

**PREVENTION OF INSULIN RESISTANCE BY SILYMARIN IN RATS****SANTOSH A. DESHMUKH, SHIVAKUMAR P. RATHOD, CHANDRASHEKHAR D. UPASANI, AMAN B. UPAGANLAWAR**

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**ABSTRACT**

**Objective:** The aim of this study was to evaluate the effect of silymarin on the dexamethasone and fructose – induced insulin resistance since its protective effects have been addressed in other models of cell damage induced by drugs.

**Methods:** Insulin resistance was induced by administration of either dexamethasone or fructose. In both models effect of concomitant administration of silymarin in two divided doses 100 and 200 mg/kg continued till end of the experiment were studied. Dexamethasone was administered to the overnight-fasted rats and continued till the end of the experiment along with silymarin 100 or 200 mg/kg p.o. At the end of the experimental period i.e. on day 11 in dexamethasone insulin resistance model, the serum glucose triglyceride and body weight was determined. Subsequently, insulin secretion from isolated perfused pancreas was determined. On day 21 in fructose induced insulin resistance model the estimation of serum glucose, cholesterol triglyceride and insulin were done. Then the animals were sacrificed, a piece of liver was dissected out for determination of glycogen as per the method described by Montgomery.

**Results:** Silymarin 200 mg/kg treatment with dexamethasone significantly inhibited the dexamethasone induced increase in serum glucose, triglyceride level, insulin secretion and reduction in body weight. After 21 days increase in serum glucose and insulin levels in fructose feeding rats was significantly decreased in group treated with silymarin 200 mg/kg. Silymarin treatment to fructose fed rats increases glycogen levels of tissue compared to fructose control group

**Conclusions:** Silymarin might improve insulin resistance through enhanced insulin sensitivity in peripheral tissues. The results obtained in the present investigation indicate that silymarin may have considerable therapeutic potential in the treatment of insulin resistance in NIDDM and its complications.

**Keywords:** Dexamethasone, Silymarin, Insulin resistance, Fructose, Diabetes mellitus

**INTRODUCTION**

Hyperglycemia or diabetes mellitus is the world's largest endocrine disease with increase rate in both developing and developed countries. Non insulin dependent diabetes mellitus primarily characterized by insulin resistance. The skeletal muscle, liver and adipose tissue become resistant to insulin action contributing to hyperglycemia. These impairments in insulin action leads to decrease in insulin mediated glucose disposal, hepatic glucose overproduction and a marked increase in lipolysis [1]. These impairments not only cause hyperglycemia of NIDDM but also cause long term complication. Therefore improvement in peripheral insulin resistance would be beneficial for the long term management of NIDDM [2].

*Silybum marianum (L.) Gaertn.* is a member of *Asteraceae* family (*Compositae*) and its seed extract contains large numbers of chemical constituents including several flavonolignans collectively known as silymarin. Silymarin has powerful antioxidant properties and protect liver against xenobiotic injury and to affect favourably the course of liver disease such as cirrhosis and steatosis. These activities of silymarin are supposed to be based mainly on its antioxidative and membrane stabilizing properties [3]. Protective effect of antioxidants in the treatment of oxidative metabolic derangement in diabetes has been reported in several experiments also several clinical trials of short duration have demonstrated that treatment with antioxidants such as vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin – resistant individuals and /or patients with type II diabetes [4].

**MATERIALS AND METHODS****Drug and chemicals**

Silymarin and Dexamethasone was obtained from olcare labs, Surendranagar and Cadilla Pharmaceuticals, Ahmadabad, Gujarat respectively. For estimation of glucose, triglyceride and cholesterol,

kits (Span diagnostics, India) were used. Serum insulin was estimated by radioimmunoassay (RIA) using RIA kit, INSIK-5 (Diasorin, Italy) with a gamma counter. All the estimations were carried out as per the instructions provided by the kit manufacturers.

**Animals**

Male wistar albino rats weighing 180-250 g were obtained from animal house, Pharmacy Department, M.S.University of Baroda. The rats were maintained under standard laboratory condition (12h light, temperature 23± 1°C). They fed dry ration *ad lib*. This study was conducted in Pharmacy Department, Faculty of Engg. & Tech., M.S.University of Baroda, Vadodara, Gujarat, India. The experimental protocols were approved by the Institutional Animal Ethics Committee of Pharmacy Department, Faculty of Engg. & Tech., M.S.University of Baroda, Vadodara, Gujarat, India and were in accordance with the guidelines of the committee for the purpose of control and supervision on experiments on animals.

