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# Antioxidant and Antidiabetic Effect of *Hibiscus rosasinensis* Flower Extract on Streptozotocin Induced Experimental Rats-a Dose Response Study

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**Abstract**

The present study was designed to evaluate the antidiabetic and antioxidant effect of *Hibiscus rosasinensis* against streptozotocin induced diabetic rats. Streptozotocin (STZ) was administered as a single dose (40 mg/kg) to induce diabetes. The hypoglycemic activity of *Hibiscus rosasinensis* extract (HRSEt) was investigated in a dose dependent manner such as (125, 250 and 500 mg/kg bwt) by evaluating various biochemical parameters. The levels of blood glucose, carbohydrate metabolizing enzymes, TBARS, enzymatic and non-enzymatic antioxidants and lipid profiles were found to be significantly increased in diabetic rats when compared to control groups. Administration of extract in the treated groups showed altered changes in the above mentioned parameters and found that among the three doseses, 250 mg/kg showed best result when compared to other two doses. HRSEt possess antioxidant, hypoglycemic and hypolipidemic activity against streptozotocin induced diabetic rats. However the detailed mechanism(s) of action will require elucidating in further studies.

***Keywords:*** antioxidant, blood glucose, carbohydrate metabolizing enzymes, *Hibiscus rosasinensis*, lipid profiles, streptozotocin

## Introduction

Diabetes mellitus is a group of metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both. The abnormalities in carbohydrate, fat and protein metabolism that are found in diabetes are due to deficient action of insulin on target tissues (Craig *et al*., 2009). The prevalence of diabetes is rapidly rising all over the world. Current estimates are that there are at least 150 million people living with diabetes worldwide of which two-thirds are in developing countries. The total number of people with diabetes is predicted to rise above 300 million by 2025 (Sicree *et al*., 2006). Animal models provide valuable clues in understanding the underlying pathological mechanisms of diabetes and are useful for the screening of drugs for the prevention and treatment of diabetes. Currently induced models have gained widespread acceptance for pathogenesis and drug screening research due to their rapid induction of diabetes. STZ is a commonly used chemical to generate diabetic animals in the laboratory for its ability to destroy insulin producing β-cells (Tjalve, 1983).

Experimental evidences demonstrate that the complication of diabetes is associated with oxidative stress induced by generation of free radicals. In the diabetic state lipid peroxidation can be induced by protein glycation and glucose auto-oxidation that can lead to formation of free radicals. The main free radicals that occur in this state are superoxide hydroxyl and peroxyl radicals. All these free radicals might play a role in DNA damage, glycation, protein modification reactions, and in lipid oxidative modification in diabetes (Sies, 1997). In diabetic state, simultaneous oxidation and glycerol processes are closely correlated with oxidized low density lipoproteins (LDL). In addition, flavonoids and phenolic compounds possessing both antioxidant and antidiabetic activity are effective in preventing the formation of reactive oxygen induced diseases (Di-Carlo *et al*., 1999).

Many synthetic drugs are used in treatment of diabetes, but plant drugs are frequently considered to be less toxic, and are free from side effects. So in in the present study it has been used *Hibiscus rosasinensis* Linn. commonly known as China rose, belongs to the family of *Malvaceae*. It is a common Indian garden perennial shrub and often planted as a hedge or fence plant (Usmanghani *et al*., 1997). Its 250 species are widely distributed in tropical and subtropical regions of the world. The plant is a large evergreen herb commonly cultivated as an ornamental plant and has several forms with varying colour of flowers. Various parts of plant used in traditional medicine. *Hibiscus rosasinensis* is commonly used as cosmetic, hedge, polish and herbal shampoo. Root is demulcent and used for cough, venereal disease and fever (Ross, 1999). Buds are used in the treatment of vaginal and uterine discharges. Leaves and stem barks are used for abortion. Leaves and flowers are good for healing ulcers, and for promoting growth and colour of hair (Adirajan *et al*., 2003). Crude extract of aerial part used in constipation and diarrhea.

*Hibiscus rosasinensis* possesses various pharmacological activities such as radical scavenging (Masaki *et al*., 1995), antibacterial, antipyretic and antiflammatory activities (Singh *et al*., 1978). It has been reported that the plant flower possesses anti-spermatogenic and androgenic (Reddy *et al*., 1997), anti-tumor (Sharma and Sulthana, 2004) and

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anti convulsant (Kasture Chopde and Deshmukh, 2000). The use of flower to treat heart disorders has also been described (Sharma, 1994). Alam *et al.* (1990) has demonstrated the anti-diabetic activity of *Hibiscus rosasinensis* in diabetic rural population. Moreover, another report also shows the effect of *Hibiscus rosasinensis* flower extract on blood glucose and lipid profile in streptozotocin induced diabetic in rats (Sachdewa and Khemani, 2003). Recently, Venkatesh (2008) demonstrated the antidiabetic activity of the flowers of *Hibiscus rosasinensis* in alloxan induced diabetic rats. In the light of above mentioned beneficial activities, *Hibiscus rosasinensis* was investigated for its antidiabetic, antioxidant and antihyperlipidemic activity against streptozotocin induced rats.

