 Determination of glucose, protein and glycogen: Glucose (Trinder et al., 1969), Protein (Lowry et al., 1951) were estimated in serum and Glycogen (Vander Vries, 1954) was estimated in liver.

2.6.2 Determination of Lipid profile: Lipid profile such as Cholesterol, Triglycerides, HDL, LDL and VLDL were estimated in serum by diagnostic kit method.

2.6.3 Determination of Liver marker enzymes: AST, ALT (Retimann et al., 1957) and ALP (King, 1934) were estimated in liver.

2.7 Statistical analysis: All the data obtained was expressed as mean ± SD. Statistical analysis was performed by using the method of distribution statistics and analysis of means using R- Statistical computing and graphical tools. A probability of $P<0.05$ was considered significant.

3. RESULTS

3.1 Blood glucose, Protein and Liver glycogen: The effect of hydroethanolic extract of *Delonix regia* on serum glucose, protein and liver glycogen in streptozotocin induced diabetic rats was given in table 1. Administration of streptozotocin led to significant ($p<0.05$) increase in serum glucose and decrease in the levels of protein and liver glycogen in diabetic rats (G2) when compared with normal control rats (G1). After the treatment with Glibenclamide and plant extract showed significantly decreased in blood glucose level and increased in the level of protein and liver glycogen in diabetic treated rats (G3,G4 and G5), when compared to streptozotocin induced diabetic rats (G2).

3.2 Lipid profile: The effect of hydroethanolic extract of *Delonix regia* on Total Cholesterol, HDL, LDL, VLDL and Triglycerides in streptozotocin induced diabetic rats was given in table 2 and 3, followed by figures 1 and 2. The table 2 and 3 showed that there was a significant ($p<0.05$) increase in the levels of Cholesterol, Triglycerides, LDL, VLDL and decreased level of HDL cholesterol in Group II diabetic rats. The above parameters are reversed on treated rats. There was no significant ($p<0.05$) difference between normal control (G1) rats and plant extract treated diabetic rats (G4 and G5).

Table 1: Effect of *Delonix regia* on serum glucose, protein and liver glycogen levels

Groups	Serum glucose mg/dl	Protein (g/dl)	Liver glycogen (mg/g tissue)
Group-I (G1)	88.05 ± 5.16	7.09 ± 0.40	65.50 ± 1.25
Group-II (G2)	317.51 ± 16.47^{a*}	2.71 ± 0.44^{a*}	31.34 ± 1.41^{a*}
Group-III (G3)	105.91 ± 8.89^{b*}	5.90 ± 0.42^{b*}	59.60 ± 1.28^{b*}
Group-IV (G4)	164.88 ± 10.88^{c*}	4.69 ± 0.36^{c*}	42.77 ± 2.02^{c*}
Group-V(G5)	144.36 ± 9.53^{d*}	3.84 ± 0.22^{d*}	52.10 ± 1.81^{d*}

Values are expressed as mean ± SD for six animals; Group comparison: G2 vs G1, G3, G4 and G5
Statistical Significance: * Significant (P< 0.05)

Table 2: Effect of Total cholesterol and Triglycerides in serum levels

Groups	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)
Group-I (G1)	154.79 ± 5.08	66.80 ± 5.47
Group-II (G2)	235.63 ± 5.35^{a*}	182.97 ± 12.84^{a*}
Group-III (G3)	160.72 ± 4.33^{b*}	87.58 ± 5.37^{b*}
Group-IV (G4)	184.23 ± 4.52^{c*}	156.55 ± 4.11^{c*}
Group-V(G5)	175.60 ± 6.08^{d*}	143.53 ± 6.97^{d*}

Values are expressed as mean ± SD for six animals; Group comparison: G2 vs G1, G3, G4 and G5
Statistical Significance : * Significant (P< 0.05)