**Experimental Design****Animal Study Protocol**

Dexamethasone induced insulin resistance [5, 6]

Thirty six rats were divided into six groups with six rats in each group.

Group 1. Normal control

Vehicles used for silymarin (carboxymethyl cellulose, 1 % orally) and for dexamethasone (saline solution s.c.)

Group 2. Silymarin control-1 (SM - 100)

Silymarin (100 mg/kg, b.w. p.o. once daily).

Group 3. Silymarin control-2 (SM 200)

Silymarin (200 mg/kg, b.w. p.o. once daily)

Group 4. Dexmathasone control (DC)

Dexamethasone sodium phosphate 10 mg/kg, once daily, s.c.

Group 5. Dexamethasone (10 mg/kg, once daily, s.c.) + Silymarin (100 mg/kg, b.w. p.o. once daily)

Group 6. Dexamethasone (10 mg/kg, once daily, s.c.) + Silymarin (200 mg/kg, b.w. p.o. once daily)

Dexamethasone was administered to the overnight-fasted rats and continued till the end of the experiment along with silymarin 100 or 200 mg/kg p.o. in two divided doses. At the end of the experimental period i.e. on day 11, the animals were anesthetized with ether; blood was collected by retroorbital puncture and serum separated for the estimation of glucose (glucose oxidase method) and triglyceride (GPO method). The animals were weighed at the beginning and at the end of the experimental period.

Insulin secretion *in vitro*: Subsequently, an isolated perfused pancreas was prepared (10). The preparation consisted of pancreas with a small residue of duodenum. The preparation was perfused via the superior mesenteric artery and the celiac trunk at a constant rate of 4 ml/min without recirculation. The perfusate was modified Krebs - Ringer bicarbonate solution with 0.25 % bovin serum albumin (RIA grade) and 4 % dextran T40. The perfusate was gassed with 95% O<sub>2</sub> and 5 % CO<sub>2</sub> to give a pH of 7.4. Flow rate was kept constant at 4 ml/min by a peristaltic pump for the entire perfusion period, which lasted for about 70 min. Glucose concentration in the perfusate was 300 mg/dl. After an equilibrium period (30 min.), we analysed insulin secretion from the isolated organ during the 30 minutes period between minutes 15 and 45. Effluent from the portal vein was collected over 1-min time intervals into tubes that were immediately frozen and stored at - 20° C until required for insulin radioimmunoassay (10, 11, and 12).

Fructose induced insulin resistance model [7,8]

Twenty four rats were divided into four groups with six rats in each group.

Group 8. Normal Control

Vehicle used for silymarin (carboxymethyl cellulose, 1 % orally) and fed with tap water

Group 9. Fructose control (FC)

Fed with 10 % w/v fructose solution *ad libitum* in feeding bottle for 20 days.

Group 10. Fructose + Silymarin (100mg/kg)

Fed with 10 % fructose solution *ad libitum* along with silymarin (100 mg/kg, b.w. p.o. once daily) for 20 days.

Group 11. Fructose + Silymarin (200mg/kg)

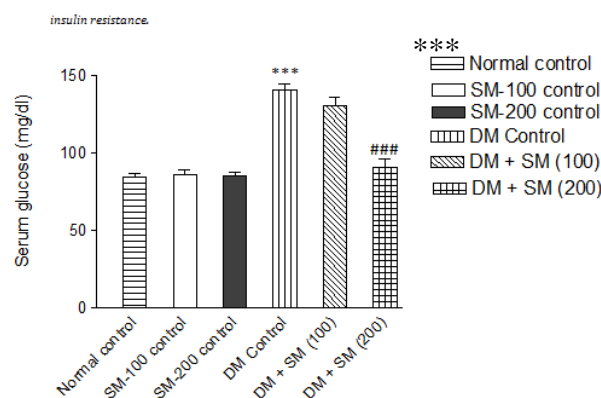
Fed with 10 % fructose solution *ad libitum* along with silymarin (200 mg/kg, b.w. p.o. once daily) for 20 days.