**Materials and methods**

*Animals*

4-7 weeks old male albino Wistar rats were purchased from the Tamilnadu Veterinary Animal Science (TANUVAS), Chennai, India. Rats weighing (180-200 g) were maintained under standard conditions of humidity temperature (28 ± 2°C) and light (12 h light/dark). The animals were housed in polypropylene cages (45×24×15cm) and were handled according to the university and institutional legislation, regulated by the ethical committee on animal care of Annamalai University. All the procedures performed on animals were approved and conducted in accordance with the National Institute of Health Guide (Reg. No.488/160/1999/CPCSEA).

*Chemicals*

Streptozotocin was purchased from Sigma chemical company, St. Louis, Mo, USA. All other chemicals and solvents used were of analytical grade.

*Plant material*

*Hibiscus rosasinensis* flowers were collected from the local area, Annamalainagar, Chidambaram, Tamil Nadu, India.

*Preparation of Hibiscus rosasinensis alcoholic flower extract*

Fresh flowers were air-dried in shade at room temperature. 50 g of this powder was mixed with 300 mL ethanol for 24 hours. The extract was filtered using a muslin cloth and concentrated under the room temperature. The solution was evaporated giving a waxy reddish residue. The yield of the extract was 15%. The residue was stored under refrigeration until further analysis.

*Experimental induction of diabetes*

The male albino Wistar rats weighing (180-200 g) were made diabetic by intraperitoneal injections of STZ. The animals were allowed to fast for 24 hrs and were given STZ injection (40 mg/kg bw), with freshly prepared aqueous solution of citrate buffer as vehicle, pH 4.5. The control animals received buffer alone. STZ treated animals were allowed to drink 5% glucose solution over night to overcome drug induced hypoglycemia. After 48 hours of STZ administration, the blood glucose range (200-300 mg/dL) were considered as diabetic rats and used for the experiment.

*Experimental design*

Animal were randomly divided into six groups of six animals each:

Group I: Control rats were given with citrate buffer (pH 4.5) intraperitoneally;

Group II: Rats were made diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg bw) with citrate buffer (pH 4.5);

Group III: Diabetic rats treated with *Hibiscus rosasinensis* alcoholic flower extract (125 mg/kg bw) daily by oral administration for 4 weeks;

Group IV: Diabetic rats treated with *Hibiscus rosasinensis* alcoholic flower extract (250 mg/kg bw) daily by oral administration for 4 weeks;

Group V: Diabetic rats treated with *Hibiscus rosasinensis* alcoholic flower extract (500 mg/kg bw) daily by oral administration for 4 weeks;

Group VI: Control rats treated with *Hibiscus rosasinensis* alcoholic flower extract (250 mg/kg bw) daily by oral administration for 4 weeks.

*Biochemical assays*

Blood glucose was estimated by the method of O-toludine using the modified reagent of Sasaki *et al.* (1972). Plasma insulin was assayed by the method of Burgi *et al.* (1988). Glucose-6-phosphatase was assayed by the method of Koide and Oda (1992). Fructose1,6-bisphosphatase was assayed by the method of Gancedo and Gancedo (1971). Thiobarbituric acid reactive species (TBARS) production was determined by Ohkawa *et al.* (1979), Niehaus and Samuelson (1968). Antioxidants such as Superoxide dismutase (SOD) in tissues was assayed by the method of Kakkar *et al.* (1984). The activity of catalase (CAT) in tissues was determined by the method of Sinha (1972). The activity of Glutathione peroxidase (GPx) in tissues was measured by the method of Rotruck *et al.* (1973). Reduced glutathione in tissues was estimated by the method of Ellman (1959). Lipids were extracted from the tissues by the method of Folch *et al*. (1957). Cholesterol were estimated by the method of Zalks *et al*. (1953). Phospholipids were estimated by the method of Zilversmit and Davis (1950).

*Statistical analysis*

All quantitative measurements were expressed as mean ± SD for control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using statistical package of social science (SPSS) Version 10.0 for Windows.

**Results**

Changes in the body weight, blood glucose and plasma insulin concentrations of rats fed with *Hibiscus rosasinensis* and streptozotocin are presented in Tab. 1. There was an increased in the bodyweight and plasma insulin in control rats and rats fed with the extracts while there was a decreased in the body weight of rats treated with STZ. Blood glucose level was increased in diabetic rats compared with control and control treated groups (group VI). *Hibiscus rosasinensis* at the doses of (125, 250 and 500 mg/kg bw) significantly attenuated the STZ-induced elevated blood glucose concentration. HRSEt (250 mg/kg) showed a sinificant ($P<0.05$) reduction in the blood sugar level compared with other two doses (group III and V).

Tab. 2 demonstrates the effects of HRSEt on the levels of hemoglobin and glycosylated hemoglobin in control and STZ-induced diabetic rats. The level of hemoglobin decreased while glycosylated hemoglobin slightly increased in diabetic rats when compared to extract treated groups. HRSEt 250 mg/kg showed significant increase in hemoglobin and decreased glycosylated hemoglobin levels compared to other doses (group III and V). There were no significant changes between control and controls treated with HRSEt.