Table 3: Effect of HDL, LDL and VLDL in serum levels

Groups	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group-I (G1)	47.70 ± 4.06	93.70 ± 6.02	13.35 ± 1.09
Group-II (G2)	17.44 ± 2.54^{a*}	181.59 ± 9.21^{a*}	36.59 ± 2.56^{a*}
Group-III (G3)	44.91 ± 2.70^{b*}	98.28 ± 6.46^{b*}	17.51 ± 1.07^{b*}
Group-IV (G4)	35.50 ± 2.89^{c*}	117.41 ± 3.86^{c*}	31.30 ± 0.82^{c*}
Group-V(G5)	39.64 ± 1.84^{d*}	104.59 ± 3.24^{d*}	28.70 ± 1.39^{d*}

Values are expressed as mean ± SD for six animals; Group comparison: G2 vs G1, G3, G4 and G5
Statistical Significance: * Significant (P< 0.05)

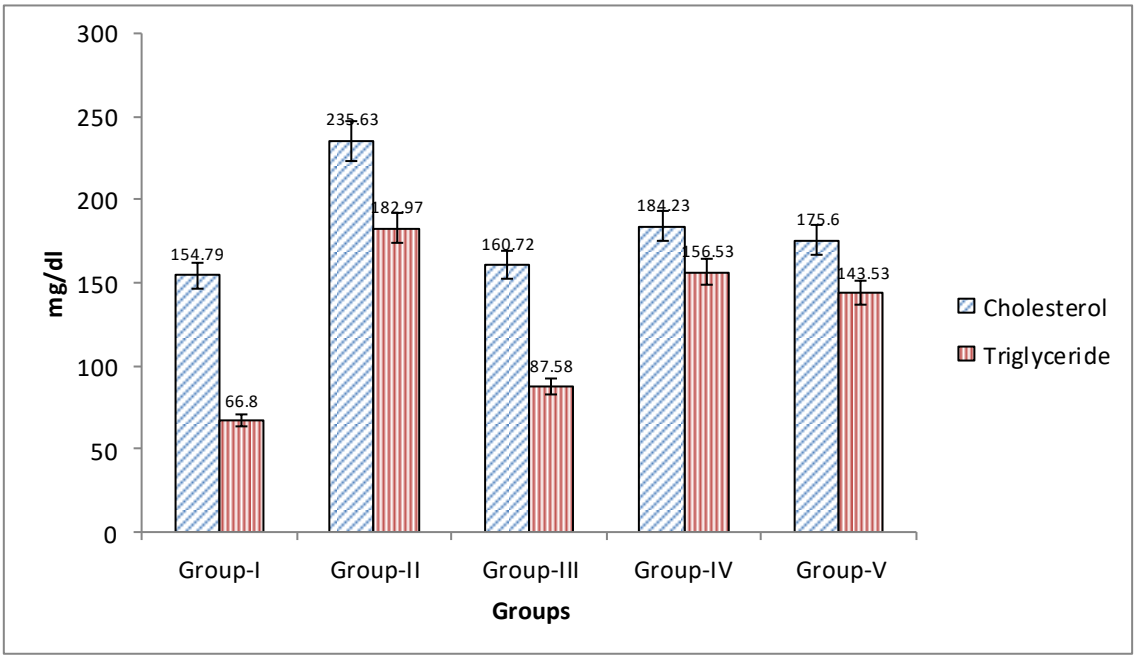

Fig.2: Levels of Total cholesterol and Triglycerides in serum of control and experimental rats