All the animals were fasted for half an hour prior to drug administrations. On day 21, the animals were anaesthetized with ether and blood was collected by retroorbital puncture and the serum separated for estimation of glucose, cholesterol (cholesterol oxidase method) triglyceride and insulin (radioimmunoassay method). Then the animals were sacrificed, a piece of liver was dissected out, weighed and immediately digested with 2 ml of 30 % KOH solution separately, for determination of glycogen as per the method described by Montgomery [9].

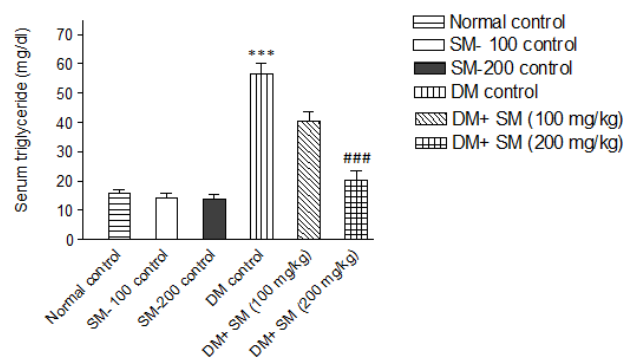
**Statistical Analysis**

The data were expressed as mean ± SE of 6 rats in each group and statistical significance between means was analyzed using one way analysis of variance (ANOVA) followed by Tukey multiple comparison test. A p < 0.05 was considered as statistically significant.

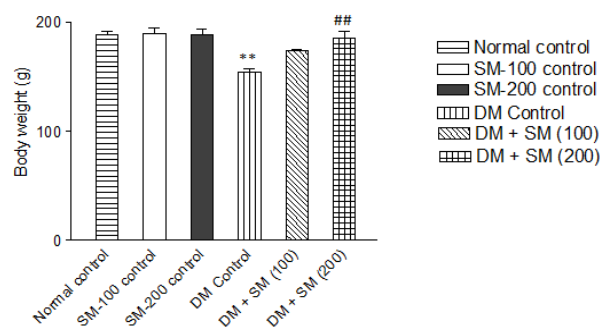
**RESULTS**



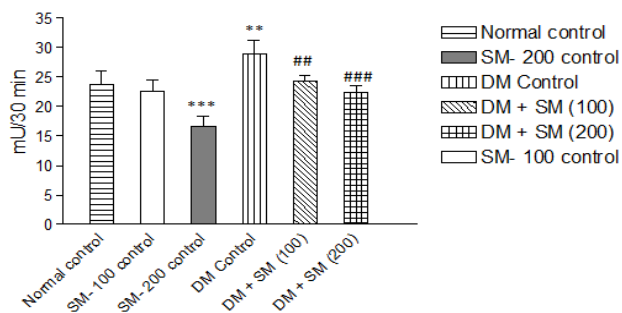
**Fig 1-A Effect of silymarin on serum glucose (mg/dl) in dexamethasone induced insulin resistance**



**Fig 1-B Effect of silymarin on serum triglyceride (mg/dl) in dexamethasone induced insulin resistance.**



**Fig 1-C Effect of silymarin on body weight (g) in dexamethasone induced insulin resistance**



**Fig 1-D Effect of silymarin on insulin secretion from the isolated perfused pancrea (mU/30 min ) in dexamethasone induced insulin resistance.**

Fig. 1-A, B, C and D

Each vertical bar represents the mean  $\pm$  S.E.M. (n=6)

\*\*\* p < 0.001 and \*\* p < 0.01 as compared to the normal control

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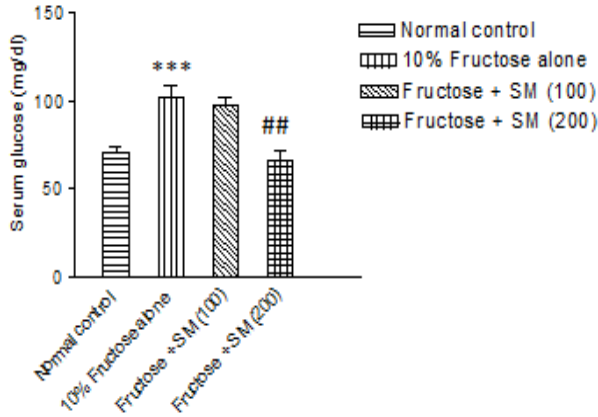