Tab. 3 and 4 depicted the levels of carbohydrate metabolic enzymes such as glucose-6-phosphatase and fructose1,6-bisphosphatase in plasma and tissues of control and experimental animals. Glucose-6-phosphatase and fructose1,6-bisphosphatase levels were markedly ($P<0.05$) elevated in STZ-induced diabetic rats compared to group I and VI. Diabetic rats treated with HRSEt (125, 250 and 500 mg/kg bw) showed a significantly decreased in the levels of carbohydrate metabolic enzymes. There is no significant difference between control and control treated groups.

The level of TBARS (lipid peroxidation) in plasma and liver tissues of control, diabetic and HRSEt treated rats in Tab. 5. The concentrations of TBARS were significantly higher in the STZ-induced diabetic rats, compared with control and control treated groups. HRSEt (125, 250 and 500 mg $kg^{-1}$) showed a significant decrease in TBARS lev-

Tab. 1. Effect of HRSEt on body weight, blood glucose and plasma insulin levels of control and experimental rats

| Group | Body weight | | Blood glucose (mg/dL) | Plasma Insulin (µL/mL) |
|---|---|---|---|---|
| | Initial | Final | | |
| Control | 198.98 ± 18.56[a] | 221.3 ± 18.01[a] | 85.26 ± 6.09[a] | 15.09 ± 1.22[a] |
| Diabetic control | 194.86 ± 18.91[a] | 150.7 ± 10.44[b] | 284.18 ± 12.10[b] | 4.46 ± 0.41[b] |
| Diabetic ± HRSEt (125 mg/kg) | 193.58 ± 14.52[a] | 170.86 ± 16.93[c] | 125.8 ± 5.44[c] | 9.05 ± 0.57[c] |
| Diabetic ± HRSEt (250 mg/kg) | 187.36 ± 15.43[a] | 212.46 ± 17.05[a] | 209.78 ± 8.51[d] | 15.24 ± 1.34[a] |
| Diabetic ± HRSEt (500 mg/kg) | 190.21 ± 17.73[a] | 193.6 ± 16.01[d] | 175.12 ± 5.99[e] | 12.36 ± 0.86[d] |
| Control ± HRSEt | 187.98 ± 15.95[a] | 219.23 ± 19.41[a] | 78.02 ± 4.78[a] | 16.02 ± 1.42[a] |

Values are given as mean + S.D (n=6) rats; Values not sharing a common superscript letter differ significantly at $P<0.05$ DMRT; HRSEt-*Hibiscus rosasinensis* ethanolic extract

Tab. 2. Effect of HRSEt on Total Hemoglobin and Glycosylated Hemoglobin levels of control and experimental rats

| Groups | Total hemoglobin (g/dL) | Glycosylated hemoglobin (mg/g of Hb) |
|---|---|---|
| Control | 14.35 ± 1.27[a] | 1.72 ± 0.14[a] |
| Diabetic control | 5.39 ± 0.32[b] | 5.39 ± 0.32[b] |
| Diabetic ± HRSEt (125 mg/kg bw) | 8.4 ± 0.38[c] | 1.4 ± 0.08[c] |
| Diabetic ± HRSEt (250 mg/kg bw) | 13.6 ± 1.15[d] | 2.61 ± 0.22[d] |
| Diabetic ± HRSEt (500 mg/kg bw) | 11.55 ± 0.95[e] | 0.55 ± 0.03[e] |
| Control ± HRSEt (250 mg/kg bw) | 14.68 ± 1.32[a] | 1.6 ± 1.11[a] |

Values are given as mean ± S.D (n=6 rats); Values not sharing a common superscript differ significantly at $P<0.05$ (DMRT); HRSEt-*Hibiscus rosasinensis* ethanolic extract

Tab. 3. Effect of HRSEt on the levels of glucose-6-phosphatase in plasma and tissues of control and experimental rats

| Group | Glucose-6-Phosphatase | |
|---|---|---|
| | Plasma (µmole of Pi liberated/min/mL) | Liver (U[@]/min/mg protein) |
| Control | 0.25 ± 0.01[a] | 0.18 ± 0.01[a] |
| Diabetic control | 0.68 ± 0.04[b] | 0.68 ± 0.04[b] |
| Diabetic + HRSEt (125 mg/kg) | 0.59 ± 0.03[c] | 0.54 ± 0.03[c] |
| Diabetic + HRSEt (250 mg/kg) | 0.38 ± 0.03[d] | 0.36 ± 0.03[d] |
| Diabetic + HRSEt (500 mg/kg) | 0.48 ±0.03[e] | 0.47 ± 0.04[e] |
| Control + HRSEt | 0.27 ± 0.02[a] | 0.20 ± 0.01[a] |

U[@] = µmole of Pi liberated/min/mg protein; Values are given as mean ± S.D (n=6 rats);Values not sharing a common superscript differ significantly at $P<0.05$ (DMRT); HRSEt-*Hibiscus rosasinensis* ethanolic extract