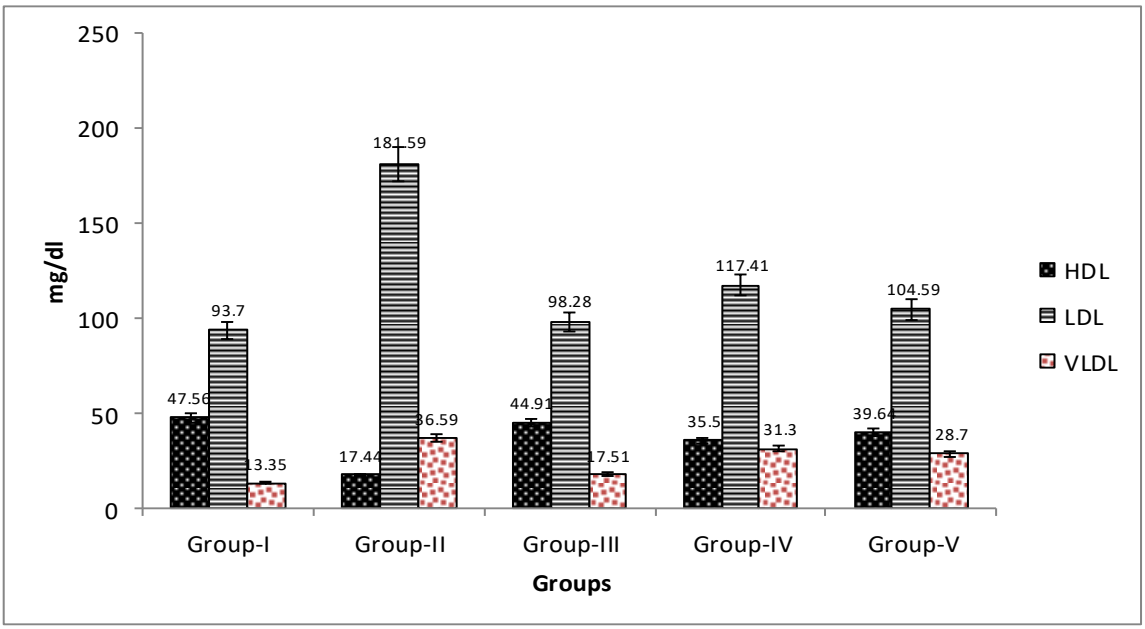

Fig.3: Effect of *Delonix regia* on LDL, HDL and VLDL levels in Control and experimental rats

Table 4: Effect of *Delonix regia* liver marker enzymes

Groups	ALT (IU/L)	AST(IU/L)	ALP(IU/L)
Group-I (G1)	47.66 ± 1.40	18.42 ± 0.72	57.45 ± 2.69
Group-II (G2)	83.50 ± 2.51^{a*}	82.85 ± 3.52^{a*}	84.56 ± 2.34^{a*}
Group-III (G3)	52.49 ± 3.52^{b*}	24.56 ± 3.64^{b*}	60.79 ± 1.02^{b*}
Group-IV (G4)	75.95 ± 4.42^{c*}	42.93 ± 3.22^{c*}	51.22 ± 1.83^{c*}
Group-V(G5)	65.52 ± 5.26^{d*}	38.67 ± 3.05^{d*}	49.22 ± 1.77^{d*}

Values are expressed as mean ± SD for six animals; Group comparison: G2 vs G1, G3, G4 and G5
Statistical Significance: * Significant (P< 0.05).

The effect of hydroethanolic extract of *Delonix regia* on liver marker enzymes such as ALT, AST and ALP levels in streptozotocin induced diabetic rats was given in table 4. Administration of Streptozotocin led to a significant ($p<0.05$) increased in the levels of Alkaline Phosphatases (ALP), Alanine Transaminase (ALT) and Aspartate transaminase (AST) in diabetic rats (G2), when compared with Normal control rats (G1). After treatment with standard Glibenclamide drug in diabetic rats (G3), *Delonix regia* extract low doze in diabetic rats (G4) and high doze in diabetic rats (G5) for a period of 21 days. There was a significant decrease in the levels of ALP, AST and ALT in diabetic treated (G 4 and G5) rats nearly to the normal level.

4. DISCUSSION

The result of the present study confirms that the administration of the hydroethanolic extract of *Delonix regia* possesses anti-diabetic activity against streptozotocin induced diabetic rats. Preliminary phytochemical screening indicates the presence of proteins, steroids, glycosides, tannins, flavonoids, phenols, saponins in hydroethanolic extract of *Delonix regia*. The present study implies that the hydroethanolic extract of *Delonix regia* leaf showed a significant decrease in the serum glucose, protein, liver glycogen, lipid profile and liver marker enzymes.

5. CONCLUSION

The study showed that the anti-diabetic activity of the ethanolic extract of *Delonix regia* against streptozotocin induced diabetic rats. Further studies are needed to identify the active principles responsible for the anti-diabetic effect and to evaluate its exact mechanism of action. Hydroethanolic extract also showed improvement in parameters like body weight, lipid profile and other biochemical parameters.

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