Fig 2-A Effect of silymarin on serum glucose (mg/dl) in fructose induced insulin resistance

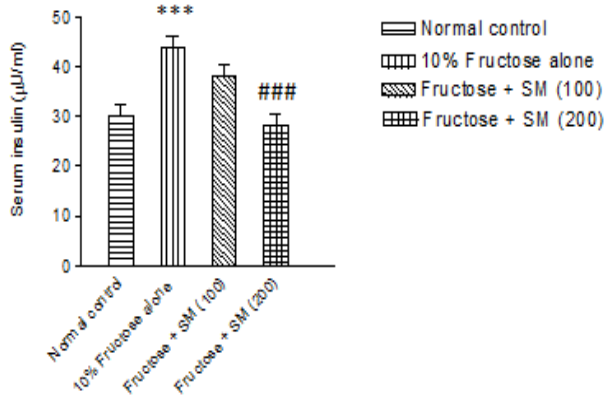


Fig 2-B Effect of silymarin on serum insulin (µU/ml) in fructose induced insulin resistance

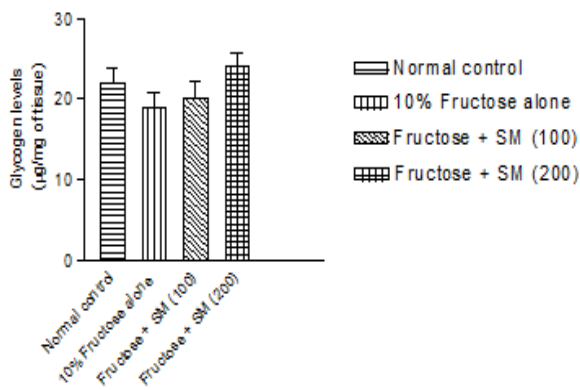


Fig 2-C Effect of silymarin on glycogen levels (µg/mg of tissue) in fructose induced insulin resistance.

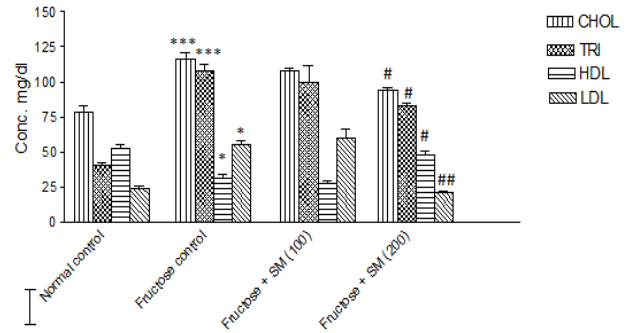


Fig 2-D Effect of silymarin on lipid profile in fructose induced insulin resistance.

Fig. 2-A, B, C and D

Each vertical bar represents the mean  $\pm$  S.E.M. (n=6)

\*\*\* p < 0.001, \*\* p < 0.01 and \*p < 0.05 as compared to the normal control

### p < 0.001, ## p < 0.01 and #p < 0.05 as compared to the normal control

Effects of SM on serum glucose, serum triglyceride and body weight in dexamethasone induced insulin resistance model.

Silymarin alone did not induce any change in serum glucose, serum triglyceride and body weight. In DM-control group there was significant increase in serum glucose level and triglyceride level (p < 0.001) when compared with normal control (Fig. 1-A, 1-B). The simultaneous treatment with DM and SM (200 mg/kg) significantly reduced (p < 0.001) the levels of serum glucose and triglyceride compared to DM - control (Fig. 1-A, 1-B). Significant reduction in body weight was observed in DM-control group when compared with normal control (Fig. 1-C). SM treatment with DM significantly inhibited the dexamethasone induced decrease in body weight (p < 0.01) when compared DM - control (Fig. 1-C).

Non significant decreased in serum glucose and triglyceride levels and inhibition of weight reduction was observed in SM (100 mg/kg) treated group along with DM compare to DM - control (Fig. 1-A, 1-B, 1-C). Reduction in glucose, triglyceride levels and inhibition of reduction in body weight by silymarin in dexamethasone treated rats indicates significant effect of silymarin on dexamethasone induced insulin resistance.