Tab. 4. Effect of HRSEt on the levels of fructose1, 6-bisphosphatase in plasma and tissues of control and experimental rats

| Group | Fructose 1, 6-bisphosphatase | |
|---|---|---|
| | Plasma (µmole of Pi liberated/min/mL) | Liver (Unit$/min/mg protein) |
| Control | 0.48 ± 0.03[a] | 0.37 ± 0.03[a] |
| Diabetic control | 0.81 ± 0.06[b] | 0.64 ± 0.04[b] |
| Diabetic + HRSEt (125 mg/kg) | 0.72 ± 0.05[c] | 0.59 ± 0.03[c] |
| Diabetic + HRSEt (250 mg/kg) | 0.59 ± 0.05[d] | 0.45± 0.04[d] |
| Diabetic + HRSEt (500 mg/kg) | 0.63 ± 0.04[e] | 0.54 ± 0.04[e] |
| Control + HRSEt | 0.50 ± 0.02[a] | 0.38 ± 0.02[a] |

U$ =µmole of Pi liberated/min/mg protein; Values are given as mean ± S.D. (n=6 rats); Values not sharing a common superscript differ significantly at P<0.05 (DMRT); HRSEt-*Hibiscus rosasinensis* ethanolic extract

Tab. 5. The levels of plasma and tissues TBARS levels in control and experimental rats

| Group | TBARS | |
|---|---|---|
| | Plasma (mmoles/dL) | Liver (mmoles/100g wet tissue) |
| Control | 0.203 ± 0.02[a] | 0.76 ± 0.06[a] |
| Diabetic control | 0.421 ± 0.03[b] | 3.33 ± 0.31[b] |
| Diabetic + HRSEt (125 mg/kg) | 0.387 ± 0.02[c] | 2.94 ± 0.24[c] |
| Diabetic + HRSEt (250 mg/kg) | 0.243 ± 0.01[a] | 1.31 ± 0.13[d] |
| Diabetic + HRSEt (500 mg/kg) | 0.306 ± 0.02[d] | 2.45 ± 0.15[e] |
| Control + HRSEt | 0.210 ± 0.01[a] | 0.79 ± 0.07[a] |

Values are given as mean ± S.D (n=6 rats); Values not sharing a common superscript differ significantly at P<0.05 (DMRT); HRSEt-*Hibiscus rosasinensis* ethanolic extract

Tab. 6. Effect of HRSEt on the activities of enzymatic antioxidants in the erythrocyte lysate of control and STZ induced diabetic rats

| Groups | Erythrocyte lysate | | |
|---|---|---|---|
| | SOD ($U^A$/mg Hb) | CAT ($U^B$/mg Hb) | GPx($U^C$/mg Hb) |
| Control | 7.8 ± 0.54[a] | 149.03 ±12.92[a] | 25.5 ± 1.39[a] |
| Diabetic control | 4.18 ± 0.27[b] | 74.16 ± 6.10[b] | 9.59 ± 0.58[b] |
| Diabetic +HRSEt (125 mg/kg bw) | 5.14 ± 0.28[c] | 92.31 ± 6.80[c] | 13.27 ± 1.26[c] |
| Diabetic + HRSEt (250 mg/kg bw) | 7.33 ± 0.56[a] | 127.82 ± 11.87[d] | 20.75 ± 1.05[d] |
| Diabetic + HRSEt (500 mg/kg bw) | 6.15 ± 0.31[d] | 110.83 ± 8.9[e] | 15.41± 1.27[e] |
| Control + HRSEt (250 mg/kg bw) | 7.85 ± 0.55[a] | 147.02 ± 11.77[a] | 24.06 ± 1.68[a] |

$U^A$ = enzyme concentration required to inhibit the NBT to 50% in one minute; $U^B$ = µmole of H2O2 consumed/minute; $U^C$ = µg of GSH utilized/minute; Values are given as mean ± S.D (n=6 rats); Values not sharing a common superscript differ significantly at P<0.05 (DMRT); HRSEt-*Hibiscus rosasinensis* ethanolic extract

Tab. 7. Effect of HRSEt on the activities of enzymatic antioxidants in the liver of control and experimental rats

| Groups | Liver | | |
|---|---|---|---|
| | SOD ($U^A$/mg protein) | CAT ($U^B$/mg protein) | GPx ($U^C$/mg protein) |
| Control | 9.32 ± 0.27[a] | 72.83 ± 4.33[a] | 7.93 ± 0.63[a] |
| Diabetic control | 4.41 ± 0.32[b] | 42.91 ± 3.2[b] | 4.13 ± 0.31[b] |
| Diabetic +HRSEt (125 mg/kg bw) | 5.63 ± 0.54[c] | 51.48 ± 1.35[c] | 5.15 ± 0.36[c] |
| Diabetic + HRSEt (250 mg/kg bw) | 7.5 ± 0.62[d] | 67.89 ± 3.32[d] | 7.54 ± 0.22[a] |
| Diabetic + HRSEt (500 mg/kg bw) | 6.86 ± 0.57[e] | 58.89 ± 2.71[e] | 6.66 ± 0.42[d] |
| Control + HRSEt (250 mg/kg bw) | 9.68 ± 0.69[a] | 76.03 ± 3.74[a] | 8.16 ± 0.78[a] |

$U^A$ = enzyme concentration required to inhibit the NBT to 50% in one minute; $U^B$ = µmole of H2O2 consumed/minute; $U^C$ = µg of GSH utilized/minute; Values are given as mean ± S.D (n=6 rats); Values not sharing a common superscript differ significantly at P<0.05 (DMRT); HRSEt-*Hibiscus rosasinensis* ethanolic extract

Tab. 8. Effect of SNFEt on the concentration of reduced glutathione in the plasma and liver of control and ethanol administered rats

| Groups | Reduced glutathione | |
|---|---|---|
| | Plasma (mg/dL) | Liver (mg/100 g wet tissue) |
| Control | 29.15 ± 1.61[a] | 46.72 ± 3.61[a] |
| Diabetic control | 13.69 ± 0.81[b] | 24.4 ± 2.12[b] |
| Diabetic + HRSEt (125 mg/kg bw) | 16.0 ± 0.99[c] | 29.38 ± 2.42[c] |
| Diabetic + HRSEt (250 mg/kg bw) | 26.15 ± 1.11[d] | 41.03 ± 2.56[d] |
| Diabetic + HRSEt (500 mg/kg bw) | 17.95 ± 1.24[e] | 34.43 ± 2.75[e] |
| Control + HRSEt (250 mg/kg bw) | 29.33 ± 1.92[a] | 48.32 ± 3.52[a] |

Values are given as means ± SD for six rats in each group; Values not sharing a common superscript differ significantly at P<0.05 (DMRT)

el. 250 mg $kg^{-1}$ dose showed better result than other two doses (Group III and V). No significant changes were observed between control and control treated groups.

The status of enzymatic antioxidants (SOD, CAT and GPx) in erythrocytes lysate and liver tissue of all experimental groups are depicted in Tab. 6 and 7. Activities of enzymatic antioxidants were markedly reduced in diabetic group compared with control and control treated groups. HRSEt (125, 250 and 500 mg/kg bw) showed a significant (P<0.05) increase in the activities of enzymatic antioxidants (SOD, CAT and GPx). HRSEt 250 mg/kg however was more effective than other two doses.

Changes in the level of non-enzymatic antioxidants (GSH) in plasma and liver of control and experimental animals are depicted in Tab. 8. In rats treated with STZ there was a statistically decreased GSH levels, when compared to group I and group VI. HRSEt 250 mg/kg showed a markedly elevated in activities of antioxidants compared

Tab. 9. Effect of HRSEt on the activities of cholesterol in plasma and liver of control and STZ-induced diabetic rats

| Group | Cholesterol | |
|---|---|---|
| | Plasma (mg/dL) | Liver (mg/100 g tissue) |
| Control | 93.35 ± 6.50[a] | 167.23 ± 9.9[a] |
| Diabetic control | 155.23 ± 12.45[b] | 334.5 ± 23.29[b] |
| Diabetic + HRSEt (125 mg/kg) | 131.87 ± 10.31[c] | 288.1 ± 11.95[c] |
| Diabetic + HRSEt (250 mg/kg) | 107.17 ± 8.98[d] | 197.93 ± 10.9[d] |
| Diabetic + HRSEt (500 mg/kg) | 120.81 ± 10.12[e] | 239.43 ± 12.97[e] |
| Control + HRSEt | 92.72 ± 7.56[a] | 174.66 ± 16.74[a] |

Values are given as mean ± S.D (n=6 rats); Values not sharing a common superscript differ significantly at P<0.05 (DMRT); HRSEt-*Hibiscus rosasinensis* ethanolic extract

Tab. 10. Effect of HRSEt on the activities of phospholipids in plasma and liver of control and diabetic rats

| Group | Phospholipids | |
|---|---|---|
| | Plasma (mg/dL) | Liver (mg/100 g tissue) |
| Control | 1.37±0.11[a] | 1.61 ± 0.12[a] |
| Diabetic control | 4.33 ± 0.37[b] | 2.71 ± 0.16[b] |
| Diabetic + HRSEt (125 mg/kg) | 3.33 ± 0.27[c] | 2.43 ± 0.13[c] |
| Diabetic + HRSEt (250 mg/kg) | 2.02 ± 0.15[a] | 1.98 ± 0.08[a] |
| Diabetic + HRSEt (500 mg/kg) | 2.74 ± 0.17[d] | 2.24 ± 0.13[d] |
| Control + HRSEt | 1.37 ± 0.11[a] | 1.71 ± 0.11[a] |

Values are given as mean ± S.D (n=6 rats); Values not sharing a common superscript differ significantly at P<0.05 (DMRT); HRSEt-*Hibiscus rosasinensis* ethanolic extract

with other two groups (group III and V). No significant difference between control and control treated groups.

Tab. 9 and 10 shows the levels of cholesterol and phospholipids in plasma and liver of normal and experimental groups. Cholesterol and phospholipids were significantly increased in diabetic rats as compared to control and control treated groups. Oral administration of HRSEt at (125, 250 and 500 mg $kg^{-1}$ bw) significantly (P<0.05) decreased