Insulin secretion from the isolated perfused pancreas (Fig. 1-D)

Insulin secretion from the isolated perfused pancreas of animals treated with silymarin alone (SM-200) was significantly lower (P < 0.001) compare to normal control group. Significant increase (p < 0.01) in insulin secretion after dexamethasone treatment was significantly inhibited by simultaneous administration of dexamethasone with silymarin treatment with lower (SM-100, p < 0.01) and higher (SM-200, p < 0.001) dose.

Effects of SM on serum glucose, serum insulin, tissue glycogen level and lipid profile in fructose induced insulin resistance model.

After 21 days of fructose feeding, significant increase (p < 0.001) in serum glucose and insulin levels was observed in fructose fed rats compared to rats from normal control group (Fig. 2-A, 2-B). However, this increase in serum glucose and insulin levels in fructose feeding rats was significantly (p < 0.001) decreased in group treated with SM (200 mg/kg) (Fig. 2-A, 2-B). Silymarin treatment to fructose fed rats increases glycogen levels of tissue compared to fructose control group (Fig. 2-C). Significant increase in serum cholesterol, triglyceride (p < 0.001) and LDL level (p < 0.05) was observed in fructose fed rats compared to normal control group (Fig. 2-D). HDL level significantly decrease (p < 0.05) in fructose fed rats compare to normal control group (Fig. 2-D). Treatment with SM (200 mg/kg) along with fructose feeding reducing the increased

levels of cholesterol, triglyceride and LDL ( $p < 0.01$ ), while increases HDL levels significantly ( $p < 0.05$ ) compare to fructose alone fed rats (Fig. 2-D).

## DISCUSSION

The present work confirms that prolonged dexamethasone administration causes a significant increase in serum glucose concentrations in rats exhibiting a hyperglycemic condition which also observed in previous studies (18, 19). One of the aims of present study was to investigate whether silymarin administration corrects dexamethasone induced hyperglycemia and improves insulin sensitivity rats. Indeed, the administration of silymarin corrects hyperglycemia in both dexamethasone and fructose induced insulin resistance in rats.

In the animals treated with both silymarin and dexamethasone restored nearly normal levels of serum glucose. The dexamethasone induced increase in the secretion of insulin *in vitro* model and serum glucose concentration *in vivo* indicating a state of insulin resistance as previously observed (16, 17) was also reversed by silymarin administration. In the present study silymarin also decreased the levels of serum triglyceride in dexamethasone treated rats.

Administration of fructose for 3 weeks significantly increased the glucose, insulin and causes hyperlipidemic condition which led to insulin resistance, hyperglycemia and hypercholesteremia in fructose fed rat. These abnormalities were prevented by concomitant administration of silymarin with fructose.

Although the mechanism of action of silymarin has not been fully clarified, many of its *in vivo* insulin-like effects have been attributed to its ability to restore peripheral tissue sensitivity to circulating insulin (22). Silymarin reduces insulin secretion without increasing blood glucose concentration. Silymarin might improve insulin resistance through enhanced insulin sensitivity in peripheral tissues as was evident from decrease in blood glucose, insulin and increase in skeletal muscle glycogen stores. It should be expected that silymarin prevented insulin resistance and hypertriglyceridemia underscores the importance of insulin resistance in causing the hypertriglyceridemia and supports the view that the improvement in insulin action was at least partly responsible for the lowering of plasma triglyceride levels (13) with these decreased lipid content could be result of diminish hepatic cholesterol and triglyceride synthesis by silymarin (15). On the other hand, present studies do not rule out the possibility that hypertriglyceridemia itself may contribute to insulin resistance.

Insulin resistance plays a major role in the onset and progression of diabetes. Many factors cause insulin resistance like obesity, high-fat diets, insufficient exercise, and stress. Combination of all effects of silymarin observed in the present study could be useful in states of hyperinsulinaemic hyperglycemia such as NIDDM. Thus the results obtained in the present investigation indicate that silymarin may have considerable therapeutic potential in the treatment of insulin resistance in NIDDM and its complications.

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