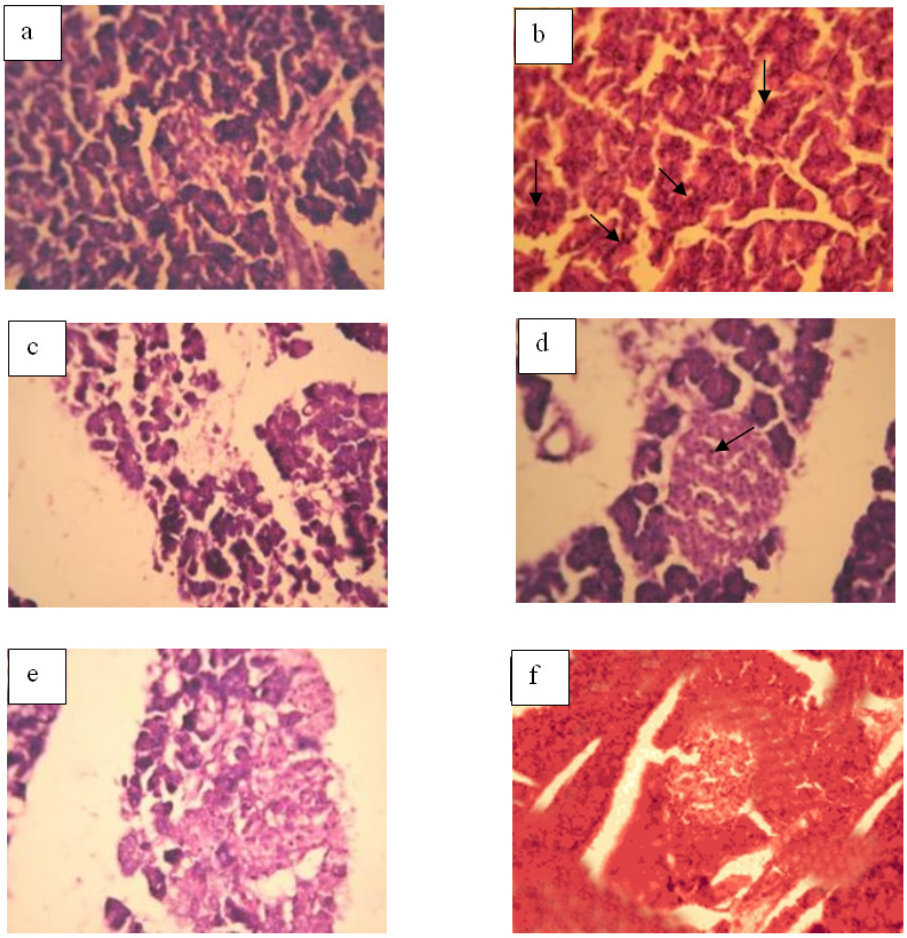


Fig. 1. Histopathological examination of Pancreatic tissues of control and experimental animals: Group I-normal islet cells (a); Group II-shrinkage of islet cells and growth of adipose tissue (b); Group III-Mild shrinkage of islet cells (c); Group IV-reduction in adipose tissue and pancreatic islets within normal limit (d); Group V-Very mild shrinkage of islet cells (e); Group VI-normal islet cells (f)

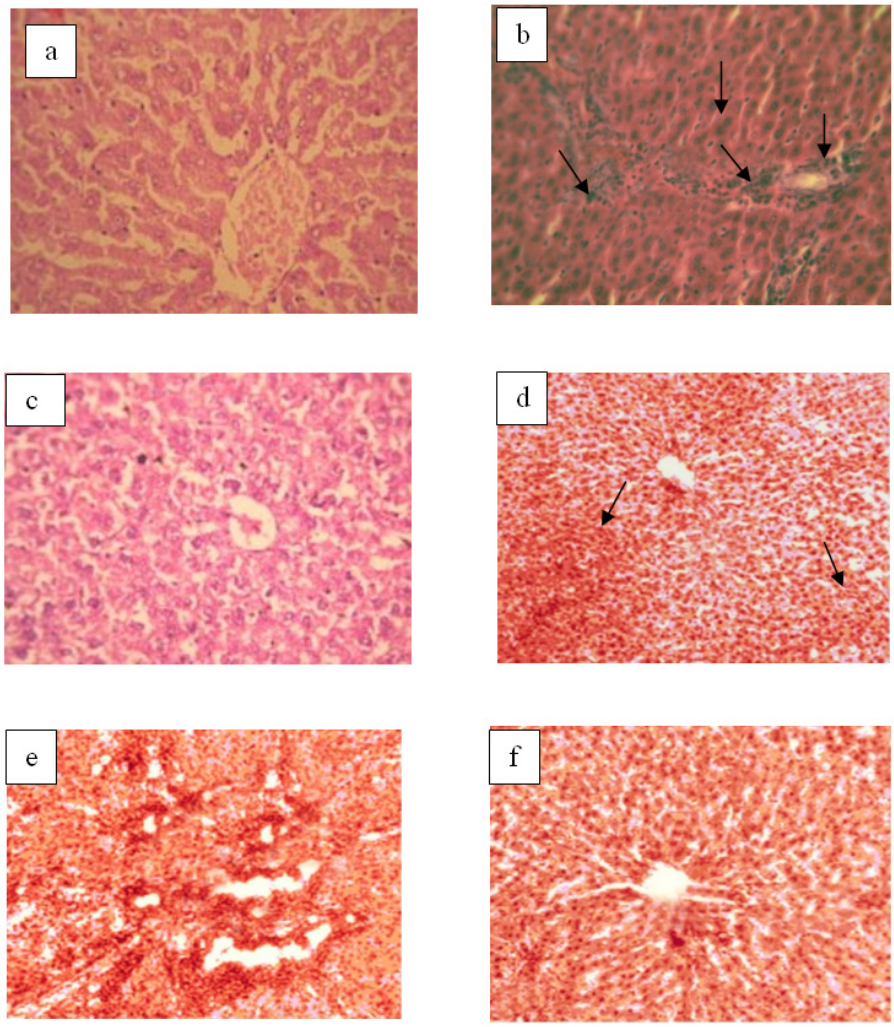


Fig. 2. Histopathological examination of Liver tissues of control and experimental animals: Group I-Normal liver around central vein (a); Group II-Fatty changes and inflammatory cell infiltration (b); Group III-Mild reduction in fatty changes and inflammatory cell infiltration (c); Group IV-Marked reduction in fatty changes and inflammatory cells infiltration (d); Group V-Very mild reduction in fatty changes and inflammatory cell infiltration (e); Group VI-No changes in liver (f)

the levels of lipid profiles. No significant changes were observed in control and control treated groups.

Histopathological changes of pancreas are showed in Fig. 1. Diabetic pancreas showed shrinkage of islet cells and growth of adipose tissue. Treatment with HRSEt (250 mg $kg^{-1}$ bw) effectively reduced pathological changes when compared to other two doses (125 and 500 mg $kg^{-1}$ bw) respectively. Mild shrinkage of islet cells was observed in the pancreas of HRSEt (125 mg $kg^{-1}$ bw) treated rats. Pancreatic tissues with very mild shrinkage of islet cells were observed in the pancreas of HRSEt (500 mg $kg^{-1}$ bw) treated rats. Pancreatic tissue showing reduction in adipose tissue and pancreatic islets within normal limit were observed in HRSEt (250 mg $kg^{-1}$ bw) treated rats.

Histopathological changes in liver are showed in Fig. 2. Diabetic rats showed fatty changes and inflammatory cell infiltration. Oral administration of HRSEt (125 mg/kg bw) showed mild reduction in fatty changes and inflammatory cells infiltration. HRSEt (250 mg/kg bw) showed marked reduction in fatty changes and inflammatory cells infiltration. Administration of (500 mg/kg bw) showed very mild reduction in fatty changes and inflammatory cells infiltration.

**Discussion**

Type I and type II diabetes is characterized by hyperglycemia and markedly diminished by insulin secretion and insulin function. Over the long term, hyperglycemia generates a larger number of ROS which induces oxidative stress. In physiological condition, antioxidant enzyme protects the cells against harmful free radicals. A number of plant derived products have been possessed hypoglycemic, hyperlipidemic as well as antioxidant properties (Vasu *et al*., 1975). *Hibiscus rosasinensis* was recently demonstrated to elicit anti-hyperglycemic and antioxidant activity. Inthe present study, untreated diabetic rats showed severe body weight loss. This characteristic weight loss in diabetic rats could be due to degradation and catabolism of fats and

proteins (Mc Nurlan and Garlick, 1979). Thus, increased catabolic reactions leads to muscle wasting which may be the major cause for weight loss in diabetic rats (Rajkumar *et al*., 1991). However, extract treated groups showed a sign of recovery in the body weight which suggest the protective effect of the extract by preventing it from muscle wastage and other macromolecular degradations.

The present study confirms the antihyperglycemic and antihyperlipidemic effects of *Hibiscus rosasinensis* in streptozotocin induced diabetic rats. Administration of HRSEt to diabetic rats reduced the blood glucose levels to near normal and the optimum activity of the extract was found at the dose of 250 mg/kg bwt. Although, the exact mechanism of action of the extract is unknown, the reduction in blood glucose level could be due to increased pancreatic insulin secretion from existing β-cell of the pancreas (Ghosh and Suryawanshi, 2001). This anti-hyperglycemic activity of *Hibiscus rosasinensis* was associated with increase in plasma insulin levels. The extents of changes in insulin levels with plant phenolic extract are insufficient to account for the obvious improvement in the glucose profile (Sachdewa and Khemani, 1999).

The liver is an important organ that plays a vital role in glycolysis and gluconeogenesis pathways. Glucose-6-phosphatase is the key enzyme in homeostatic regulation of blood glucose level (Massillon *et al*., 1996). It catalyzes the hydrolysis of glucose-6-phosphate to glucose and phosphate. Glucokinase activity was decreased in the liver of diabetic rats, which may be due to deficiency of insulin. Decrease in the enzymatic activity of hexokinase and increase in the level of glycogen phosphorylase observed in the present study are responsible for the depletion of liver glycogen. In addition fructose1,6-bisphosphatase catalyzes on the irreversible step in gluconeogenesis and serves as a site in the regulation of the process (Gupta *et al*., 1999). Glucose is transported out of the liver to increase in blood glucose concentration. Normally insulin inhibit the hepatic glucose production by glucose-6-phosphatase and fructose-1,6-bisphosphatase activity. Administration of *Hibiscus rosasinensis* ethanolic extract (HRSEt) decreased both enzymes activities in diabetic rats in a dose dependent manner thereby decreasing gluconeogenesis.

Lipid peroxidation is a free radical induced process leading to oxidation of polyunsaturated fatty acids. It is found to be an important pathophysiological event in variety of diseases including cancer, diabetes and cardiovascular disorders. The present results showed a significant increase in lipid peroxidation in plasma as well as in tissues of rats treated with STZ. This confirms the earlier report on the ability of this diabetogenic compound to induce oxidative damage through generation of free radicals (Sakudelski, 2001). In addition, the increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels which upon autoxidation generates free radicals (Wolf and Dean, 1987). The formation of reactive oxygen species (ROS) from different sources such as enzymatic reaction and xenobiotic metabolism may lead to lipid peroxidation with subsequent cell injury and toxicity (Yamasaki *et al*., 1996). The increase in liver and kidney tissues may contain relatively high concentration of easily peroxidizable fatty acids. The administration of *Hibiscus rosasinensis* ethanolic extract (HRSEt) reduced MDA, a marker of lipid peroxidation in the tissues of diabetic rats suggesting that the extract possesses potent antioxidative properties. Recent studies show that various plants and plant extracts can also stimulate the synthesis of cellular antioxidants. *Hibiscus rosasinensis* and its phenolic compounds, which are widely distributed in plant have been considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living system (Essa and Subramanian, 2006). The strong anti-lipidperoxidative effect shown by the extract is in accordance with the earlier findings (Ahmed and Sharma, 1997).

The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic scavenger system (Halliwell and Gutterridge, 1994). SOD, CAT and GPx constitute a mutually supportive of defense against ROS. Functions of these antioxidant enzymes are interconnected and a decrease of their activities resulting in the accumulation of lipid peroxides and increase oxidative stress in diabetic rats (Sen and Hanninen, 1994). The level of enzymatic antioxidants (SOD, CAT and GPx) was significantly restored to near normal after treatment of HRSEt. This indicates the modulatory activity of HRSEt since antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase which have a direct role in scavenging free radicals, were also partly restored by the plant extract (Panneerselvam and Govindasamy, 2004). Parallel to these effects reduction in the peroxidation of lipids and formation of hydrogen peroxide content occurred. Inhibition of hydrogen peroxide implies that further generation of hydroxyl radicals is also reduced which protects the cells by making them less prone to attack by these damaging moieties (Amina and Hamza, 2006). The phytochemical screening of *Hibiscus rosasinensis* showed the presence of flavonoids and phenolic compounds which have been reported to antioxidant activity. It is believed that phenolic antioxidants can coverage harmful free radicals and thus inhibit their oxidative reaction with vital biological molecules (Rice-Evens *et al.,* 1996 ).

GSH plays a crucial role in cell defense against ROS. The decreased in GSH level may be associated with reactive oxygen species (ROS) generation by mechanisms creating oxidative stress in chronic hyperglycemia (Scholz *et al*., 1997). Furthermore the depletion in GSH level may be related to the apparent increased in lipid peroxidation in the tissues of rats exposed to STZ. Recent studies have revealed lowered antioxidant enhanced peroxidative status diabetic condition particularly in the liver and kidney (Gumieniczek, 2005). In the present study HRSEt is a source of many potentially active antioxidants and active

constituents, such as flavonoid, flavonoid glycosides, hibiscetin, cyandine, cyanine glycoside. This would be the reason for the enhanced activities of antioxidant levels in extract treated groups.

The present investigation shows that the plasma lipid level is usually raised during diabetes, and also a risk factor for coronary heart disease. The lowering of plasma lipid levels through dietary and drug therapy appears to be associated with a decrease in the risk of vascular disease (Brown *et al*., 1993). Furthermore the present data indicate an increased in lipid profile levels in the liver and kidney of rats treated with streptozotocin. Increased cholesterol is one of the risk factor for many cardiovascular diseases. Hypercholesterolemia is directly proportional to the severity of diabetes. During diabetic conditions lipogenesis is decreased while lipolysis is increased in the hepatic tissue. The increased level of cholesterol in tissues is due to the decreased level of high density lipoprotein (HDL) cholesterol. The observed hypolipidemic effect may be due to decreased cholesterogenesis and fatty acid synthesis (Bopanna *et al*., 1997). The strong antihyperlipedemic effect shown by the HRSEt extract was also in accordance with earlier findings. Significantly lowering of total cholesterol and rise in HDL-Cholesterol in biochemical state for prevention of atherosclerosis (Schwab, 2000). Phospholipids are vital compounds of biomembrane and cholesterol is response for the enhanced synthesis of ischemic phospholipids levels. Hence the increase in the level of tissue lipids may be the result of an increased uptake and mobilization of lipids from the portal system (Yadav *et al*., 2005). The results are consistent with a recent study that ability of the aqueous extract of *Hibiscus sabdariffa* to possess hypolipidemic properties.

Therefore, among three doses of *Hibiscus rosasinensis* extract, medium dose (250 mg/kg bwt) exhibited possible antidiabetic activity, on the other hand diabetic rats treated with low dose (125 mg/kg bwt) did not show any remarkable changes when compared to control whereas diabetic rats treated with maximum dose (500 mg/kg bwt) developed diarrhea and weight loss.

## Conclusions

Thus from the above findings it is illustrated that, *Hibiscus rosasinensis* extract exhibited antioxidant, antihyperglycemic and antihyperlipidemic activities mainly at the dose of 250 mg/kg bwt without showing any toxic effects. Further studies on the characterization and the active principles responsible for antidiabetic activity of *Hibiscus rosasinensis* are highly warranted.

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